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Nanoghosts for therapeutic applications

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VOLUME THREE

ADVANCED NANOFORMULATIONS

Theranostic Nanosystems

Edited by
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Advanced Nanoformulations

Theranostic Nanosystems, Volume 3

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Preface

Advanced Nanoformulations

Recently, numerous theranostic nanomaterials have emerged as promising tools in healthcare disciplines because of their multifunctional features that help in early diagnosis, imaging, and effective therapy. As a result of nanotechnological progress, the next-generation theranostic nanoformulations that combine imaging and therapeutic functions on a single platform have already been produced. These sophisticated theranostic nanoformulations enable us to diagnose, distribute drugs, and monitor therapy responses in real time, thereby minimizing the risk of over- and underdosing. As a result, they look forward to have a bright future in the era of personalized therapeutics. An ideal theranostic nanoformulation not only accurately diagnoses diseases at their early stages but also delivers the most effective therapy. Recently, there have been a number of advanced nanoformulations as theranostic nanosystems (such as nanosuspensions, nanoemulsions, self-nanoemulsifying systems, polymeric micelles, dendrimers, nanogels, nanocrystals, niosomes, cubosomes, aquasomes, nanophytomedicines, nanostructured lipid carriers, nanofibers, nanovectors, nanoprobes, nanorobots, nanoghosts, nanoconjugate formulations, multifunctional nanocomposites, self-assembled protein-drug nanoparticles, biomimetic nanosystems, stimuli-responsive nanosystems, and cell-penetrating peptide-modified nanosystems) developed for efficient diagnosis and therapy of various diseases. In this context, the current book volume, "*Advanced Nanoformulations: Theranostic Nanosystems, Volume 3*," serves as a valuable resource for readers, providing detailed information about advanced nanoformulations as theranostic nanosystems and how they function as both diagnostic and therapeutic tools in the effective management of various complex diseases.

The current book volume covers the recent innovations in the development of advanced theranostic nanoformulations with a collection of 28 chapters by leading academicians and researchers across the world. To provide readers with a clear overview of the current book, a concise synopsis of each chapter's contents has been provided.

Chapter 1 highlights some of the recent developments in nanoformulations possessing theranostic properties for critical conditions, including cancer, cardiovascular, autoimmune, and neurodegenerative diseases. Finally, the challenges and prospects of nanoformulations in theranostic applications have also been discussed.

Chapter 2 presents nanosuspensions as nanoformulations, with a special focus on the synthesis of chitosan-based nanosuspensions and their potential uses for translational ophthalmic applications.

Chapter 3 discusses various essential aspects of nanoemulsions, including their design and development, with a focus on their theranostic applications.

Chapter 4 focuses on the types of nanoemulsion-based systems and their applications in drug delivery. Moreover, the methods of preparation and characterization of the most common nanoemulsion-based systems have been summarized.

Chapter 5 discusses recent advances in the development and utilization of block copolymer micelles and related nanoparticulate systems for selective and targeted drug delivery and diagnosis. In addition, several examples from the literature have been used to elucidate the properties and multifunctionality of polymeric micelles in theranostic applications.

Chapter 6 aims to comprehensively discuss different theranostic systems based on dendrimers or dendrons bound to nanoparticles.

Chapter 7 covers different important aspects of nanogels, such as polymers for nanogels' composition, types of nanogels, and preparation methods, with a special emphasis on their theranostic applications in drug delivery, targeting, and imaging.

Chapter 8 reviews the advancements in the field of nanocrystals for theranostic applications, with a focus on the most promising application pathways, namely, therapeutic drug monitoring and image-guided therapy in cancer nanotheranostics. This includes platforms for the delivery of sparingly water-soluble drugs, targeted drug delivery platforms, nanotheranostic platforms for chemotherapy, photodynamic therapy, and photothermal therapy, with a discussion on the stabilization, functionalization, and decoration of nanocrystals for theranostic applications.

Chapter 9 deals with comprehensive discussions on the methods of niosome preparation, characterization techniques of niosomes, preclinical and clinical trial reports, and their numerous types of drug delivery applications.

Chapter 10 spotlights the background of cubosomes' discovery, their composition, methods of preparation, and applications in drug delivery, targeting therapeutics, and theranostics.

Chapter 11 presents the preparation, properties, applications, and potentials of aquasomes as effective drug delivery nanocarriers.

Chapter 12 discusses the drug delivery potential of nanostructured lipid carriers for the delivery of a variety of drugs as well as their biomedical applications.

Chapter 13 highlights the fabrication methods for self-assembly, the factors involved in the formation dynamics of protein nanoparticles, and the implications and challenges of self-assembled protein-drug nanoparticles related to their applications for enhanced drug delivery and targeting therapeutics.

Chapter 14 summarizes the most relevant physicochemical aspects of novel stimuli-responsive nanotheranostics. In addition, recent examples of functional and clinically relevant nanotheranostics have also been addressed.

Chapter 15 covers the basics of nanoconjugation and nanoconjugates, with a focus on nanoconjugate formulations for improving drug delivery and therapeutic efficacy.

Chapter 16 provides a brief overview of nanophytomedicines as advanced nanoformulations for the delivery of herbal medicine as well as their therapeutical applications.

Chapter 17 describes different multifunctional theranostic nanocomposites designed for imaging and drug delivery applied in different diseases, including cancer, osteoporosis, cerebral ischemia, and cardiovascular diseases.

Chapter 18 overviews the uses of nanofibers as advanced nanoformulations in biomedical applications, including diagnosis, drug delivery, and therapy.

Chapter 19 encompasses the preliminary introduction of nanovectors, their classifications, and theranostic applications in the effective management of different diseases.

Chapter 20 summarizes the current advancement of several nanocarriers as nanoprobes for advanced nanotheranostic applications and their preclinical and clinical successes.

Chapter 21 gives a general overview of nanorobots, including their parts and the different types of nanorobotic systems. The focus is on their possible benefits and uses, such as targeted drug delivery, precise surgery, medical diagnosis, and detoxification.

Chapter 22 shows what nanoghosts are, how they are made, how they are characterized, and how they can be used in therapy.

Chapter 23 mainly highlights and discusses evolving progress in the fabrication and application of biomimetic nanoparticles, with an emphasis on clinical impact and translation. The challenges and limitations associated with the emerging technology have been addressed to contribute to further innovations in the field.

Chapter 24 discusses the advances in the applications of different nanoformulations for cardiovascular therapy.

Chapter 25 addresses the current status of therapeutic nanomaterials and nanoformulations for neurological therapeutics. Future directions, such as improving their permeability and reducing their neurotoxicity, have also been addressed.

Chapter 26 presents important insights into the development and application of multifunctional nanoformulations and nanotheranostics in terms of targeted imaging and therapy of diseases such as cancer.

Chapter 27 describes the different methods of peptide synthesis, the role of enzymes, and the applications of cell-penetrating peptides conjugated to theranostic nanocarriers for the effective treatment of cancer.

Chapter 28 gives an overview of the development of polymeric hydrogel systems based on natural and synthetic polymeric components (alginate, gelatin, and 2-hydroxyethyl methacrylate) as well as inorganic agents (nanographene oxide and nanotitanium dioxide).

To help us publish this book, the editors would like to express their heartfelt gratitude to all of the accomplished authors for their timely and valuable contributions. We especially acknowledge the tremendous help with editing provided by *Elsevier Inc*., *Erin Hill-Parks*, and *Andre Gerhard Wolff*. We want to sincerely thank *Ivy Dawn Torre* and *Howi De Ramos* (Editorial Project Manager) for their important support while the book was being created. We sincerely thank *Mohan Raj Rajendran* (Copyright Coordinator) for her essential help in obtaining clearances and *Swapna Srinivasan* (PM) for providing the finished book. All permissions for the reprinting of intellectual property from different sources have been gratefully appreciated. Finally, we must recognize the efforts and contributions made by our family members to the creation of the book. We owe a debt of gratitude to all of our friends, coworkers, and students who contributed to the creation of this book. We will be happy, along with our contributing authors and publishers, if our work meets the needs of students, academics, scientists, drug delivery formulators, pharmacy professionals, polymer engineers, biomedical scientists, and others.

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CHAPTER

Polymer-based nanotheranostics: current status and challenges

1

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1.1 Introduction

Even though the terms nanomedicine and/or nanobiotechnology are synonymous, many scientists are not sure what they represent. The discipline of nanomedicine is concerned with creating all the nanosized tools for diagnosing diseases, their prevention, and therapy. This includes outdoor patients for diagnostics, surgical and biosensor instruments as well as sophisticated biomaterials for tissue engineering and their repair regarding nanomedicine. The patient is at the heart of nanomedicine and nanotechnology plays a major role in clinical trials. The term "nanobiotechnology" refers to all the biomedical engineering currently focused fundamentally on the biologically associated with the physicochemical aspects of cellular nanoscale as well as nanomaterials. Nanomedicine includes theranostics, nanopharmaceuticals, and nanoimaging agents. Polymeric micelles, nanoliposomes, nanocrystals, carbon nanotubes, nanoparticles (NPs) like polymeric NPs and inorganic NPs, etc., are the nanocarriers, which recently have received extensive attention (Kumar, Aadil, Ranjan, & Kumar, 2020). However, it is worth mentioning that over the past two decades, more than 40 kinds of nanomaterials have been licensed for routine human usage, and several of them are in clinical trials, today (Duncan & Gaspar, 2011; Prabhu & Patravale, 2012; Suresh, 2007). Pharmaceutical nanotechnologies (with sizes from 1 to 1000 nm) are rationally developed for a certain route of administration and to provide treatment for specific ailments.

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With the advent of nanotheranostics and an evolving discipline of nanotechnology, there is dramatic hope in the health sector for slower delivery of drugs, imaging modalities, and diagnosis. This new branch of nanotechnology intends to combine diagnostics and therapies to produce more effective results in the treatment of many deadly diseases (Chen, Gambhir, & Cheon, 2011; Lammers, Aime, Hennink, Storm, & Kiessling, 2011; Prabhu & Patravale, 2012). However, NPs may act as multifunctional agents due to their unique features; for example, gold (Au) has numerous unique qualities, such as surface functionalization, plasmon resonances, photothermal (PT) ablation, and detecting ease (Day et al., 2010; Shin et al., 2009; Sironmani & Daniel, 2011). The different components related to polymeric theranostics are depicted in Fig. 1.1. The optical and quantitative phenomena of surface plasmon resonance (SPR) can occur when the input light is transformed into scattered and absorbed components. The scattered part provides the optical qualities, whereas the absorbed portion provides the thermal effect. It is used to determine the real-time molecule binding kinetics. It is a label-free, extremely sensitive detection approach that just needs a small amount of the sample for handling, whereas other techniques, such as ELISA can only provide the binding affinity; SPR provides the binding kinetics or the on-and-off phenomenon caused by the molecular attachment and dissociation. The primary drawback the plasmon resonant NPs is that they have low sensitivity due to the background scattering from the cells and tissues (Homola, Yee, & Gauglitz, 1999; Jiang & Török, 2014; Pattnaik, 2005).

The procedures for PT can help to increase image quality. Electromagnetic radiation, usually near-infrared (NIR) wavelengths, is utilized in photothermal treatment (PTT). Heat is produced when NIR radiation is absorbed and the heat destroys the cells in the area. This method has effectively been employed to eliminate local malignant cells and cancer cells that have spread throughout the body

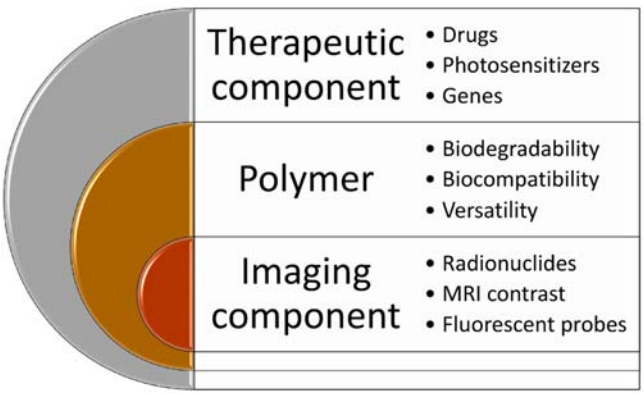

FIGURE 1.1

Different components related to polymeric theranostics.

(Huang, Jain, El-Sayed, & El-Sayed, 2008). PTT is a type of photodynamic therapy that has been extended for practical use. A photosensitizer is utilized in the photodynamic treatment and is administered directly into the bloodstream. Photosensitizers are taken up by all the body cells. Normal body cells, on the other hand, release photosensitizers more quickly than cancer cells. Only diseased cells retain most of the photosensitizers after 24−72 h. On being bombarded with a laser beam on the human body, using a specific wavelength, specifically cancerous invaded cells are exposed to radiation. Reactive oxygen species (ROS) are produced by a photosensitizer, which damages the adjacent cells (Lu et al., 2021). PTT, on the other hand, has benefits over photodynamic therapy. PTT has more substantial penetration and can be utilized to treat deep cancer as well as cancer metastasis. However, PT procedures demand higher laser-induced temperatures, which can be harmful to cells and molecules (Doughty et al., 2019).

The therapeutic index of drugs and substances imaged at the desired target can be improved using the targeted drug delivery systems. However, the convergence of therapeutics, diagnostics as well as nanotechnology might play a crucial role in customized and precision medicine enabling on-demand pharmaceutical delivery (Dai, Liu, Luo, Li, & Cai, 2016; Muro, 2012). Thus, nanotheranostics offer a once-in-a-lifetime opportunity to integrate numerous components, such as specific therapeutic chemicals, controlled-release mechanisms, targeting methods, and reporting capability into a nanoscaled architecture for the therapeutic detection and visualization (Alnasser, Shaw, & Santra, 2019). A broad overview of polymeric nanotheranostics with current status and challenges is presented in this chapter.

1.2 Polymeric theranostics

The polymer-drug conjugates as nanotheranostics are being researched using a variety of polymers. The polymeric chains can open functional groups that permit them to be combined with various agents like therapeutics, imaging, and targeting moieties, making them a viable material for nanotheranostics applications (Lee, Ponta, & Bae, 2010). Polymeric NPs are a type of nanocarrier system used in various medication delivery applications (Al-Jamal et al., 2009). These can be generated through the copolymer self-assembly or multiple functional units are conjugated to soluble macromolecules in this method. The nanoparticle cores can indeed be loaded with a diverse array of therapeutic or imaging substances in a typical self-assembled formulation. In a typical self-assembled formulation, the agents are released in a sustained and controlled manner via surface or bulk erosion, diffusion over the polymeric matrix, swelling accompanied by diffusion, or stimulation by the surrounding environment (Peer et al., 2007).

Drug molecules can be conjugated to a polymeric backbone, allowing for specific loading of the drug control release of a drug profile (Aryal, Hu, & Zhang, 2011).

Stealth materials like PEG shield the polymer cores to provide stability while decreasing immunogenicity. Targeting moieties can be presented to the surface in a more detailed manner. Biocompatible polymeric NPs have been developed using polymers obtained from natural resources like cyclodextrin and chitosan (Liu, Jiao, Wang, Zhou, & Zhang, 2008). In the meantime, a wide range of synthetic polymers with excellent biocompatibility and biodegradability are accessible to formulate polymeric NPs. Polymeric NPs are increasingly being used in the development of theranostic platforms (Hoskins, Thoo-Lin, & Cheng, 2012; Hu et al., 2009; Miller, Batrakova, Waltner, Alakhov, & Kabanov, 1997).

Polymeric NPs-based delivery methods have already shown a significant impact on biomedical applications that can combine therapy and diagnostics. In recent years, nanotechnology, biotechnology, and polymer science are combining the foundation for developing new nanocarriers for diagnostic and therapeutic uses. Compared to the typical therapeutic nanocarriers, polymeric NPs facilitate better solubility of hydrophobic drugs, increased circulation of drugs in the bloodstream, and site-specific delivery to the target regions (Elsabahy & Wooley, 2012; Fornaguera & Solans, 2016; Hasnain et al., 2019; Owens & Peppas, 2006; Park, Lee, et al., 2008; Vauthier & Bouchemal, 2009). Because of the multiple benefits of nanocarriers, medical science is now focusing on the development of multifunctional polymeric hybrid systems at the nanoscale. Polymeric micelles, liposomes, biodegradable polymeric NPs, etc., are all covered by polymeric nanocarriers. Because of the flexibility and biodegradability of polymeric nanocarriers, these can be utilized to deliver the encapsulated biomolecules including hydrophilic/hydrophobic drugs and biomolecules like peptides, proteins, DNA, and RNA in a controlled manner (Alexis, Pridgen, Molnar, & Farokhzad, 2008; Cohen et al., 2000; Yuan et al., 2006). Designed NPs should possess particularly favorable properties to create an efficient and versatile polymeric nanocarrier system for therapeutic use. The size of polymeric nanocarriers is well acknowledged to play a significant role in defining the fate of the particles *in vivo*. Polymeric nanocarriers are typically between 10 and 200 nm in size, allowing for particle biodistribution in the human system (Ishihara, Chen, Liu, Tsukamoto, & Inoue, 2016; Sironmani & Daniel, 2011).

1.3 Polymers for nanotheranostics

Synthetic and natural polymers that have been approved by the Food and Drug Administration (FDA) have already reached the medical and pharmaceutical markets (Bhojani, Van Dort, Rehemtulla, & Ross, 2010). Polymers provide a great deal of flexibility in terms of tweaking their physicochemical properties based on the number of monomers and functional groups present. The applications of polymeric nanotheranostics in pharmaceutical technology are represented in Fig. 1.2.

Few biocompatible polymers, including poly(lactic acid) (PLA), poly(lactic-*co*-glycolic acid) (PLGA), poly(ethylene glycol) (PEG), polycaprolactone (PCL), and

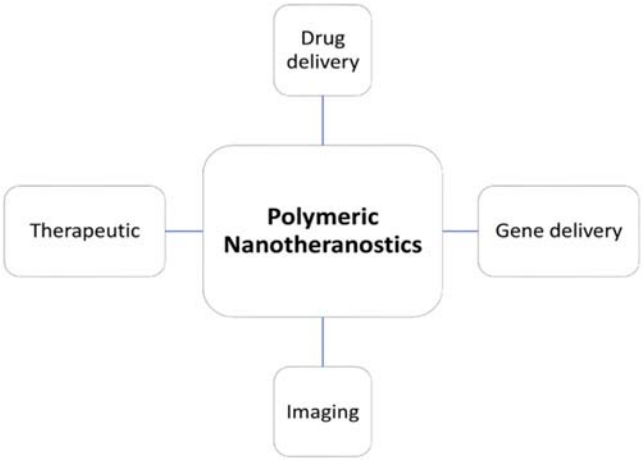

FIGURE 1.2

Applications of polymeric nanotheranostics in pharmaceutical technology.

chitosan, etc., deserve a special mention among the many biocompatible polymers utilized for cancer theranostics. With the rise of polymer conjugate systems, the diversity of such polymer-based systems has reached unprecedented heights.

A contrast agent for the identification and imaging, a pharmacotherapy agent, a polymeric matrix to carry both, and an additional polymeric interface for enhancing the stability and biodistribution can also be used in specific situations for integrating the targeting, which is the primary complementary components in a typical polymer-based nanotheranostic construct.

1.3.1 PEG

PEG is a water-soluble polymer candidate that is commonly found in numerous theranostic platforms to lengthen their residence time in the biological domain and inhibit reticuloendothelial system (RES) enzymatic degradation of encapsulated moieties (Patel & Mirosevich, 2019). It also allows the system to invisibly combat the host immune system by imitating them as indigenous molecules (Goodson & Katre, 1990; Hoskins et al., 2012). PEG serves as a carrier material for therapeutic and diagnostic agents by covalently or electrostatically coupling with them or surrounding them within the micelle systems by another polymer attached. The hydrophobic interaction between the conjugated polymeric blocks and the immediate environment as well as therapeutic molecules can be substantially responsible for the development and stability of polymeric conjugate-based micelle systems. Moreover, these PEG-based micelles possess high chain mobility in an aqueous environment, resulting in a large exclusion volume around the polymers, and prohibiting interaction with biological fluid components.

PEG is being utilized to modify proteins as well as peptides such that they can be delivered to the cancerous cells more effectively. The PEG entity is coupled with proteins at specific locations, like the −SH group in cysteine and the amino group at the N terminus. These systems have been found to overcome biological activity loss and reduce immunogenicity (Goodson & Katre, 1990). Cancerous cells are auxotrophic for certain amino acids, including arginine, asparagine, and methionine, which cannot be synthesized. PEG has been used to tag the enzymes in cancer therapy. PEG-tagged methionine is also used in certain circumstances (Guo & Huang, 2011; Hofmann, Pressman, Code, & Witztum, 1983). PEGylated proteins for the treatment of cancers can combine the anticancer effects of many drugs by functioning as carriers for all of them simultaneously (Pasut et al., 2008). Besides this, various poly(amino acid)-PEG micelle systems are currently undergoing phase I clinical trials.

1.3.2 PLGA

PLGA is amongst one of the most widely utilized biocompatible polymers for developing nanoscale theranostics. Drugs, proteins, peptides, RNA, and DNA are among the therapeutic components of such formulations (Bouissou, 2006; Ruhé et al., 2006). Shi et al. developed an innovative PLGA-based theranostic system in which anticancer medication paclitaxel and magnetic resonance imaging (MRI) contrast magnetic NPs were encapsulated by using PLGA (Shi et al., 2020). Fluorescent quantum dots (QDs) were grafted onto the surface to help track it under the in vivo settings. Apart from providing contrast in MRI, the iron oxide nanoparticles in the complex are also used for hyperthermia, thereby, enhancing the therapeutic effects of paclitaxel on the cancer cells. The theranostic system was then coupled with an antiprostate-specific membrane antigen antibody to achieve single-cell delivery to the cancer cells. For cancer-specific theranostic uses, the system was validated, in vitro and in vivo (Wang & Thanou, 2010).

Theranostic applications of PLGA NPs date back to 2005 when McCarthy et al., employed to encapsulate and transport meso-tetraphenyl porpholactol, which served as imaging and cytotoxic substance after being activated by an externally applied source (McCarthy, Perez, Brückner, & Weissleder, 2005). With simple stimulation by an externally visible source of light, the compound was found to be efficient in treating malignancies in animal models.

1.3.3 Chitosan

Chitosan is a biodegradable natural polymer with an ionizable amino group well suited for functionalization with medicinal and/or diagnostic compounds. Because of the solubility of chitosan, numerous additional water-soluble derivatives of chitosan, such as glycol chitosan and carboxy methyl chitosan have been synthesized. Lee et al., employed glycol chitosan to deliver chlorin e6 (Ce6), which might be used for both in vivo imaging and photodynamic treatment (Lee et al., 2011). The Ce6

molecules were attached to hydrophobically modified glycol chitosan, that is, glycol chitosan-5-cholanic acid to increase the Ce6 release profile, circulatory half-life, and passive accumulation in the tumors. Theranostic system buildup was seen at the tumor locations using in vivo NIR imaging in mice models. The following prognosis of the tumor after the laser irradiation revealed tumor necrosis and decreased tumor volume. Kyung et al. used the same carrier molecule (i.e., 5-cholanic acid-modified glycol chitosan) to encapsulate and deliver hydrophobic anticancerous medication camptothecin (Park, Bae, Joung, Shin, & Park, 2008). When tested in nude mice implanted with MDA-MB231 human breast cancer xenografts, the carrier offered a prolonged and sustained release profile, lasting for 5−6 days as well as it showed the tendency to accumulate at the tumor site passively. During the duration of treatment, NIR fluorescence confirmed a significant reduction in tumor size. In a separate study, glycol chitosan-cholanic acid NPs were employed to encapsulate and distribute doxorubicin and Bcl-2 siRNA to overcome drug resistance and facilitate the effective use of anticancer action by doxorubicin (DOX). A noninvasive NIR fluorescence imaging system was used to track the in vivo fate of both glycol chitosan-based NPs (Yoon et al., 2014). The identical amphipathic form of chitosan was proven to deliver tumor-targeted delivery (Lee et al., 2016). The surface functionalization of tumor cells with azide groups was possible by the targeted delivery of precursor molecules by a glycol chitosan-cholanic acid carrier molecule.

Chitosan, which has many cationic amine groups, is commonly utilized to transport nucleic acid to cancerous cells. Despite the promising outcomes in the in vitro transfer of siRNA as well as DNA to cancerous cells, the in vivo potential application of chitosan is constrained because it interacts with the extracellular matrix (ECM) (Ki et al., 2014).

1.3.4 Polymeric micelles

Polymeric micelles have received much attention, in recent decades, as a multifunctional nanosystem aimed at cancer treatment. These nanocarriers are currently being used in preclinical and clinical investigations with great success. Polymeric micelles, to put it simply, are nanoplatforms with a hydrophilic shell and a hydrophobic core. Due to their various thermodynamic and kinetic stability as well as their large payload and small size, they are specifically suitable for disease therapeutics (Sonali et al., 2018). Polymeric micelles containing superparamagnetic NPs and DOX were investigated as multifunctional MRI and therapeutic delivery agents (Guthi et al., 2010). PEG−polylactic acid micelles were also used as a 3-in-1 NP-based delivery of drugs of hydrophobic nature like 17-allyamino-17-demethoxygeldanamycin, paclitaxel, and rapamycin to be used as therapy in cancerous patients. Micelles of polyethylene glycol-block-poly-caprolactone (PEG-b-PCL) with carbocyanine drug were employed as a NIR optical imaging agent (Cho & Kwon, 2011). Therefore, as consequence, these techniques show how to manage polymeric micelles for many theranostic implementations.

1.4 Concluding remarks, future implications, and prospects

With the advent of new equipment facilities that can test the material properties on a nanoscale scale, researchers have shifted their focus to designing materials that are only a few nanometers thick to meet therapeutic needs. Furthermore, with a significant increase in the prevalence of cancer, hazardous adverse effects of the newly produced medications, and the need for delivery of drugs to the proper diseased site at a specific time, the right dosage form and quantity of dosage are important for researchers to consider alternate cancer treatment options. In this sense, polymeric nanocarriers have found a prime position at this crossroad because of their potential applications in disease imaging, diagnosis, and therapy monitoring. It is occasionally possible to create multifunctional NPs that can serve as both a therapy and a diagnostic tool. These materials hold great promise for developing next-generation theranostic agents and tailored medicine to meet clinical challenges. The biological aspects, nanoparticle properties, and chemical as well as physical properties of the novel pharmaceuticals have created tremendous anticipation to challenge from a large number of options to build and change polymeric nanocarriers. For an effective medication delivery, biological barriers, such as muscle, skin, cellular, and mucosal parts must be traversed in addition to biological characteristics. Furthermore, diversity, stability, nonspecific toxicity, and half-life of NPs must be finely tuned to avoid identifying the RES and body parts such as the kidney. Although polymeric nanocarriers have shown high promise, they still face many obstacles in terms of biological applications and future translation to clinical applications.

The rapid advancement in nanotechnology has geared enormous relevance in therapeutic applications, particularly in cancer. Because the traditional therapeutic methods are yet to overcome these limits, many adverse effects have resulted. Nanotechnology has proven to be a successful method of overcoming these constraints. The inherent features of NPs allow them to combine diagnostic as well as therapeutic applications onto a solitary platform. As a result, this encourages focused drug distribution by improving biocompatibility and pharmacokinetics potential for cancer treatment. Diagnosis allows clinicians to emphasize and obtain accurate data for clinical purposes. At the moment, nanotechnology is making much progress toward real-time monitoring of illness therapy, which is a bigger accomplishment than therapeutics. The nanotechnology-based cancer therapy is now progressing toward disease treatment without the use of medicines. These could boost nanotechnology to new heights. Liquid metal NPs, neat NPs, and QDs are currently being studied extensively for theranostics applications. Even if people are debating their usefulness and effectiveness in a variety of disciplines at the moment, nanotoxicological issues are a bigger concern than their uses. Then, in biological systems, physiochemical properties like shape, size, stability as well as surface functionality, may produce toxicity, which is influenced by extrinsic factors like organismal characteristics, such as age, nature of targeting sites, and internal environmental pH to which NPs are sensitive. As a result, appropriate formulation and manipulation could greatly assist nanotechnology in progressing to the next level of treatment.

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CHAPTER

Chitosan-based nanosuspensions for ocular diagnosis and therapy

2

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2.1 Introduction

Throughout the past decades, the introduction of nanotechnology in the biomedical and pharmacology field (nanomedicine) has awakened a revolution around its potential application as diagnostic and therapeutic agents. That is to say, nanosuspensions allowed the use of tools to analyze tissue structures at both molecular and cellular scales, as well as allowing the design of compatible biomaterials that can be used in various therapies. Nanosuspensions can be defined as dispersions consisting of very fine colloidal solid drug particles which are biphasic in nature, in an aqueous vehicle, and stabilized through surfactants. These systems present several advantages such as reduced toxicity, improved bioavailability, targeted drug delivery to a specific site, reduced dosing frequency, sustained and controlled release effects, high patient compliance, better stability, ease of administration through different routes, etc. (Agrawal & Patel, 2011; Goel, Sachdeva, & Agarwal, 2019).

Nanomedicine has also made possible the development of techniques to release molecules that have difficulty reaching their sites of action, as well as protect them from degradation or avoiding an immune response in the body by promoting the controlled release of the drug. These systems are formed by nanosuspensions between 1 and 1000 nm in diameter and have been designed to increase the bioavailability of drugs at the site of action or reduce their toxicity, among other advantages (Özsoy, Güngör, Kahraman, Ezgi, & Durgun, 2019). This projection is not just a "science fiction dream," on the contrary, it is a reality nowadays. In this regard, scientists have been able to mold nanosuspensions into tools with a reliably efficient ability to transport, protect and improve the bioavailability, solubility, permeability, stability, and therapeutic efficacy of drug molecules in the specific organ with sustained release, especially for diagnosis.

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Particularly, regarding the latter, most of the reports focus on their use in cancer, for example, when nanosuspensions are laden with imaging probes, this type of system offers the chance to develop magnetic or optical resonance imaging.

Polymeric materials are typified by a variety of functionalities in which therapeutic and diagnosis could be combined, providing a dual innovative platform (Özsoy et al., 2019). This is the case for both synthetic polymers (e.g., polyethylene glycol, polyglutamic acid, polylactic acid, polycaprolactone, poly-D,L-lactide-*co*-glycolide, and *N*-(2-hydroxypropyl)-methacrylamide copolymers), as well natural polymers (e.g., dextran, alginate, collagen, chitosan). Now, why polymers? Because of their striking qualities: the adaptability to different formulations, resistance to mechanical stress, biocompatibility, and the option to adapt their properties such as stability, solubility, degradation, and swelling (Ilaria Parisi, Scrivano, Stefania Sinicropi, Picci, & Puoci, 2016). One of the natural polymers that have gained high popularity in the last decade is chitosan, which is produced by the deacetylation of chitin. A wide range of methods have been established in the preparation of chitosan nanosuspension involving emulsification, covalent cross-linking or coupling, polyelectrolyte complexation, complex coacervation, emulsion solvent diffusion evaporation, self-assembly, polyelectrolyte complex, spray drying, microemulsion, and ionic gelation, among others (Jacob, Nair, & Shah, 2020; Vichare et al., 2020) (Fig. 2.1).

The eye is a complex organ with two central anatomical segments: the anterior segment and the posterior segments. Hence, ocular therapy and diagnosis have been a classic challenge without a reliable solution for pharmacologists and ophthalmologists. That is to say, multiple barriers especially hamper the drug

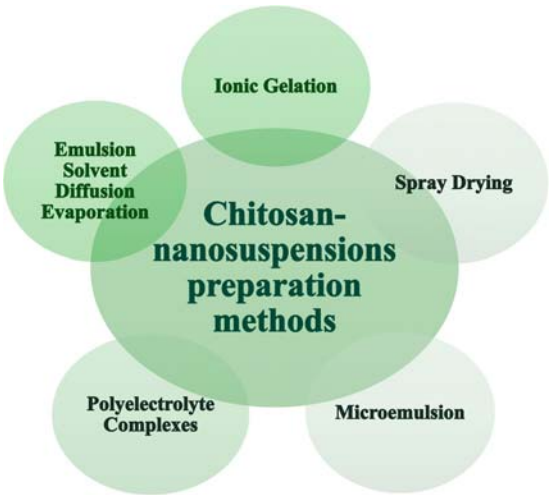

FIGURE 2.1

Schematic description of different methods of chitosan-based nanosuspension design.

delivery and treatment from the cornea to the choroid. In this regard, nanotechnology joins into this landscape as a crucial player to improve the two obstacles mentioned above. Particularly, chitosan-based nanoparticles are getting much attention and several reports demonstrated their promising results in the ophthalmic field (Gorantla et al., 2020; Özsoy et al., 2019).

In the present chapter, chitosan-based nanosuspensions synthesis and its potential use for translational ophthalmic applications are discussed here in the following sections.

2.2 Chitosan, the "biopolymer of the 21st century"

Henri Braconnot, a French chemist, and pharmacist, described in 1811 a polysaccharide in an edible fungus containing a considerable proportion of nitrogen, which would later be called chitin (Muzzarelli et al., 2012). Chitin is considered the second most abundant polysaccharide after collagen and cellulose on Earth. Unlike the latter, chitin has a source of nitrogen as well as carbon. The main natural sources of this organic polymer are the exoskeleton of crustaceans (e.g., crabs, shrimps, lobsters, krill, prawns) and 'mollusks' feathers or leaves (e.g., squid and cuttles), insects' cuticles (e.g., ants, spiders, cockroaches, beetles, scorpions); also, chitin is found in polychaete annelids worms' chaetae, Coelenterata and in the cell walls of certain fungi, green and browns algae. Notably, and less well known, is that bony fish *Paralipophrys trigloides* (*Blenniidae*), has a chitinous epidermal cuticle. Therefore, chitin is not only an exclusive component of invertebrates, but it can also be present in vertebrates. Among all the sources described, 70% are related to marine species (Jiménez-Gómez, Cecilia, Guidotti, & Soengas, 2020) (Fig. 2.2).

Despite the various natural sources, the main ones used are those of crustaceans. In this sense, there is a very well-described and fine-tuned protocol. The procedure to obtain chitin consists of the following main steps: (1) *Deproteinization and lipolysis*, (2) *demineralization*, and (3) *bleaching*. The first stage is carried out using an alkaline treatment (NaOH, KOH, Na_2SO_3, Na_2CO_3), by means of which lipids and proteins are hydrolyzed. The second step normally occurs in the presence of acids (HCl, HNO_3, CH3COOH, HCOOH). The last stage requires oxidative treatment (Acetone, ethyl alcohol, diethyl ether, $KMnO_4$, $NaClO/H_2O_2$). Nonetheless, despite the reduced applications of chitin, there is an expanding interest in the pharmaceutical and biomedical areas since it can be converted into chitosan. Both polysaccharide polymers have the same chemical structure; however, chitin, a white color nitrogenous polysaccharide, consists of a linear homopolysaccharide formed by *N*-acetyl-β-D-glucosamine units. On the other hand, chitosan, whose pigmentation varies from pale yellow to white, comprises β-1,4-linked 2-amino-2-deoxy-β-D-glucose (deacetylated D-glucosamine) and *N*-acetyl-D-glucosamine units (Jiménez-Gómez et al., 2020; Pillai, Paul, & Sharma, 2009; Yeul & Rayalu, 2013) (Fig. 2.2).

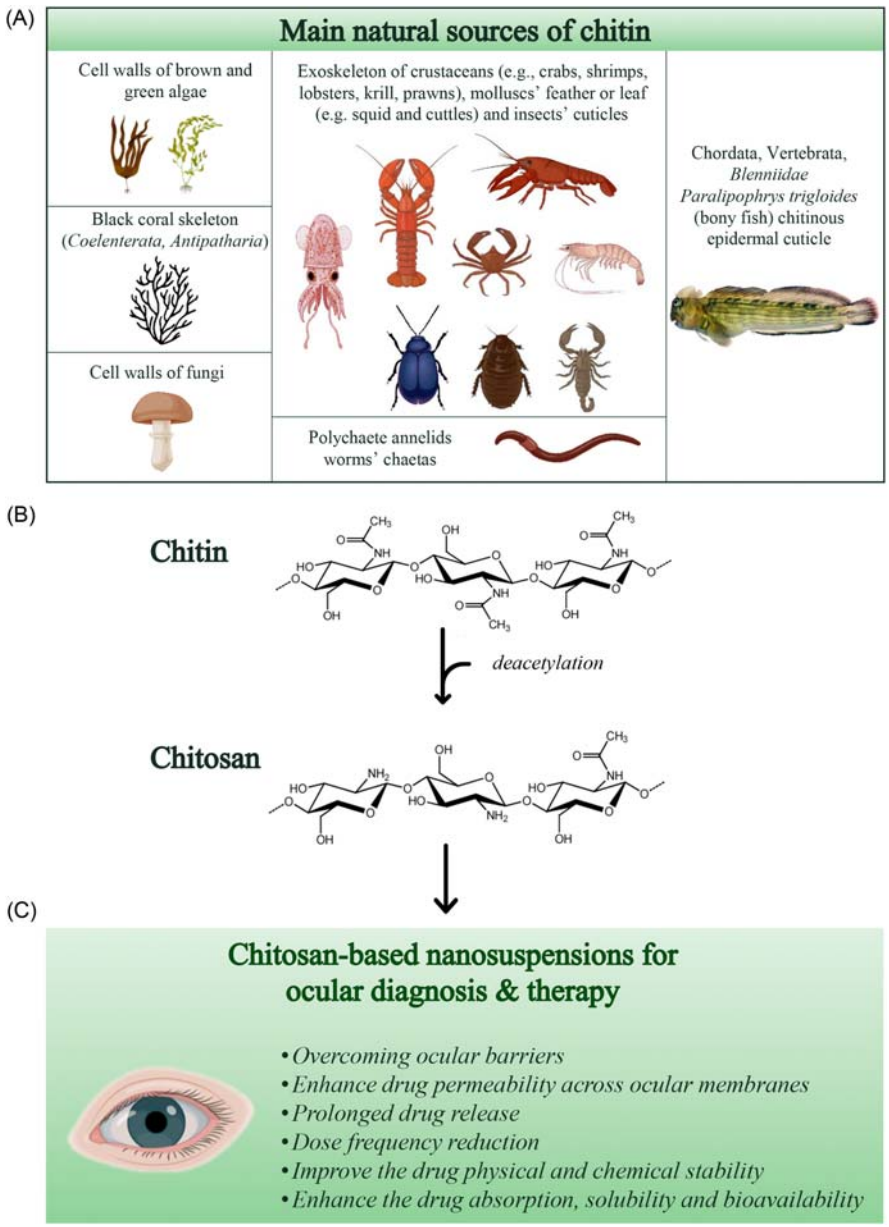

FIGURE 2.2

(A) Main natural sources of chitin. (B) Chitin and chitosan structures. (C) List of limitations of drugs for therapy and diagnosis that can be overcome by the application of chitosan-based nanosuspensions.

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The term chitosan was first introduced in 1874 by the German physiologist and chemist Felix Hoppe-Seyler, who has also been a founder of disciplines such as biochemistry and molecular biology. Chitosan is nowadays considered the biopolymer of the 21st century since the research and subsequent employment has grown exponentially in recent decades.

Chitosan is obtained after the removal of a large number of acetyl groups from the chitin molecule through an alkaline treatment (NaOH/KOH). The latter chemical process is called *deacetylation* and rise amine groups. These chemical groups give the chitosan molecule the cationic characteristic and are responsible for the versatility properties of this biopolymer in diverse areas such as the food industry, agriculture, medicine, and biotechnology. In particular, chitosan is an attractive biomaterial in the pharmaceutical field such as being an excipient for drug protection and delivery. The main reason for this application is due to its intrinsic properties, such as being, biodegradable, biocompatible, low toxicity, antimicrobial, and low toxicity, among others (Buosi et al., 2020; Di Santo, Alaimo, Dominguez Rubio, De Matteo, & Pérez, 2020; Yeul & Rayalu, 2013).

The degree of deacetylation (DD), molecular weight (MW), solubility, and viscosity are the most remarkable characteristics of this polysaccharide structure and dictate the polysaccharide's possible applications (Dutta, 2015). Next, there will be a brief description of all of them:

DD: to determine the DD, many methods from simple (pH-metric titration, UV-Visible spectroscopy, infrared spectroscopy, elemental analysis) to complex analytical techniques (^{1}H NMR spectroscopy and ^{13}C NMR spectroscopy) were applied. The obtained DD value can vary depending on the nature, the level of impurities, and the source of the chitin. In general, the DD between 55% and 70% is considered a chitosan with a low-grade DD; 70%−85% would correspond to a medium level; 85%−95% is considered high grade, while 95%−100% is ultra-high level of DD. On the other hand, chitosan with 100% DD is called "full chitosan", which is difficult to produce industrially (Buosi et al., 2020). In the 70s, Muzzarelli et al. (1977) explained that DD has a noticeable effect on the solubility and solution properties. There are two great advantages of chitosan over chitin. On the one hand, chitosan has free amino groups, which makes them an active site in various chemical reactions. On the other hand, chitosan is easily soluble in 1% acetic acid in the water while chitin must be dissolved in highly toxic media (e.g., dimethylacetamide, lithium chloride) (Muzzarelli & Chitin de Muzzarelli, 1977).

MW: is one of the most fundamental parameters in characterizing a polymer. Similar to DD, the MW of chitosan varies according to raw material sources and preparation methods. This characteristic can be determined by chromatography, light scattering, or viscometry. The MW of native chitin is usually greater than 1000 kDa, while commercial chitosan products have a wide MW range from 5 to 375 kDa. Notably, temperatures above 280°C can generate a thermal degradation of the chitosan, breaking its chains, and, consequently, reducing its MW. Likewise, depolymerization can be generated by the presence of concentrated strong acids (e.g., hydrochloric acid and sulfurous acid).

Solubility: Several critical factors affect chitosan solubility, including deacetylation temperature and time, alkali concentration and pretreatments applied for chitin isolation, chitin to alkali solution ratio, and particle size. As previously mentioned, chitosan is easily soluble in weak acids (e.g., acetic, formic, and lactic) below pH 6.0. The most frequently used is 1% acetic acid in the water, generating a solution with a pH of 4.0. In certain circumstances, chitosan can be dissolved in 1% hydrochloric acid as well. It is important to note that above pH 7.0, the solubility stability of chitosan is poor. At basic pH, chitosan tends to precipitate. On the other hand, solubility is regulated by DD and it is estimated that deacetylation must be at least 85% complete to achieve the desired solubility.

Viscosity (η): the η is considered a measure of the volume of a single polymer molecule in an ideal condition, represented by the Mark–Houwink–Sakurada equation:

$$\eta = K.M^{a}$$

where M is the mean viscometry molar mass, meanwhile, K and α are both constants determined by evaluating a plot of log [η] versus log MW. Various parameters affect the intrinsic viscosity of chitosan, such as concentration, molar mass, solvents, temperature, shear, the chemical structure of the polymer, and the DD of chitosan. In the case of chitosan in acid medium and polyelectrolytes in general, the degree of dissociation of the ionic groups is also a factor to consider. That is, the presence of ionic groups in its structure leads to the expansion of the polymeric chains by electrostatic repulsions, causing an increase in viscosity; however, when a salt is added to the aqueous polyelectrolyte solution it causes a reduction in electrostatic repulsion, resulting in a shape that does not spread or tangle excessively, which reduces the viscosity.

2.3 Chitosan at the service of nanotechnology

Nanotechnology is an innovative science that positively impacts a large array of disciplines. In recent years, pharmacology and biomedicine adopted nanotechnology as a potential tool to generate and/or improve devices, drugs, and other utilities for the early diagnosis or treatment of a wide range of diseases, being the main objective, the improvement of the patient life quality. Particularly, a variety of parameters that therapeutic drugs must comply such as solubility, room temperature stability, compatibility with solvents, excipient, and photostability play a critical role in the successful formulation of a drug. To date, most drugs are lipophilic or poor water-soluble compounds. In this sense, and as previously announced, nanotechnology comes into the scene to solve the drug challenges of low solubility and bioavailability (Mitchell et al., 2021).

The applications of chitosan-based nanosuspensions are presented as potential tools capable of overcoming obstacles in the transport of drugs, thus

improving their efficacy. Chitosan-based nanosuspensions are highly effective in targeted drug therapy. In this regard, chitosan and its derivatives have been explored for various biomedical applications due to their unique chemical and biological characteristics. The amine groups present in the chitosan molecule allow chemical modification to change the physical properties. Based on hydrophobic or charge interactions with this chitosan polymer backbone, aqueous stable self-assembled nanoparticles can be manufactured. These nanometric structures allow their delivery efficiently for use in diagnosis and therapy (Zhao et al., 2018).

2.4 Methodologies for chitosan-based nanosuspension synthesis

The main two groups of strategies for chitosan-based nanosuspension construction can be namely Bottomup technology and Topdown.

The *bottomup approach* is an assembling method to form nanoparticles like precipitation, microemulsion, and melt emulsification methods. The tiny size material is assembled for the constitution of more complex structures. The *top-down approach* involves the disintegration of larger particles into nanoparticles, examples of which are high-pressure homogenization and milling methods. Thus the process starts with materials at the macroscopic level, which is reduced up to the required particle size is achieved.

The more common processes associated with the topdown strategy are milling, trituration, and extrusion, which involve the solid material disruption or the formation of small droplets, which implies high-intensity compression, impact, and shear forces application (Merisko-Liversidge, Liversidge, & Cooper, 2003) This strategy is used in the industry for the biopolymer-based NP formation. The application of these procedures results in a complex for obtaining particles with well-defined structural properties and safeguarding the physic-chemical integrity of the bioactive compound at the same time (Joye & McClements, 2014). The bottomup strategy implies atoms or molecule manipulation. It is characterized by the NP design through molecular self-assembly as a consequence of changes in the environment surrounding them as pH, ionic strength, temperature, or intrinsic characteristic of solution as the biopolymer concentration. Small particles can be obtained in this way with a high degree of properties control (size, morphology, surface charge, etc.). Among the natural polymers, carbomer, hyaluronic acid (HA), gelatine, alginate, cellulose, and its derivates, collagen, polyethylene-glycol, and chitosan, are known to prolong the residence time and bioavailability of the encapsulated drug in the ocular surfaces. CS can be used to achieve NP formation by applying different methods of self-assembly principle which is framed into the bottomup strategies (Agrawal & Patel, 2011; Buosi et al., 2020).

2.5 The eye is "the sensory organ responsible for the visual perception of the world"

The following sections aim to compile the various types of nanosuspensions used for ocular diagnosis and treatment, being this topic the central part of this contribution. For this purpose, it is essential to know the aye anatomy as well as the natural barriers that drugs must overcome to reach the place of action and the limitations presented for the release systems.

2.5.1 Anatomy

The ocular structure is a set of components whose function is vision. These elements will be detailed below.

The *eyeball*, whose shape is approximately spherical and measures 2.5 cm in diameter, is a receptor organ made up of a light-sensitive membrane called the retina and an optical system that directs light beams to it. The *optical route* comprises a conduction system to transport the nerve impulses generated in the retina to the visual cerebral cortex for their analysis and correlation. The *extraocular muscular system* allows the movement of the eyes to expand its field of reception. Further, the eyeball is divided into an anterior segment and a posterior segment. The former is located between the cornea and the lens. It is filled with aqueous humor and is divided into two chambers communicated by the pupil anterior chamber and posterior chamber. The former is bounded by the cornea and the iris, meanwhile, the latter is delimited by the posterior part of the iris, the lens, and the zonule. On the other hand, the posterior segment of the eye is located between the lens and the sclera. It is filled with vitreous humor and includes the anterior hyaloid membrane and all of the optical structures behind it, the retina, the choroid, and the optic nerve. Remarkably, near the retina center region, there is a yellowish oval structure due to the presence of xanthophyllic pigment which is called *macula*. It is an area with a large grouping of photoreceptors, specifically cones. Inside the macula, there is a small depression called the *fovea*. The retina is made up of two basic regions that consist of the retinal pigment epithelium (or outer layer) and the neural layer of the retina (or inner layer). The retinal pigment epithelium is attached to Bruch's membrane and consists of a hexagonal cells that is densely packed with pigment granules. The presence of tight junctions between these cells is essential in controlling the diffusion of substances through the paracellular spaces. These pigmented epithelial cells host and participate in the metabolism of the outer segments of the photoreceptors and avoid interference due to light reflection. On the other hand, the neural layer of the retina is formed by retinal neurons and neuronal support cells. Retinal neurons, in turn, are divided into photoreceptor cells (rods and cones), cells of the afferent fibers that enter the optic nerve (ganglion cells), and a group of neurons interposed between the two previous types that make up the entrance sensory from the photoreceptors before their transmission to the cerebral cortex (bipolar,

horizontal and amacrine cells). The neuronal support cells (astrocytes and Müller cells) perform functions of support and support to the whole (Forrester, Dick, McMenamin, Roberts, & Pearlman, 2016).

2.5.2 Natural ocular barriers

There are many challenges when attempting to effectively deliver drugs to the eye. Many of these are the result of this organ's natural barriers and mechanisms present within it specifically designed to protect the microenvironment from foreign particles and substances. Among the active drugs most frequently administered are miotics, antiinflammatories, antivirals, antifungals, antibiotics, and adjuvants for diagnosis and surgery. Notably, the application of ophthalmic drugs to the ocular surface may be for a local or intraocular target of action. Regarding the latter, despite the possibility of administering drugs intraocularly (e.g., intravitreal injections), this is not considered the most recommended option. Aside from their inherent adverse effects, such as retinal detachment, hemorrhage, endophthalmitis, and traumatic cataract, they require frequent injections, which are not always well tolerated by the patient, in addition to their high cost. On the other hand, systemic administration has also been considered as another route for a drug that must reach the posterior segment of the eye: however, the concentration of such active substances is often so high that leads to unwanted side effects. After this brief introduction, the main ocular defense mechanisms are described below (Forrester et al., 2016; Nettey, Darko, Bamiro, & Addo, 2016) (Fig. 2.3):

Tear film: is the eye layer that is in intimate contact with the air. It comprises a first lipid layer, an intermediate aqueous layer, and the deepest, the mucin layer. The latter contributes to the stability of the tear film, as well as to the adhesion to the cornea. Any alteration of the tear can destabilize it and thus, alter the residence time of any drug. Simultaneously, whether the pH of the tear changes, it can also cause a variation in the ionization state of the drug. Hence, the diffusion capacity of such a drug can additionally be influenced.

Cornea: consist of a thick first layer that the drug delivered has to pass through when administered topically. It is divided into five layers: the epithelium, Bowman's membrane, the stroma, Descemet's membrane, and the endothelium. Notably, the main layer that restricts drug penetration through the cornea is the epithelium. This layer is conformed with cells closely joined through tight junctions and with a paracellular pore of 2 nm. Hence, this barrier causes the diffusion of the drug from the tear film to the anterior segment of the cornea in a very low manner. Moreover, the lipophilicity of the drug must be considered, since while the epithelium and endothelium would allow the passage of lipophilic molecules, the stroma allows the passage of hydrophilic ones, preventing the passage of hydrophobic substances. This implies that only substances with a suitable degree of lipophilic characteristic are able to pass across this tiny layer. Even more, the molecule charge of any drug also influences its diffusion through the cornea, being those anionic species more permeable.

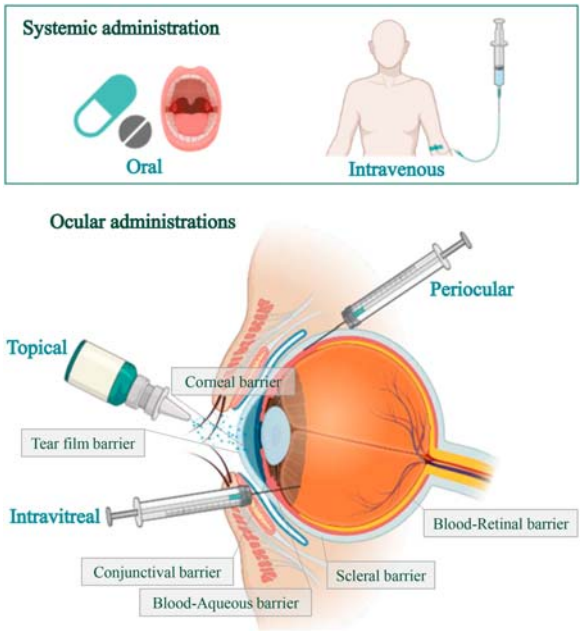

FIGURE 2.3

Routes of drug administrations (systemic and ocular) and eye-barriers.

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Conjunctiva: is a vascularized mucosa that is divided into three sections. That is to say, the conjunctival sac, the bulbar conjunctiva, and the palpebral conjunctiva. It is composed by up of two layers, one more superficial, which is the epithelium, and another more internal, the stroma. The pore formed between these cells is wider than in the cornea, being 3 nm in the bulbar conjunctiva, and 4.9 nm in the palpebral, so the permeability of the conjunctival barrier is greater than in the mentioned case. Nevertheless, this passage continues to be low due to the presence of blood and lymphatic vessels, which trigger a rapid elimination of aqueous-soluble drugs.

Blood-aqueous barrier: the eye structure formed by the capillary endothelium of the iris and the nonpigmentary ciliary epithelium separate the anterior and posterior segments. This layer controls the passage of solutes from the anterior segment to the posterior segment and is poorly permeable due to the presence of tight junctions between cells. As consequence, the topical administration results are not effective for the treatment of posterior segment eye diseases.

Blood-retinal barrier: especially narrow and restrictive, this barrier regulates the flow of nutrients, metabolic waste products, ions, water, and proteins in and out of the retina. The blood-retina barrier is crucial to preserving the eye as a privileged environment and is vital for normal visual function. The blood-retina

barrier consists of two barriers: inner and outer. Both barriers present tight junctions between adjacent endothelial and retinal pigment epithelial cells. The inner barrier regulates transport across retinal capillaries; meanwhile, the external barrier is composed of the tight junctions that are established between the cells that make up the monolayer of the retinal pigment epithelium. Among other functions, this barrier regulates the passage of nutrients and solutes from the choroidal vasculature to the neural retina.

2.5.3 Administration routes in ophthalmology

Although it is not intended to make a detailed description of the ocular routes of administration, mention is made below of the most used in the ophthalmic field since it will be pertinent information for the next section (Gorantla et al., 2020; Nettey et al., 2016) (Fig. 2.3).

Topical administration: is the most preferred route for drug delivery in ophthalmology, due to the simplicity of the technique and its economic cost. More than 90% of the ophthalmic commercial formulations exist in the form of drops. However, this route is considered ineffective for treating posterior segment diseases. When a drug is administered topically to the eye (in the form of drops, suspensions, or ointments), different mechanisms are stimulated such as tearing, blinking, dilution, and tear film turnover, which contributes to the clearance and/or washing of the substance of the ocular surface so that the amount of drug that can pass through the cornea is much lower than the administered dose. In this sense, less than 5% of the total dose administered by the ocular topical route reaches the aqueous humor. Ocular penetration of drugs administered topically can occur in two ways, corneal and noncorneal. Preference for one way or another penetration will depend on the physical-chemical properties of the substance.

Systemic administration: is used for the treatment of posterior segment diseases; however, the doses required to reach therapeutic levels are very high, which implies a greater risk of generating unwanted adverse effects.

Intravitreal administration: direct drug deposition in the posterior compartment of the eye by intravitreal injection is a useful alternative since effective concentrations of the active ingredient in the vitreous humor are achieved from the outset with minimal secondary effects at the level systemic level. Thus, intravitreal administration has gained popularity in recent years thanks to the application of antivascular endothelial growth factor () drugs in the treatment of age-related exudative macular degeneration. VEGF is a dimeric glycoprotein of 40 kDa that stimulates endothelial cell proliferation, migration, and tube formation leading to angiogenic growth of new blood vessels. However, intravitreal injections are an invasive method, with risks at the ocular level, such as the formation of cataracts, retinal detachments, vitreous hemorrhages, endophthalmitis, and/or increased intraocular pressure.

Periocular administration: Drug delivery to the posterior segment of the eye requires the search for less harmful routes of administration than intravitreal

injection. In this sense, the periocular administration approach has many advantages over the other routes. Unlike the systemic route, it allows reaching high concentrations of the drug in the vitreous, since the sclera, being a fibrous tissue, offers less resistance than the hemato-aqueous and hemato-retinal barriers, to the penetration of substances, being permeable even to large molecules. In addition, the periocular route provides a virtual space where the active principle is stored, as a reservoir, for its progressive release. Within the periocular route, we can differentiate different injection modalities, according to anatomical criteria: peribulbar, retrobulbar, posterior juxtascleral, subtenon and subconjunctival.

2.6 Chitosan-based nanosuspension for ocular therapy and diagnosis

Nowadays, the main challenge faced by pharmacologists and scientists is the ocular administration of drugs. As noted in the previous section, drug delivery to target ocular tissues is restricted by a variety of static, dynamic, and precorneal ocular barriers. Furthermore, the therapeutic drug levels are not maintained for longer times in the target tissues. The idea of employing nanosuspensions to target the anterior and posterior segments of the eye, and to overcome the limitations of conventional formulations, has gained considerable attention. That is, advances in nanomedicine have facilitated the controlled and specific delivery of drugs in specific tissues to treat eye diseases. Therapeutic administration via nanosuspensions can reduce the frequency of general administration of dyes, ointments, intraocular injections, etc., to improve therapeutic efficacy, substantially reduce treatment costs, and enhance the patient's life quality. Additionally, technological advancements related to ease of scalability and reproducibility provide further hope for the profitable commercialization of nanomedicines. Several methods have been developed for chitosan-based nanosuspensions synthesis for ophthalmological purposes (Harikumar & Sonia, 2011; Jacob et al., 2020). These reports will be mentioned in this section (Fig. 2.4):

2.6.1 Glaucoma

This disease is characterized by a pathological increase of the intraocular pressure, due to a lack of aqueous humor drainage. Its final condition is optic neuropathy characterized by the progressive loss of the nerve fibers of the optic nerve and changes in its appearance. The following briefly describes the mechanism of action of some ophthalmic drugs used to treat glaucoma, which has also been encapsulated in chitosan nanosuspensions:

Pilocarpine is a class of medicine called miotics. This drug acts on the cholinergic receptors present in the eye causing the contraction of the ciliary muscle and the sphincter of the iris. Contraction of the ciliary muscle facilitates the flow

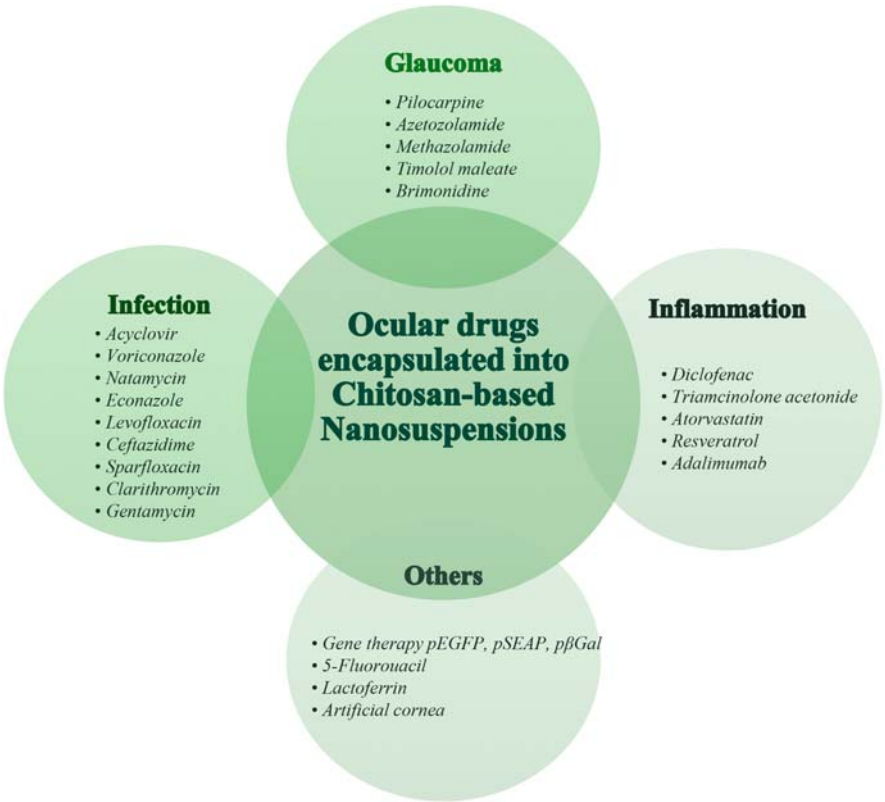

FIGURE 2.4

List of the more commonly used ocular drugs that are encapsulted into chitosan-bases nanosuspensions and discussed in the present chapter.

of aqueous humor. Currently, pilocarpine is no longer used in general due to its side effects. In 2007 (Lin, Yu, Kuo, & Kao, 2007) successfully prepared chitosan-poly (acrylic acid) nanosuspensions to be used as an ophthalmic drug carrier for pilocarpine by using template polymerization. When the polymerization was done at 70°C with chitosan: acrylic acid weight ratio of 1:1 of acrylic acid, the authors achieved particles with a mean diameter of 92 nm and a stable ζ-potential (+25 mV) in an acidic (pH 4.5) buffer solution since the particle size was significantly affected by the pH value of the medium. Additionally, the encapsulation efficiency of pilocarpine resulted in 71%. In an in vitro drug release assay at 37°C in simulated tear fluid (NaCl 0.67 g, $NaHCO_3$ 0.20 g, $CaCl_2 \cdot 2H_2O$ 0.008 g in distilled deionized water to 100 g; pH 7.4) and commercial eye drops were similar. Particularly, the pilocarpine release practically in its entirety (95%) within the first 5 min. On the other hand, the release profile of pilocarpine-loaded nanosuspension showed an initial burst of the release of about 40% of the drug within

the first 15 min followed by a slow release. After that, 80% of the drug was released within 360 min. Additionally, in vivo drug release performed in rabbits showed that the whole miotic response of the chitosan-poly (acrylic acid) nanosuspension was the best. Three years later (Lin, Yu, Lin, & Wang, 2010). experienced this time the use of hydrogen peroxide before the polymerization to cut down the MW of chitosan to improve its solubility and tolerance of pH values in the physiological condition. In this case, the surface charge of the modified chitosan-poly (acrylic acid) nanosuspensions was 20 mV with a pH tolerance increased up to 7.1 and a particle size of about 140 nm. Overall, the in vitro and in vivo studies of both reports revealed that obtained nanosuspensions either unmodified or modified have an improved ability in the sustaining release of pilocarpine than simulated tear fluid and commercial eye drops.

Acetazolamide derived from sulfonamides and is a carbonic anhydrase inhibitor. This enzyme catalyzes the interconversion of carbon dioxide and hydroxyl ion to bicarbonate ion and plays an important physiological role in the proximal tubules of the kidney and the ciliary processes of the eye. Particularly, in the eye, acetazolamide reduces the formation of aqueous humor and, consequently, intraocular pressure. As (Manchanda, Sahoo, & Majumdar, 2016) explained, high oral doses of acetazolamide are given to effectively lower intraocular pressure, ultimately resulting in a variety of complications, for example, diuresis, systemic acidosis, and occasionally severe dyscrasias. As a consequence, most patients discontinue their therapy. Harmful systemic side effects of acetazolamide could be avoided by using topical ocular formulations; however, limitations appear for the latter due to the low solubility of the drug and limited corneal penetration. The chitosan-acetazolamide dextran sulfate nanoparticles were formulated using the ionic gelation technique. Among the formulations tested, the most optimal had a mean particle size of 172 nm and a ζ-potential of $+36$ mV. The particles had a spherical shape and a polydispersity index of 0.25. Using excised goat cornea, it was possible to determine that the corneal permeability of the drug increased approximately 2.5 times more compared to the aqueous solution of the drug and without causing any corneal damage. Likewise, the authors carried out mucoadhesion studies using pig mucin suspension. The chitosan-dextran suspension showed high mucoadhesion effectiveness. On the other hand, in vivo studies involving ocular hypotensive activity in rabbits revealed significantly higher hypotensive activity than the simple drug solution, and notably, no signs of ocular irritation.

Methazolamide, similarly to azetozolamide, is a carbonic anhydrase inhibitor that lowers the intraocular pressure by reducing the production of aqueous humor. Its mechanism of action is not yet known but it is assumed that it is related to the decrease in the concentration of bicarbonate ions in the ocular fluid. Wang et al. (2018) refer to the development and optimization of chitosan-coated solid lipid nanoparticles for the encapsulation of methazolamide. In this case, the nanosuspensions were performed by the oil-in-water emulsification solvent evaporation method modified with glyceryl monostearate as solid lipid and phospholipid as

surfactant. The optimized formula achieved a mean particle size of 248 nm and a ζ-potential of +33 mV, as well as an encapsulation efficiency of methazolamide of 58%. Transmission electron microscopy showed homogeneous spherical particles in the nanometer range. An in vitro methazolamide prolonged release profile was obtained in nanosuspension compared to methazolamide solution. Remarkably, no eye damage was observed in the rabbit susceptibility test.

Timolol maleate is a beta-adrenergic blocker that is nonselective between β-1 and β-2 adrenergic receptors. The exact mechanism of action by which timolol reduces intraocular pressure has not yet been clearly established, although many studies indicate that its predominant action may be related to a reduction in the production of aqueous humor. Unlike miotics, timolol reduces intraocular pressure with little or no effect on accommodation or pupil size. In clinical trials, timolol maleate was generally effective in more patients and produced fewer milder adverse reactions than pilocarpine; however, timolol maleate is usually administered 4−6 times daily for prolonged therapeutic effect due to its short time of acting and its elimination half-life of 2.5−5 h. Additionally, the conventional ophthalmic eye drops are quickly eliminated by blinking reflex, lacrimation, and drainage; consequently, this leads to a brief precorneal residence and poor bioavailability. Tan et al. (2017) developed and characterized a colloidal system formed with chitosan-coated liposomes to enhance the ocular permeation, precorneal residence time, and bioavailability of the timolol maleate. The most auspicious formulation presented a mean particle size of 150 nm, a polydispersity index of 0.19, a ζ-potential of +16 mV, and an entrapment efficiency of 76%. Notably, animal studies performed in New Zealand white rabbits demonstrated that this nanoformulation displayed an increased adhesion as well as an enhanced corneal permeation versus the commercial eye drops. Additionally, the ocular irritation analysis indicated that the developed liposomes did not produce any irritant effects. Moreover, pharmacodynamics results showed the chitosan-based colloidal system was more effective in reducing intraocular pressure. These results demonstrate that CHL is a potentially useful carrier for ocular drug delivery, which could improve the efficacy of TM. Two years later, (Mittal & Kaur, 2019) aimed to overcome timolol maletato limitations by increasing the residence time and improving its therapeutic effectiveness. In their report, formulate timolol maleate loaded-polymeric nanoparticles of flaxseed gum and chitosan for ocular delivery using the ionic gelation method. Flaxseed gum is a hydrocolloid extracted from flaxseed (*Linum usitatissimum* L.) and consists of a heterogeneous polysaccharide (xylose, arabinose, glucose, galactose, and rhamnose) present in the outermost layer. Along the process of nanosuspension preparation, the optimal formulation generated particles with a minimum particle size around 267 nm with an encapsulation efficiency of 75%. In addition, those nanoparticles showed considerable bioadhesive strength and exhibited sustained release of the timolol maleate. Notably, this report verified the capability of the nanoparticles to penetrate deeper layers of the cornea and its biocompatibility in such tissue. Both ex vivo and in vivo studies demonstrated a higher corneal penetration of this drug compared

to commercial eye drops as well as a prolonged period of residence when compared to conventional eye drops, respectively.

Brimonidine is an $\alpha 2$ receptor agonist adrenergic drug. This drug reduces elevated intraocular pressure by decreasing the degree of aqueous humor production, as well as by producing an increase in uveoscleral efflux. Finally, the report by (Barwal, Kumar, Dada, & Yadav, 2019), cited various reported methods for the entrapment of brimonidine to improve its efficacy and diminish their side effects (e.g., itching, stinging, blurred vision, puffy eyes, nausea, irregular heartbeat, shallow breathing, and numbness in the hands and feet). However, what the authors are going to aim at in this case is to achieve monodispersal and ultrasmall chitosan-based nanoparticles (28 nm) prepared by a modified ionotropic gelation process. Unlike other papers, in this report, the chitosan polymer dissolved in 1% acetic acid is first subjected to a water bath sonicator at room temperature. The following steps consisted of dropwise a mix of sodium tripolyphosphate and brimonidine into the chitosan solution to form the nanoparticles. As consequence, brimonidine was successfully entrapment with 39% of efficiency. The obtained formula called nanobrimonidine did not show any toxicity in the L-929 cell line. Notably, ex vivo studies showed that nanobrimonidine-treated trabeculectomy tissue from glaucoma patients exhibited a better dilation of the trabecular meshwork. Those results evidence that this nanobrimonidine may have a better bioavailability after topical administration, as well as improve efficacy by reducing the dosage and frequency of the brimonidine.

All the presented reports agree that chitosan-based nanosuspension, regardless of the synthesis method used, has a promising role for ocular drug delivery in the treatment of glaucoma.

2.6.2 Ocular infection caused by microorganisms

Eye infections are an ophthalmologic emergency that threatens its integrity, which may result in a poor visual outcome; therefore, it needs prompt treatment. Ocular infections can be caused by several different microorganisms, including bacteria, viruses, ameba, yeast, and fungi. In addition, some eye coinfections can occur, bacteria-bacteria, bacteria-fungus, bacteria-virus, fungus-yeast, fungus-virus, parasite-bacteria, etc. Current limitations in the administration of ophthalmic treatments for many infections include the incapability to exert a long-term drug delivery without compromising ocular structures and/or systemic drug exposure. In the present section, the potential applications of chitosan-based nanosuspensions as a new-found vehicle for the improvement of drug delivery to overcome infections produced by microorganisms (Forrester et al., 2016; Watson, Cabrera-Aguas, & Khoo, 2018).

Herpes keratitis is an eye viral infection caused by the herpes simplex virus. When the infection is superficial, involving only the cornea's outer layer, it will typically heal without scarring. Nevertheless, whether involves the deeper layers of the cornea, the infection can lead to scarring of the cornea, loss of vision, and

occasionally blindness. Acyclovir is commonly used to treat an eye infection caused by the herpes simplex virus. The topical application of acyclovir, either in the form of drops or ointments, is limited by the low corneal penetration and the poor ocular bioavailability of the drug due to the drainage of the nasolacrimal. Therefore, Rajendran, Natrajan, Kumar, and Selvaraj (2010) developed acyclovir-loaded chitosan nanoparticles for effective ocular drug delivery to improve bioavailability. Chitosan nanoparticles were prepared by ionic gelation with tripolyphosphate as a crosslinker agent. The formulation that achieved the maximum encapsulation (80%) of acyclovir consisted of particles whose mean size was 495 nm. They presented a smooth spherical morphology with a ζ-potential of +42 mV. The in vitro diffusion profile of acyclovir from the nanoparticles showed a sustained release of the drug for 24 h. Therefore, the results suggest that the suspension of chitosan nanoparticles loaded with acyclovir appears to be an effective solution for the treatment of eye viral infections such as herpes keratitis.

Fungal endophthalmitis is a rare but serious intraocular infection with devastating visual sequelae usually caused *Aspergillus* spp. *Fusarium* and *Candida albicans*, etc. The presence of fungi should be suspected when there is contact with plant matter, which is why it is more frequent in rural environments. On the other hand, fungal keratitis is an infection of the cornea. Voriconazole is a lipophilic candidate, effective against fungal infections like ocular endophthalmitis and keratitis. The antifungal drug voriconazole has broad fungicidal activity, for example, is highly potent against all *Aspergillus* species, molds such as *Scedosporium* species, *Fusarium* species, and fluconazole-resistant *Candida* species (*Candida krusei*, *Candida glabrata*, and *C. albicans*). Bhosale, Bhandwalkar, Duduskar, Jadhav, and Pawar (2016) synthesized water soluble chitosan microemulsion as sustained voriconazole carriers for ophthalmic application. Microemulsion can be a possible ocular delivery system for a lipophilic candidate, as is the case with voriconazole. The authors successfully generated microemulsions with acceptable physicochemical properties, that is, characterized by a mean droplet size of less than 250 nm for all formulations with a good polydispersity index (<0.5) and positive zeta potential. The latter is very necessary as it allows the precorneal residence time to be prolonged. The voriconazole microemulsion containing chitosan (0.25% w/v) was able to provide sustained release. The selected voriconazole microemulsion formulation was able to produce maximum antifungal activity against *C. albicans* compared to the commercialized formulation. An in vivo study carried out in rabbit eyes allowed for to detection of a higher concentration of voriconazole in aqueous humor versus the free drug, thus, this type of emulsion could be useful for use as eye drops in the treatment of ocular fungal keratitis. On the other hand, natamycin is another used drug in the treatment of fungal keratitis despite its disadvantages such as low drug bioavailability and poor water solubility, which hinders its penetration into deep corneal layers and the anterior chamber. As consequence, this situation limits its use as monotherapy only for superficial keratitis. The chitosan-tripolyphosphate

nanoparticles prepared by Liu et al. (2021) exhibited good physicochemical properties (polydispersity index: 0.3, encapsulation efficiency: 93%, ζ-potential: +35 mV) and strong antifungal effect on *C. albicans*. Additionally, nanoparticles allowed for sustained release of the natamycin for 12 h with an initial burst release in the first hour. In parallel to this report (Verma et al., 2021), developed trimethyl chitosan coated mucoadhesive cationic niosomes by a modified thin-film hydration method. That is to say, natamycin, cholesterol, span 60, and diacetyl phosphate were used to synthesize those niosomes which were incubated with trimethyl chitosan to obtain mucoadhesive cationic natamycin-loaded niosomes. This type of vesicle formed presented a mean size of 1031 nm exhibited a spherical shape and managed 80% of the natamycin entrapment. The drug release profile showed gradual drug release from the coated niosomes. Also, the in vivo assays performed in rabbits' eyes demonstrated a mucoadhesive property and prolonged local drug release which improved the safety and efficacy of natamycin, suggesting that the obtained niosomes could be an emerging system for effective treatment of fungal keratitis. Another classic antifungal is econazole nitrate, which is usually used for the treatment of fungal infections caused by *C. albicans* and *Tinea* sp. Econazole nitrate is a water-insoluble drug; hence, its introduction into nano-vehicles has shown many advantages in improving aqueous drug solubility, increasing drug bioavailability, reducing drug toxicity, and increasing drug selectivity to certain eye layers. Maged, Mahmoud, and Ghorab (2016) applied the nano-spray drying technique to encapsulate econazole nitrate into a nanosuspension formulation for ocular use. The particularity of the methyl-β-cyclodextrin and hydroxypropyl-β-cyclodextrin nanoparticles is that Tween 80 was added to preserve the econazole nanosuspension from aggregation during storage at room temperature alongside improving drug release from the nanosuspension. Also, this formulation was added to the chitosan solution to increase drug release and bioavailability. In vivo evaluation of Albino rabbit's eyes demonstrated markedly improved bioavailability, viscosity, and mucoadhesive of the selected formulation suspended in chitosan compared to its counterpart formulation suspended in the buffer. Hence, nano-spray drying constituted an advantageous one-step technique for formulating stable and effective nanosuspensions.

Postoperative bacterial endophthalmitis is an eye disease that is difficult to manage and treat. It is serious, with a significant compromise of visual function and the anatomical integrity of the eyeball in most patients. Most cases of endophthalmitis are caused by gram-positive bacteria, such as *Staphylococcus epidermidis* or *Staphylococcus aureus*. Particularly, levofloxacin is an antibiotic with high inhibitory activity against *S. aureus*. The dosage regimen comprises one drop in every 1−2 h for 3 days and then every 4−5 h (Gupta et al., 2011). Bao et al. (2021) carried out the encapsulation of levofloxacin, a hydrophobic drug, to prolong the ocular drug delivery system and reduce the frequency of dosing. Levofloxacin-loaded hydrogel films, prepared by crosslinking HA with glycol chitosan, were not cytotoxic for human corneal epithelial cells, as was indicated by MTT assay and Live/Dead staining assay. The antibacterial successfully

antiproliferative activity of levofloxacin-loaded hydrogels was confirmed in two different bacterial strains, namely gram-negative *Escherichia coli* and gram-positive *S. aureus* by the disc diffusion method. On the other hand, ceftazidime is one of the most frequently used antibiotics to cover practically all of the bacteria responsible for postoperative endophthalmitis. In this sense, Silva et al. (2017) investigate the mucoadhesive of novel nanoparticle eye drops containing ceftazidime to treat endophthalmitis. The eye drop nanoformulation comprised a polymer hydroxypropylmethylcellulose (HPMC) in an isotonic solution incorporating chitosan/tripolyphosphate-HA. HPMC is added to increase the ophthalmic solution viscosity, that is tear mimic. The ionotropic gelation method allowed the formation of nanoparticles loaded with ceftazidime with a particle size of around 350 nm, a ζ-potential of −3 mV, and polydispersity index values < 0.4. In vitro release and permeation studies showed a prolonged drug release profile of the antibiotic drug from the nanoparticles gel formulation. Furthermore, the latter were not cytotoxic in an in vitro assay performed in ARPE-19 pigment epithelial cells of the human retina. In addition to the fact that they did not induce the generation of reactive oxygen species. The formulation has shown antiantimicrobial activity against *Pseudomonas aeruginosa*. In conclusion, chitosan-HA nanoparticle formulations are a hopeful platform for eye topical drops with enhanced mucoadhesive properties.

Conjunctivitis is a harmful, sight-threatening eye infection that requires immediate ophthalmic treatment. Bacterial conjunctivitis, although a less frequent cause of this disease is more common in children. Bacterial conjunctivitis requires treatment with antibiotics for a week or more with a frequency of drug administration of 2−3 drops every 2−3 h. The most common bacteria are *Haemophilus influenza*, *Streptococcus pneumoniae*, and *S. aureus*; however, are commonly treated empirically with broad-spectrum antibiotics. Sparfloxacin is a fluoroquinolone antibiotic effective to treat eye infections against pathogens such as *S. aureus*, *S. pneumoniae*, and *S. epidermis*. Ambhore, Dandagi, and Gadad (2016) prepared sparfloxacin nanosuspensions by combining HPMC E5 and chitosan by using the solvent diffusion method followed by ultrasonication. Additionally, Poloxamer 407 and Kolliphor P188 were used as a surfactant. The average particle size of the best-obtained nanosuspension presented 400 nm, polydispersity index values < 0.6, ζ-potential of −39 mV, and entrapment efficiency of sparfloxacin more than 90%. The in vitro drug release study showed that the best nanosuspension formula sustained drug release for up to 9 h. Also, the sparfloxacin nanosuspension exhibited optimal ocular tolerance and biocompatibility as determined by hen's egg test chorioallantoic membrane assay and MTT assay in Vero-2 cells. Moreover, the microbiological studies performed in rabbits' eyes revealed a high antimicrobial activity against *S. aureus*. As the authors indicate, this new formulation would improve patient compliance by reducing the frequency of administration and therapeutic effectiveness of conjunctivitis treatment. Clarithromycin is a broad-spectrum macrolide antibiotic, efficient against gram-negative and gram-positive microorganisms, as well as intracellular pathogens. It is typically utilized

to treat experimental *Mycobacterium fortuitum*, *Mycobacterium chelonae*, and *S. aureus*. It is a poorly water-soluble drug and has a half-life of 3−4 h, hence frequent dosing is mandatory. Bin-Jumah et al. (2020) prepared clarithromycin-loaded chitosan-tripolyphosphate nanoparticles by ionic gelation method. The optimized formulation presented a mean particle size of 152 nm, a ζ-potential of +35 mV, and a spherical shape. High encapsulation efficiency of the clarithromycin (70%) was achieved as well as a sustained drug release profile. Furthermore, concerning the commercial clarithromycin solution, its nanoencapsulated form exhibited about 3 times more corneal permeation with the advantage of also not being harmful as well as exhibited significantly higher antibacterial activity. In the same way, with the same ionic gelation methodology, the above-mentioned research group also entrapped gentamicin. This is a wide-spectrum aminoglycoside antibiotic used to treat infections caused by gram-negative and Gram-positive bacteria. The optimized formulation obtained by Alruwaili et al. (2020) showed a particle size and encapsulation efficiency of 135 nm and 60%, respectively. The optimized gentamycin-loaded chitosan nanoparticles were designed for the stimulus-responsive sol-gel system using pH-sensitive carbopol 974P. The resulted nanogels exhibited good gelling strength and a sustained release among the first 12 h (58%). Those findings revealed that chitosan nanogels could be considered good candidates for bacterial conjunctivitis treatment.

2.6.3 Ocular inflammation

Topical antiinflammatories are drugs that act locally. They are classified into two main groups whose structure and mechanism of action and indications are different: corticosteroids and nonsteroidal antiinflammatory drugs.

Corticosteroids are involved in a wide variety of physiological mechanisms, including those that regulate inflammation, the immune system, carbohydrate metabolism, protein catabolism, electrolyte levels in plasma, and, finally, the stress response. Synthetic corticosteroids repeat the chemical action of the hormone cortisone and are used mainly for their antiinflammatory and immunosuppressive properties and their effects on metabolism. Regarding the eye and visual function, we will see below that corticosteroids are among the most useful, beneficial, and decisive drugs to treat the pathologies that affect this organ. Undoubtedly, one of the most valued properties of corticosteroids in ophthalmology is their particular benefit in the control and treatment of inflammatory ophthalmopathy. That is to say, there is practically no inflammatory disease in ophthalmology that escapes the use of corticosteroids as a drug of the first choice, except in very few cases. On the other hand, although the use of topical nonsteroidal antiinflammatory drugs (NSAIDs) is increasingly widespread in ophthalmology, an NSAID can never compete in antiinflammatory activity with a corticosteroid. Precisely, diclofenac belongs to the NSAIDs group of medicines. Two reports attempted to encapsulate diclofenac in different chitosan-based

nanosuspensions. The first case was reported by Kouchak and Azarpanah (2015), in which this NSAID was entrapped into chitosan-tripolyphosphate nanoparticles by the ionic gelation method. The suspensions with chitosan: tripolyphosphate ratios 1:1, 2:1, and 3:1 showed undesirable turbidity. This was possibly due to the spontaneous formation of microparticles in the presence of adequate concentrations of tripolyphosphate. The physic-chemical analysis revealed that the increased chitosan: tripolyphosphate ratio (4:1, 5:1, and 10:1) led to larger nanoparticles. The ζ-potential of the obtained formulations were all positive with values ranging from +19 to 25 mV. Particularly, the optimum size distribution was obtained at a chitosan: tripolyphosphate ratio of 4:1, for which drug loading efficiency was within a range of 65%. Additionally, a gradual release pattern in an acidic medium and a rapid release at higher pH shows that chitosan-based nanosuspensions could be applied for the enteric delivery of diclofenac to diminish the side effects of the diclofenac in the stomach after oral delivery. However, despite the rapid diclofenac release measured at pH 7.4, the prepared nanosuspensions could be optimal for topical ophthalmic delivery. In parallel to the aforementioned report, in the same year, (Shi et al., 2015) performed nanosuspensions of chitosan grafted methoxy poly (ethylene glycol)-poly (ε-caprolactone) to encapsulate diclofenac. The nanosuspension presented a mean particle size of 105 nm with a low ζ-potential of +8 mV; however, resulted in stability without apparent physical property changes after storage at 4°C or 25°C for 20 days. A sustained release of the encapsulated diclofenac occurred over 8 h. Remarkably, the nanosuspension did not exert any ocular irritation in the rabbit's cornea after 1, 6, and 24 h of instillation. Moreover, enhanced penetration and retention in corneal tissue were detected. Finally, the delivery from de chitosan-based nanosuspensions allowed to reach higher concentrations of diclofenac in the aqueous humor compared with commercial eye drops.

Turning to corticosteroids there is a study in which triamcinolone acetonide, a poorly soluble drug, has been encapsulated into a chitosan matrix for ocular delivery. Likewise, dexamethasone and triamcinolone acetonide are often indicated in the treatment of ocular disorders including cataracts, allergic processes, conjunctivitis, scleritis, uveitis, diabetic macular edema, and diabetic retinopathy. In such a study, triamcinolone acetonide was successfully entrapped in poly (D,L-lactide-*co*-glycolide)-chitosan nanoparticles (mean size: 176 nm; ζ-potential: +20 mV). In vitro assays performed in human corneal epithelial (HCE) cells lead to conclude that the nanoformulation exhibited better antiinflammatory activity, revealed by the decreased secretion of both interleukin-6 and tumor necrosis factor. In an in vivo model, nanoparticles did not produce clinical signs of inflammation. Triamcinolone acetonide-loaded carriers significantly improved inflammatory symptoms compared with free triamcinolone acetonide suspension, 24 h after a single dose in rabbits' eyes. Additionally, after 24 h, the authors detected a substantially higher concentration of triamcinolone acetonide. Overall, the chitosan-bases nanosuspensions bring a brand-new approach to the treatment of ocular inflammatory diseases with corticoids.

Now, corticosteroid and noncorticosteroid drugs are not the only components with antiinflammatory capacity. One example is the statins (atorvastatin, Fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin), drugs usually used to decrease the amount of cholesterol and other fats in the blood. Arafa, Girgis, and El-Dahan (2020) employed atorvastatin calcium, usually used as an ocular antiinflammatory; however, with limited bioavailability after oral intake due to its poor solubility at low pH. To overcome these problems, authors entrapped atorvastatin calcium in polymeric nanoparticles (chitosan-coated poly-lactic-*co*-glycolic acid) to improve surface properties and sustained release, as well as to delivery with site-specific action. In this report, the atorvastatin calcium entrapment was prepared by a single emulsion and solvent evaporation technique (formula 1). Formula 1 presents a mean size of 194 nm and a positive surface charge (+17 mV); moreover, a second formula was created when formula 1 was embedded in thermosensitive Pluronic127-HPMC to form thermosensitive gels. The formulations were biocompatible, nonirritant and prolonged atorvastatin calcium retention was achieved in the corneal tissue. Furthermore, the thermosensitive gel (formula 2) possessed better antiinflammatory activity compared to the other formulation as well as exhibited a more efficient controlled drug delivery.

Inflammation and oxidative stress play a key role in many ocular pathologies. Resveratrol, a natural polyphenol has gained interest in the scientific community for its antioxidant and antiinflammatory characteristics. However, its therapeutic potential is restricted by its low solubility and photosensitivity. In the report from (Buosi et al., 2020), chitosan-based nanosuspension was synthesized by ionic gelation to overcome the obstacles that the resveratrol presented. The spherical morphology of the particles presented a mean size of 140 nm with a ζ-potential value of 32 mV. In addition, the resveratrol encapsulation efficiency was 59%. Biocompatibility tests revealed that the nanosuspension was not cytotoxic nor immunogenic for human retinal pigment epithelial ARPE-19 cells. On the hand, those carriers were successfully internalized in those cells, opening the chance of its application for ophthalmic use.

Corticosteroids are often prescribed for uveitis. However, steroids present long-term side effects systemically and topically. Hence, a novel research work brings up the encapsulation of a monoclonal antibody for the treatment of uveitis, which occurs when the middle layer of the eyeball becomes inflamed. Adalimumab is a fully human antitumor necrosis factor (TNFα) monoclonal antibody. TNFα is involved in the immune system and is found at high levels in inflammatory diseases. Adalimumab provides a promising strategy for patients to significantly reduce the risk of decreased visual acuity from noninfectious uveitis. Although eye drops, a noninvasive topical treatment, could be a potential strategy to reduce the effects, it remains difficult to apply due to limited bioavailability mainly related to poor retention time and permeability of ocular biological barriers, as it was detailed in previous sections.

Thus (Chen et al., 2021) propose the generation of hydrogel eye drops composed of chitosan and β-glycerophosphate as adalimumab carriers. The authors

verified the low cytotoxicity in epithelial cells (HCEC), retinal pigment epithelial cells (ARPE 19) cells, and mouse fibroblast cells (L929) provoked by the nanosuspensions administration. Likewise, the use of Sprague-Dawley rats and male Japanese white-eared rabbits were required to support optimal tolerability, intraocular patency, and efficacy of uveitis treatment. Hence, the published research shows a friendly noninvasive strategy to improve the drug permeability rate and the efficacy of uveitis treatment.

2.6.4 Others

The use of gene therapy in the target tissue is considered more than a promising alternative for the treatment of various chronic diseases. Gene therapy aims to introduce nucleic acid-based drugs as they would be more efficient and safer than those currently available. These predictions are based on the fact that genes are capable of expressing their products for periods that greatly exceed the duration of action of currently available drugs. However, despite the recognized value of DNA-based therapies, there are limitations such as poor cell uptake, rapid degradation in vivo, and limited transport to the target, which remain to be resolved before this technology becomes therapeutically viable. Among the strategies developed to overcome these challenges, viral vectors have received great attention. Unfortunately, these vectors can also elicit strong immune responses and cause harmful reactions. The use of synthetic nanocarriers has recently been proposed as a promising alternative for gene delivery to the target tissue. de la Fuente, Seijo, and Alonso (2008) focused on gene therapy for ocular surface affections, for example, dry eye, corneal dystrophies, corneal neovascularization, and ocular allergy. Those researchers have shown that polymeric nanoparticles are capable of adhering to the ocular surface and penetrating through the corneal and conjunctival epithelium, thus achieving specific genes (pEGFP, pSEAP, pβGal) delivery after topical administration. Among the different polymers for the design of ocular gene nanocarriers, polysaccharides, and chitosan, are particularly attractive biomaterials. A mix of chitosan and HA with the crosslinker tripolyphosphate was used for nanoparticle design. The ionic gelation method allowed to obtain nanoparticles with a size from 100−215 nm, being this parameter is affected by the plasmidic deoxyribonucleic acid (pDNA) loading. Notably, pDNA did not exert any variations on the nanoparticle surface charge; also, the ζ-potential range from +40 to −30 mV upon the addition of increasing amounts of HA. Authors reported that HA-chitosan nanoparticles enter the corneal and conjunctival epithelial cells and effectively deliver pDNA, reaching high transfection levels. Therefore, we conclude that these nanoparticles may represent a new strategy for gene therapy for various eye diseases.

5-Fluorouracil is a pyrimidine analog and is a common drug used to treat epithelial cancers. It acts by interacting with the S phase of proliferating abnormal cells that form squamous carcinomas. It has limited side effects on the normal ocular surface epithelium. Currently, a high concentration (1%) of 5-Fluorouracil

is used for ophthalmic application; however, this therapy could be improved by reducing toxicity and facilitating the specific accumulation in the tumor regions with prolonged exposure of those cells to this reagent. Nagarwal, Singh, Kant, Maiti, and Pandit (2011) developed 5-fluorouracil loaded chitosan-tripolyphosphate nanoparticles by the ionic gelation method for ophthalmic delivery. The best formulation of nanoparticles exhibited a spherical morphology with 192 nm of mean diameter and a positive ζ-potential of +30 mV. The encapsulation efficiency of the 5-fluorouracil resulted in 34%. Importantly, the drug crystallinity was not changed in drug-loaded nanoparticles. The mucoadhesive property of chitosan nanoparticles allows for an increase in the residence time of the encapsulated 5-fluorouracil observed in New Zealand rabbits. Additionally, nonirritant signs and inflammation were detected.

Keratoconus occurs when the cornea thins and gradually protrudes into a cone shape. A cone-shaped cornea causes blurred vision and can cause sensitivity to light and glare. Furthermore, there is a significant increase in the levels of proinflammatory cytokines in the tears of patients with keratoconus. In the early stages of keratoconus, one may be able to correct vision problems with glasses or soft contact lenses.

Lactoferrin is a milk-based globular protein belonging to the transferrin family. This group of proteins shows a high affinity for iron ions, this protein is present in body fluids, such as blood, and is abundant in mucous fluids (e.g., tears, semen, saliva, bronchial secretions, etc.). Lactoferrin has antimicrobial, antioxidant, antiparasitic, antiallergic, antitumoral, and antiinflammatory. Also, is considered a component of innate immunity. Additionally, lactoferrin protects the corneal epithelium from the effects of ultraviolet radiation, direct airflow, and chemical agents. Varela-Fernández et al. (2021) attempted to encapsulate lactoferrin by ionic gelation into chitosan-tripolyphosphate nanoparticles to use them therapeutically in the keratoconus condition. The authors obtained carriers with a mean size of 181 nm and positive ζ-potential values (∼18 mV). In vitro release profiles revealed lactoferrin enhanced, prolonged, and controlled delivery from the polymeric nanoparticles. Those noncytotoxic nanocarriers presented appropriate mucoadhesive properties with a high permanence time on the ocular surface. Hence, this formulation could be considered for the controlled release of lactoferrin as a brand-new topical ophthalmic drug delivery system. On the other hand, Sunkireddy, Kanwar, Ram, and Kanwar (2016) manufactured algae-derived chitosan nanoparticles for bovine lactoferrin topical administration to prevent carbendazim-induced toxicity. Carbendazim is a commonly used fungicide and is an environmental contaminant that impacts animal and human health. In this report, the authors performed highly stable, nonaggregative, and monodispersed algal chitosan-tripolyphosphate nanoparticles by ionic gelation.

The average particle size of the synthesized nanoparticles as determined by DLS was around 30−50 nm and the lactoferrin encapsulation efficiency was 81%. A burst release of 40% was quantified within the first 6 h at neutral pH. Then, the release rate persisted and sustained until 72 h. Algae-derived chitosan nanoparticles did not

induce any cytotoxic effect on human lens epithelial cells (HLEB-3) cells. Also, the nano-encapsulated lactoferrin protein efficiently prevented the carbendazim-induced oxidative stress and apoptosis in HLEB-3 cells. Moreover, this nanoformulation when administered topically in rat and bovine eyes revealed an efficient internalization within the first hour, being especially abundant in corneal and lens regions versus retina. To conclude, the authors suggest that chitosan from an algal source could be employed for topical administration of lactoferrin preventing carbendazim-induced toxicity in ocular tissue. However, this nanoformulation could be utilized for the targeted delivery of other eye diseases, for example, age-related macular degeneration, glaucoma, and cataract.

The cornea transplant is surgery to replace the cornea with tissue from a donor and is one of the most common transplants performed. However, there is limited access to compatible donors, in addition to the surgical cost. Therefore, artificial corneas are considered alternative tools with promising prospects. In the study carried out by (Bakhshandeh et al., 2021), a polymeric artificial cornea was designed and developed. On the other hand, an extract of the human amniotic membrane was prepared that contained growth factors, cytokines, antiinflammatory factors, and antiangiogenic factors. In this way, those factors were encapsulated into chitosan-dextran nanocarriers and physically decorated on poly (ε-caprolactone) -polyvinyl alcohol from the artificial cornea. Fortunately, characterization tests demonstrate that the prepared artificial cornea had similar biocompatible and structural characteristics relative to a natural cornea. Likewise, it was found that bioactive loaded chitosan-dextran nanoparticles improved the antiangiogenic property of the artificial cornea through the sustained release of antiangiogenic factors such as thrombospondin-1, endostatin, and heparin sulfate proteoglycan. Thus, the aforementioned study indicates that the use of the developed artificial cornea could be a promising strategy in corneal transplantation, without the need to depend on postmortem donors.

2.7 Conclusions

Nanosuspensions are novel, practical, and industrially viable tools to solve the limitations of hydrophobic drugs, such as low solubility and bioavailability Their physicochemical characteristics, that is, size and charge, make them appropriate for ocular illness treatments and their application in diagnostics.

The improved characteristics of many drugs, such as dissolution speed, greater solubility, better bioadhesives, versatility in the surfaces modifications, and easily postproduction processing allow affirming the success of nanosuspensions applications for various ocular routes of administration. In this way, the obtained advances in understanding the interaction of nanosuspensions with ocular barriers should translate into ocular nanomedicines innovations with a significant impact on clinical ophthalmology.

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CHAPTER

Nanoemulsions in theranostics

3

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3.1 Introduction to emulsion systems

Emulsions are a class of colloidal and coarse disperse systems comprised of particles of one liquid dispersed in another immiscible liquid (Tadros, 2016). These two immiscible liquids require the presence of emulsifying agents (emulsifiers) or surface-active agents (surfactants) for sufficient mixing. The choice of surfactant has a critical role in emulsion formation and long-term stability (Tadros, 2016). Surfactants are amphiphilic molecules comprised of a polar, hydrophilic head and a nonpolar, hydrophobic tail that orient at the interface of water and oil, respectively, to reduce the interfacial tension and promote miscibility (Tadros, 2016).

In general, there are two types of emulsion systems: oil-in-water (O/W) and water-in-oil (W/O). O/W emulsion systems are comprised of droplets of oil dispersed in a water continuous phase, while W/O emulsion systems consist of droplets of water dispersed in an oil continuous phase. Fig. 3.1 depicts the

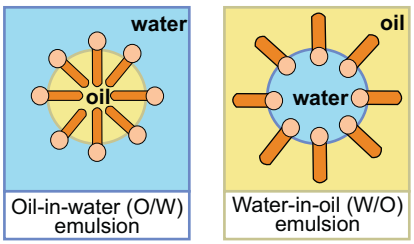

FIGURE 3.1

Orientation of surfactant molecules (*orange*) at oil and water interfaces. In O/W systems, surfactant molecules orient with their hydrophilic heads projecting outward into the aqueous phase (around the droplet) while their hydrophobic tails are contained in the organic phase (within the droplet). In W/O systems, the orientation is reversed. These surfactant-covered droplets are called micelles.

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orientation of a typical surfactant molecule at the interface of oil and water in both O/W and W/O emulsion systems.

The type of emulsion formed is best predicted using the Bancroft rule (Bancroft, 1913). According to Bancroft (1913), the continuous phase of the emulsion is dictated by the phase in which the surfactant is most soluble (Bancroft, 1913). Therefore, hydrophilic emulsifiers result in the production of an O/W emulsion while hydrophobic emulsifiers result in the production of a W/O emulsion. The hydrophilic-lipophilic balance (HLB), as proposed by Griffin in 1949 (Griffin, 1949) is one way to classify hydrophilic and hydrophobic emulsifiers. It relates the structure of each surfactant to its emulsification potential. The ratio of the hydrophilic portion of the surfactant to that of the hydrophobic portion is classified quantitatively on an arbitrary scale of 0−20 where surfactants with an HLB value >10 are considered hydrophilic and those <10 are considered hydrophobic.

Emulsion systems are diverse and exist in a variety of types. The emulsions described above are most commonly referred to as "macroemulsions," given that droplet sizes are typically above 400 nm in diameter (Rosen & Kunjappu, 2012). As a result, these emulsions are often opaque in nature (Rosen & Kunjappu, 2012), as evident in food products such as mayonnaise or in cosmetic products such as lotions. Macroemulsions are kinetically stable in nature, due to their energetic profile. The interfacial Gibbs free energy for macroemulsion formation is positive, resulting in a steep energy barrier that must overcome prior to emulsion formation (Weiner, 2020). This results in temporary stability with inevitable phase separation. Therefore, surfactants provide a steric (and sometimes, electrostatic) barrier around droplets that simply delays phase separation for some period of time.

3.2 "Nano" emulsion systems

In principle, the terms "nano" and "micro" refer to entities in the size range of 10−1000 nm and 10−1000 μm, respectively. However, these definitions vary by field and this is certainly evident in the case of emulsion science, where the terms "nano" and "micro" have very distinct and counterintuitive meanings.

In emulsion science, nanoemulsion droplets may be larger than microemulsion droplets, possessing sizes up to 400 nm in diameter compared to microemulsions which are 10−100 nm in diameter (Callender, Mathews, Kobernyk, & Wettig, 2017). The reason for this historical "mix-up" has been well documented by McClements (2012) but beyond size differences, important energetic and compositional distinctions set nano- and microemulsions apart.

With respect to the energetics of formation, nano- and microemulsions possess varying energy profiles. The energy profile of nanoemulsions is most similar to that of macroemulsions where the interfacial Gibbs free energy is

positive and an energetic barrier must be overcome before formation (McClements, 2012). This results in the nanoemulsions possessing kinetic stability, similar to their macroemulsion counterparts. Therefore, phase separation in nanoemulsions is also inevitable (McClements, 2012). Countering this, the smaller sizes of nanoemulsions as compared to macroemulsions affords a greater advantage with respect to stability and so nanoemulsions may possess significantly longer stability profiles than that of macroemulsions, sometimes in the range of months to years. In contrast, the energy profile of microemulsions is such that the interfacial Gibbs free energy of formation is negative. This means that microemulsion formation is a spontaneous and forward-driven process. It also means that in contrast to both macro- and nanoemulsions, microemulsions are thermodynamically stable and therefore, phase separation is only possible with the addition of energy to the system or with a change in compositional elements due to factors such as evaporation. The differing energy profiles of nano- and microemulsion formation mean that nanoemulsions often require high energy input (e.g., homogenization) for formation whereas microemulsions may be formed by a simple stirring of the starting ingredients. Fig. 3.2 depicts the energy profiles of microemulsions.

Concerning composition, the energetic profiles of both nano- and microemulsions result in slightly different compositional requirements. The starting ingredients of surfactant, oil, and water are still required—just in slightly varying amounts. For nanoemulsions, the higher interfacial energy results in a greater variety of surfactants that may be used for formation. In contrast, the low interfacial energy of microemulsions requires that only certain surfactants, specifically small-molecule surfactants, be used for formation to ensure efficient packing at the interface (McClements, 2012). Cosurfactants (discussed below), if present in microemulsion systems, are typically shorter in length than in nanoemulsions for this reason as well (Rosen & Kunjappu, 2012). Nano- and microemulsions also differ slightly with respect to their surfactant-to-oil ratios, as microemulsions require a slightly higher ratio to ensure that all oil droplets are sufficiently solubilized in the hydrophobic core of the surfactant-coated droplets (McClements, 2012). Finally, the lower interfacial tension observed in microemulsions typically requires greater adsorption of surfactant molecules at the interface and thus, a higher surfactant content than in nano- and macroemulsions. In general, nanoemulsions most closely resemble macroemulsions with respect to the energetics of formation, stability, and composition, and microemulsions with respect to droplet size. Table 3.1 depicts these similarities and differences.

3.3 Nano- and microemulsion use in theranostics

Nanotechnology tools with the potential to simultaneously diagnose and treat the disease have received an unprecedented amount of attention since the term was

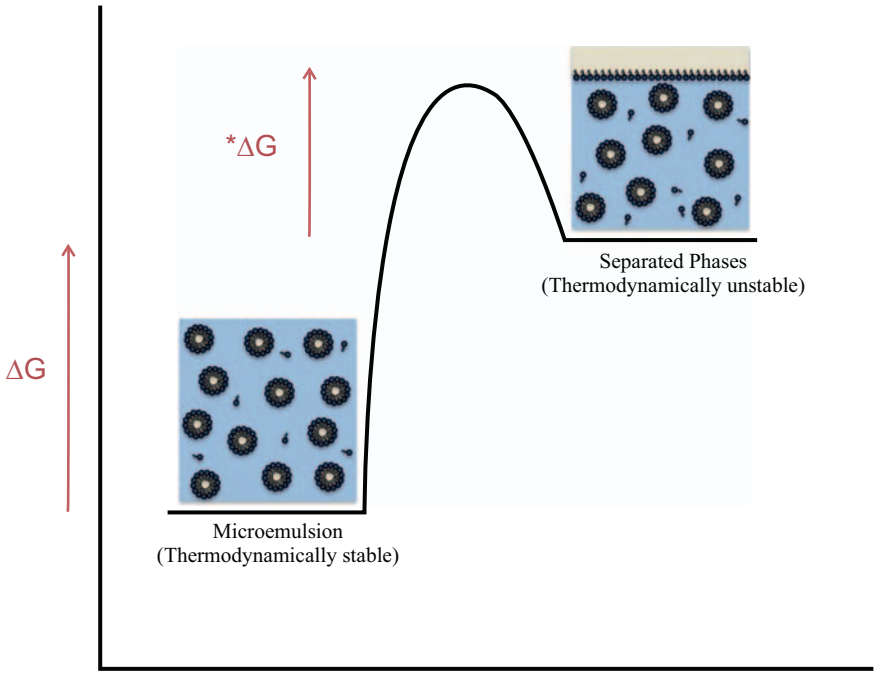

FIGURE 3.2

Schematic of the energetics involved in a microemulsion system. The microemulsion product is at a lower energy state than that of its separated phases or starting components, contributing to its thermodynamic stability. Nanoemulsions have a reversed profile with the nanoemulsion product at a higher energy state than the starting components. This not only results in an input of energy to form the nanoemulsion but also in a kinetically, rather than thermodynamically, stable state.

Taken from Callender, S., Mathews, J., Kobernyk, K., & Wettig, S. (2017). Microemulsion utility in pharmaceuticals: Implications for multi-drug delivery. International Journal of Pharmaceutics, 526, 425–442. https://doi.org/10.1016/j.ijpharm.2017.05.005 *with permission. Tadros, T. F. (2016). Chapter 1: Emulsions: Formation, stability, industrial applications. In* Emulsions: Formation, stability, industrial applications *(pp. 1–6). Berlin, Germany: De Gruyter.*

first coined by PharmaNetics CEO, John Funkhouser in 1998 (Langbein, Weber, & Eiber, 2019). Nevertheless, the principles of theranostics have been used since the 1940s, particularly in the treatment of thyroid cancer using ^{131}I for radioactive iodine therapy (Langbein et al., 2019). Since then, nano theranostics have been used for the treatment of myriad neurological, inflammatory and cardiovascular conditions.

Over the past 15 years, the use of nano- and microemulsions in theranostics has generally increased as depicted in Fig. 3.3. The increased use of nano- and

Table 3.1 Physical and energetic differences between macro-, nano-, and microemulsions.

	Macroemulsion	Nanoemulsion	Microemulsion
Composition	Low surfactant	Low surfactant; Lower surfactant-to-oil ratio	High surfactant; Higher surfactant-to-oil ratio
Droplet size (diameter)	> 400 nm	< 400 nm	10–100 nm
Interfacial Gibbs free energy of formation	Positive	Positive	Negative
The energy required for the formation	High	Typically high	Low
Stability	Kinetic	Kinetic	Thermodynamic

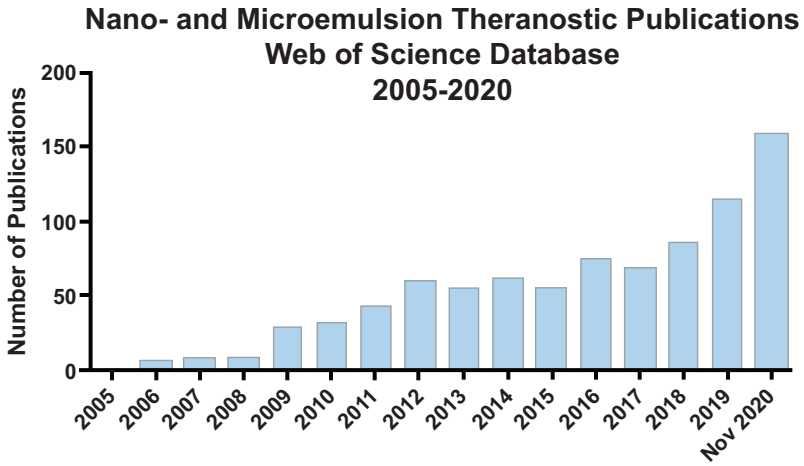

FIGURE 3.3

Publications regarding nano- and microemulsion theranostics available on the database.

Web of Science from January 1, 2005 to November 27, 2020. [Search criteria: ((“nanoemulsi ”) AND (“theranostic* ” OR “theragnostic* ”)) and ((“microemulsi* ”) AND (“theranostic* ” OR “theragnostic* ”)) in “Topic” field]. Results include research and review articles, proceedings papers, meeting abstracts and book chapters.*

microemulsions in theranostics is particularly evident in the last three years, and may be attributed to an increasingly comprehensive understanding of their advantages.

3.4 Advantages of nano- and microemulsions in theranostic applications

The use of nano- and microemulsions for theranostic purposes offers many unique advantages, especially with respect to (1) droplet size, (2) starting ingredients, (3) stability, and (4) the delivery of both hydrophilic and hydrophobic compounds. These advantages are discussed below.

3.4.1 Droplet size

As previously noted, nano- and microemulsions generally possess droplet sizes ranging from 10 to 400 nm in diameter (McClements, 2012). These small droplet sizes increase the surface area to volume ratio for drug absorption leading to improved bioavailability. In theranostics, this is particularly advantageous for diagnostic imaging or treatment monitoring where a tagging molecule is orally consumed. This is also advantageous from a therapeutic delivery standpoint as the small droplet sizes and large surface area to volume ratio have the potential to speed up this process- particularly when targeting molecules are employed (McClements, 2012).

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3.4.2 Starting ingredients

In contrast to other nanotechnology systems, nano- and microemulsion systems are constructed from relatively simple starting ingredients. A functional emulsion system need only be comprised of three basic ingredients- an organic phase, an aqueous phase, and a surfactant or amphiphile of some sort. In general, oil and surfactant materials are relatively economical, and there are a wide variety of types to choose from. Furthermore, the surfactants can self-assemble, and in the case of microemulsion systems, require little to no energy input for formation (Callender et al., 2017). These economic benefits are particularly advantageous in the field of theranostics, as the use of metallic particles such as iron or gold, for example, is already a high-cost factor in diagnostic imaging.

3.4.3 Stability

Nano- and microemulsions may be resistant to gravitational separation, but as noted in Fig. 3.2, the energy profiles of both emulsions are very different. Nanoemulsions are kinetically stable while microemulsions are thermodynamically stable. This means that nanoemulsions will inevitably, over time, phase separate into their original aqueous and organic components. This may occur over a few weeks, months, or even years (McClements, 2012). Microemulsions, however, remain stable for an indefinite period of time, and for all intents and purposes, are considered infinitely stable.

This difference in stability has implications with respect to manufacturing. The energy profile of nanoemulsions means that a homogenization method must often be employed. This is often an unfavorable step in theranostics design and development, as sensitive targeting or therapeutic molecules (i.e. proteins) may not be able to withstand this type of preparation process. In cases like this, microemulsions are the obvious choice.

3.4.4 Delivery of hydrophilic and hydrophobic compounds

In addition to the small droplet sizes, stability, and relative simplicity of nano- and microemulsion systems, one of their primary advantages is the delivery of both hydrophilic and hydrophobic compounds. While other nanotechnology systems are limited in the type of compound that can be delivered, the innate nature of emulsion systems and the fact that both organic and aqueous phases are present allow delivering both hydrophilic and hydrophobic compounds, even simultaneously (Callender et al., 2017). In the context of theranostic delivery, this is particularly advantageous should the therapeutic compound and diagnostic compound differ in solubility. Both compounds can still be delivered using nano- and microemulsion technology without making modifications to the formulation itself. The many advantages of nano- and microemulsion systems in theranostic applications have led to increased use, particularly in the last three years (Fig. 3.3). However, their design and development require careful consideration of several factors.

3.5 Design and development of theranostic nanoemulsions

The design and development of theranostic systems depend on a variety of factors including the type of diagnostic imaging to be employed, the type of therapeutic to be delivered, the unique characteristics of the organ or tissue targeted, and the route of administration or delivery and more. In addition to these factors, the design and development of nano- and microemulsions for theranostic applications depends on the following: (1) surfactant choice, (2) oil choice (3) the type of compound to be encapsulated or delivered and (4) the type of targeting molecule

employed (if any). A fundamental understanding of these characteristics and their role in nano- and microemulsion formation can equip researchers with the tools they need to build a functional, nano- or microemulsion system for their unique theranostic application

3.5.1 Surfactant choice

Surfactants and/or emulsifying agents are critical components of an emulsion system. As noted earlier, surfactants are amphiphilic molecules that reduce the interfacial tension between two immiscible phases such as an aqueous and organic phase, to promote miscibility. Without surfactants, it would be near impossible to sufficiently stabilize such a system. In general, all surface-active compounds, or compounds that act at a surface or interface between two immiscible phases to reduce the surface or interfacial tension, are amphiphilic in nature. However, not all amphiphilic compounds are surface-active or have the ability to reduce surface tension. We have discussed earlier that one way to select an appropriate surfactant for an emulsion system, is using the Bancroft rule (Bancroft, 1913) and HLB (Griffin, 1949). In other words, a hydrophilic surfactant of high HLB can be selected to deliver hydrophobic compounds, and a hydrophobic surfactant of low HLB can be selected to deliver hydrophilic compounds (Griffin, 1949). Another important consideration in surfactant selection is the nature of the head group. Surfactants may be broadly classified by their head groups as ionic or nonionic. Ionic surfactants possess a charged head group while nonionic surfactants possess an uncharged polar head group. Surfactants may be further classified as positively or negatively charged, or cationic or anionic, respectively. A special case of surfactant also exists where both negative and positive charges are present, but cancel each other to result in an electrostatically neutral compound. These surfactants are known as zwitterionics. Examples of each are listed in Table 3.2.

Table 3.2 Classification of surfactants based on the nature of the hydrophilic head group.

	Surfactant type (net charge)	Examples
Nonionic	Nonionic (0)	Polyoxyethylene (POE)-based compounds for example, polysorbates/tweens sorbitan-based compounds for example, Spans Tri-block copolymers for example poloxamers/ pluronics
Ionic	Anionic (–)	Sulfates, carboxylates, phosphates
	Cationic (+)	Quaternary ammonia compounds
	Zwitterionic (+/ – , Net 0)	Betaines

From a drug delivery perspective, nonionic surfactants are preferred for various reasons. Their uncharged nature not only results in a favorable safety profile, especially for oral administration but also affords resistance to charge effects, such as pH changes (Myers, 2005). Nonionic surfactants also possess a lower critical micelle concentration or concentration at which surfactant micelles are formed, than ionic surfactants (Myers, 2005). This is because the charged surfactant head groups of ionic surfactants tend to electrostatically repel each other, interfering with the surfactant's ability to concentrate at the interface and reducing the interfacial tension to promote miscibility. In the context of emulsion preparation for theranostic purposes, this means that lower concentrations of nonionic surfactants can be used, ameliorating toxicity concerns.

Although nonionic surfactants are advantageous for the above reasons, ionic surfactants confer their own unique advantages with respect to theranostic applications. For diagnostic imaging involving the use of metals with magnetic properties, ionic surfactants may be the preferred choice. Ionic surfactants of the opposite charge may be coupled to metal particles for use in magnetic resonance imaging (MRI) for example. They may also be coupled to therapeutic compounds or target molecules of a charged nature for other theranostic applications.

It should be noted that, particularly for microemulsion systems, cosurfactants may be used. Cosurfactants act in consort with surfactants to further reduce interfacial tension and introduce an element of flexibility into the interfacial film. This flexibility allows the emulsion system to adapt to a wider variety of curvatures, including smaller droplet sizes, across a wider range of conditions (Talegaonkar & Negi, 2015). Common cosurfactants are medium-chain (6−12 carbons) alcohols (Talegaonkar & Negi, 2015). Cosurfactants may also act as scaffolds to which targeting molecules are attached or coupled for theranostic purposes.

One final consideration when selecting a surfactant is its safety and toxicity. Ionic surfactants tend to be toxic at lower concentrations than nonionic surfactants (Attwood & Florence, 1983). This is because charged surfactants are better able to electrostatically interact with structural components of the cell membrane causing a disruption in their structural integrity (Attwood & Florence, 1983). Therefore, for theranostic purposes, the type and concentration of surfactant used must be carefully controlled. One useful resource to note is the Inactive Ingredient database created by the United States Food and Drug Administration (United States Food & Drug Administration US FDA, 2021). This database provides tolerability limits for many ionic and nonionic surfactants that have been previously used in approved therapeutic products.

3.5.2 Oil choice

There is a variety of oils to choose from when it comes to preparing nano- and microemulsions. Oils with varying alkyl chain lengths such as hexane or

dodecane, aromatic hydrocarbons such as toluene, or even triglycerides consisting of fatty acids such as oleic and stearic acid, may be used. Concerning microemulsion formation, triglycerides are common (Hauss, 2007; Mullertz & Grove, 2007).

Triglycerides are weakly polar oils that consist of three fatty acids attached to a glycerol backbone. They exist as short- (<6 carbons), medium- (6−12 carbons), and long- (>12 carbons) chain triglycerides (Shah & Limketkai, 2017). Medium and long-chain triglycerides are often used in microemulsion formation for the delivery of hydrophobic or lipophilic compounds (Čerpnjak, Zvonar, Gašperlin, & Vrečer, 2013). These weakly polar oils possess greater water solubility than conventional oils, allowing for faster mobility to the oil-water interface (Hauss, 2007; Mullertz & Grove, 2007). This surface activity may afford an even greater reduction in interfacial tension and promote small droplet formation. Medium chain triglycerides have also been reported to enhance the stability of therapeutic compounds (Čerpnjak et al., 2013).

In selecting an oil for nano- and microemulsion formation for theranostic applications, it is important to consider the compatibility of the oil with the therapeutic, targeting, or diagnostic molecule used, especially when such molecules are hydrophobic. Hydrophobic interactions between the oil phase and any of these molecules may be advantageous when functioning as a drug carrier, but disadvantageous when considering the release of these molecules from the emulsion droplet.

3.5.3 Type of compound for encapsulation and delivery

Nanosized emulsion systems are effective drug delivery tools given their ability to solubilize a variety of compounds, regardless of their solubility preferences. As mentioned previously, O/W nano- and microemulsions are best suited for the encapsulation and delivery of hydrophobic compounds, as these can be effectively contained in the hydrophobic core of O/W emulsion droplets. W/O nano- and microemulsions, on the other hand, are best suited for the encapsulation and delivery of hydrophilic compounds, as these can be effectively contained in the core of W/O emulsion droplets.

Though nano- and microemulsions are well suited for the delivery of hydrophobic and hydrophilic compounds, such compounds are not always localized in the core of emulsion droplets. Molecules localize in different regions of the droplet depending on their polarity and composition. In O/W systems, the presence of certain functional groups in a compound may encourage interactions with the hydrophobic surfactant tails present within the droplet, causing the compound to localize near the surface of the droplet in the palisade layer. Hydrophobic compounds demonstrating weakly hydrophilic characteristics may even concentrate in the upper palisade layer, near the hydrophilic surfactant head groups. This is also true in the case of W/O

emulsion systems. The presence of certain functional groups in hydrophilic compounds may encourage interactions with the hydrophilic surfactant head groups, causing localization in the palisade layer. Hydrophilic compounds demonstrating weakly hydrophobic characteristics may localize in the upper palisade layer in an effort to interact with the hydrophobic surfactant tail groups. It is therefore evident that the extent of these interactions and the localization of a compound within an emulsion droplet directly depends on a number of factors. These include, but are not limited to, the polarity of the compound, surfactant/oil characteristics such as alkyl chain length, geometric restraints, and environmental conditions such as temperature and pH (McClements, 2012). These factors also have direct implications on the amount of material that can be incorporated into nano- and microemulsion droplets (McClements, 2012).

Nano- and microemulsion systems are not only suitable for the delivery of compounds that are strictly hydrophilic or hydrophobic; amphiphilic compounds may also be delivered. In this case, the amphiphilic compound is unlikely to concentrate within the droplet core of the emulsion, or even within the palisade layer. Instead, these compounds are most likely to concentrate at the oil-water interface in a similar fashion to that of a surfactant, with the hydrophilic portion of the molecule projecting into the aqueous phase and the hydrophobic portion molecule projecting into the organic phase. Therefore, it is important to note that although the delivery of amphiphilic compounds is possible, its concentration at the interface may directly impact the surfactant molecules located there. Depending on the composition and size of the amphiphilic compound, it may be able to fit itself in between the surfactant molecules at the interface, acting in consort with the surfactant to further reduce the interfacial tension between oil and water and promote emulsion formation. Conversely, it may disrupt the surfactant layer at the oil-water interface, destabilizing the emulsion system. Microemulsion systems generally require a higher ratio of surfactant to oil for formation, as compared to nanoemulsion systems (McClements, 2012). Thus, in microemulsion systems, a greater degree of competition may arise with respect to space at the interface when attempting to incorporate amphiphilic compounds. Nevertheless, it is evident that the type of stabilizing or destabilizing effect is a direct result of the type of amphiphilic compound, surfactant, and oil used.

In theranostic applications, the hydrophilicity, hydrophobicity, or amphiphilicity of the compounds used for diagnostic imaging, targeting, and therapeutic purposes must be carefully considered. in combination with the type of surfactant and oil selected for microemulsion formation. Therefore, the design and development of theranostic nano- and microemulsions must begin with solubility studies in the desired organic or aqueous phase, as well as interaction studies amongst components. Fig. 3.4 illustrates the localization of hydrophilic, hydrophobic, and amphiphilic compounds in O/W and W/O nano- and microemulsion droplets.

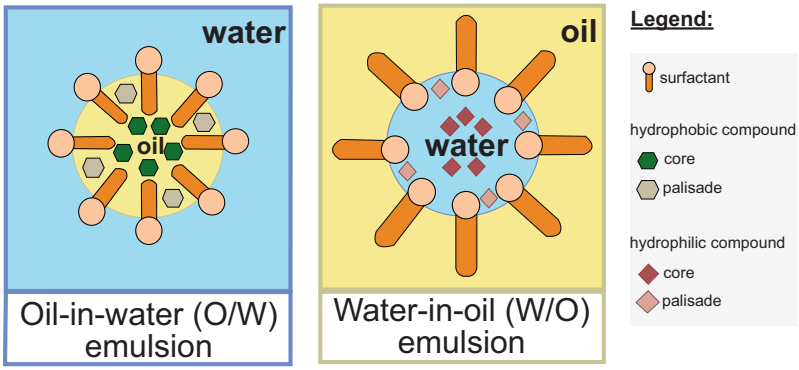

FIGURE 3.4

Localization of therapeutic and diagnostic molecules for theranostic purposes in O/W and W/O emulsion systems. Hydrophobic and hydrophilic compounds may concentrate in the core of O/W and W/O emulsions, respectively, or the palisade layer near the surfactant molecule.

3.5.4 Targeted delivery

In addition to the encapsulation and delivery of hydrophobic, hydrophilic, and amphiphilic compounds in nano- and microemulsion systems, whether for imaging or therapeutic purposes, the use of targeting molecules may also be employed. Such targeting molecules may be used for both diagnostic imaging purposes in the form of tagging, and for therapeutic purposes in the form of binding to induce a therapeutic effect or binding to release therapeutic material from the emulsion droplet. Proteins and peptides are typically used to target cell-surface receptors or markers for many diseases. Antibodies may also be used to generate an immune response. In addition to proteins, peptides, and antibodies other components such as lipids and polymers may also be used in targeting. These lipids and polymers may act as targeting molecules themselves, or as linkers to which targeting molecules are attached.

Given that the surface of nano- or microemulsion droplets is covered with surfactant molecules, attaching targeting molecules to the surface of these droplets means directly coupling such molecules to the surfactant. In O/W emulsion systems, the targeting molecule must be compatible or interact with, the hydrophilic head of the surfactant while in W/O emulsion systems, the targeting molecule must interact with the hydrophobic tails of the surfactant. In addition to direct coupling the target molecule to the respective surfactant moiety, if amphiphilic, the targeting molecule may concentrate at the oil-water interface like a surfactant, with a portion of its structure in the droplet core and the other on the droplet surface. Linker molecules may also be used to directly attach the targeting molecule to the surfactant head or tail group, or they may be localized at the interface

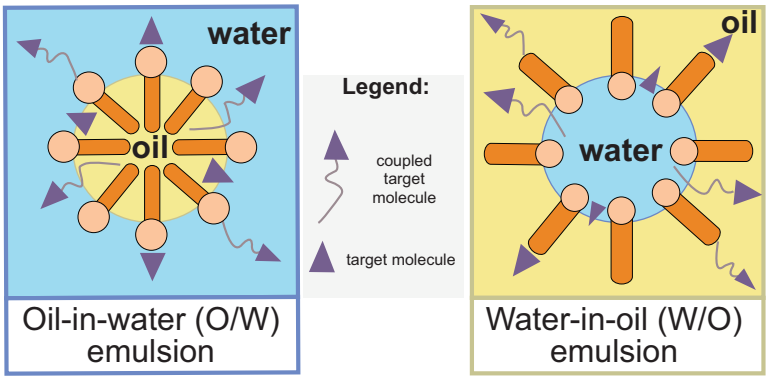

FIGURE 3.5

Localization of target molecules for theranostic purposes in O/W and W/O emulsion systems. Amphiphilic target molecules may concentrate at the oil-water interface, between surfactant molecules. They may also be directly attached to the surfactant head group (in O/W systems) or the surfactant tail group (in W/O systems). Target molecules may be coupled to lipids, polymers, and other linker compounds.

between the surfactant molecules. Fig. 3.5 illustrates the localization possibilities of targeting molecules that may be used in nano- and microemulsions designed for theranostic applications.

3.6 Applications of nano- and microemulsions in theranostics

The simultaneous encapsulation and delivery of diagnostic imaging and therapeutic molecules using nano- and microemulsions has been achieved for a variety of theranostic applications. From the encapsulation of magnetic particles for MRI diagnostics to the targeted delivery of therapeutics via molecules attached to emulsion droplet surfaces, the landscape of nano- and microemulsion development for theranostic purposes is rapidly expanding. The following sections discuss the use of diagnostic, therapeutic, and targeting molecules incorporated into nano- and microemulsion systems for theranostic applications.

3.7 Diagnostic purposes

3.7.1 Metals

The use of metals, particularly with magnetic properties, has afforded the advancement of diagnostic imaging tools such as MRI and magnetic particle

imaging (MPI). It is well-known that the metal of choice in theranostic applications depends on several factors including the type of imaging desired, the route of administration, and course, the metallic properties of the particle itself.

For positive (T1) contrasts in MRI, paramagnetic ion complexes such as gadolinium (Gd^{3+}) and manganese (Mn^{2+}) are most common while for negative (T2) contrasts, superparamagnetic contrast agents such as iron oxide or iron platinum are popular. Metals such as dysprosium (Dy^{3+}) and holmium (Ho^{3+}), though paramagnetic, have also been used as T2 contrast agents (Pellico, Ellis, & David, 2019). Depending on the target organ, contrast agents may also include mineral oils, oil emulsions, and sucrose polyester, as documented previously (Ballinger, Magin, & Webb, 1991; Gach, 2019). Nonmagnetic metals may be used in heat or light-responsive theranostic applications.

Regardless of the type of metallic contrast agent employed, the most important consideration concerning use in nano- or microemulsion theranostics remains where each metal particle localizes in an emulsion droplet. In contrast to therapeutic agents or target molecules that are "affixed" to the droplet core, droplet surface or oil-water interface, metal particles may localize in one of two areas: (1) at the oil-water interface of each droplet, resulting in a Pickering emulsion or (2) within an emulsion droplet as in typical nano- or microemulsion systems.

3.7.1.1 Pickering emulsions

Solid compounds, including metals, may be delivered using a special type of emulsion called a Pickering emulsion. A Pickering emulsion consists of a film of solid particulates around each droplet, affording stability against coalescence, or droplet merging (Yang et al., 2017). Concerning theranostic applications, these solid particulates are likely to comprise metal particles for diagnostic imaging purposes. The formation of Pickering emulsions is dictated by the "wettability" of the solid particle at an oil-water interface; the phase better able to "wet" the solid metal particle, becomes the continuous phase of the emulsion while the other phase becomes the dispersed phase (Gonzalez Ortiz, Pochat-Bohatier, Cambedouzou, Bechelany, & Miele, 2020). In addition, if the contact angle of the particle with water or oil is less than 90 degrees, the emulsion is of the O/W or W/O type, respectively (Gonzalez Ortiz et al., 2020). Although Pickering emulsions are typically larger in size than nano- and microemulsions due to their thicker interfacial layer which can confer a greater degree of physical stability compared to a surfactant (Zhao, Wang, Xiao, Chen, & Cao, 2020), they often go through a homogenization or ultrasonication process to allow for the formation of nanosized emulsion droplets.

The manufacture of a Pickering emulsion involves the mixing of the solid particle with either the organic or aqueous phase. Typically, oil is used as the increased viscosity enables the solid particles to remain suspended in solution (Josefczak & Wlazlo, 2015). The organic mixture is then combined with water and external energy in the form of ultrasonic waves or high-pressure homogenization, for example, is applied (Josefczak & Wlazlo, 2015). This homogenization

process can result in metal-containing Pickering emulsions of nanoscale proportions, useful for numerous biomedical imaging purposes. However, stability- particularly sedimentation, is a key consideration for this type of solid-stabilized emulsion system. Emulsion systems containing magnetic particles are also susceptible to separation in the presence of a magnetic field (Zhou et al., 2011). The amount of solid material necessary for incorporation into a Pickering emulsion varies depending on the application. For example, in MPI, concentrations of as low as 40 μmol Fe/L have been reported as sufficient (Wu et al., 2019). Therefore, provided that Pickering emulsions are sufficiently able to accommodate the required concentration of metal particles for the required application while demonstrating stability, their use in theranostics is possible.

Pickering emulsions comprised of iron oxide magnetite, Fe_3O_4, remain one of the most common technologies used in the biomedical field, especially for MRI purposes (Yang et al., 2017; Zhou, Wang, Qiao, Binks, & Sun, 2012). The hydroxyl groups present in magnetite particles result in a degree of hydrophilicity that has direct implications on the type of emulsion that can be formulated and used for imaging purposes. According to Zhou et al. (2011), these hydrophilic magnetite particles in their native state can be used to stabilize emulsions comprised of nonpolar or weakly polar oils such as dodecane and silicone, rather than strongly polar oils like butyl butyrate and 1-decanol (Zhou et al., 2011). This is because the contact angle between the particle and the hydrophobic phase is sufficiently large (close to 90 degrees) (Zhou et al., 2011). Modifying the magnetite particles with silane coupling agents to render them more hydrophobic also afforded suitability for emulsion formation with strongly polar oils (Zhou et al., 2012). Other surface modifications of magnetic particles, such as sulfonation or carboxylation, were also found to improve the stability of Pickering emulsions (Zhou et al., 2012).

In the past few decades, a number of promising Pickering emulsion formulations for potential theranostic purposes have been formulated. Most notably are Fe_3O_4-coupled nanocrystals, graphene oxide, and titanium oxide. In 2017, Xie et al. reported the preparation of dual-responsive light-sensitive and magnetic Pickering emulsions suitable for imaging purposes (Xie et al., 2017). In addition, more recently, Low et al. (2019) reported the use of Fe_3O_4 and cellulose nanocrystals, prepared to contain curcumin, for cancer theranostic purposes (Low et al., 2019). Interestingly, Liu et al. (2019) reported the assembly of amphiphilic Janus particles via ultrasonication, is suitable for multiple cancer theranostic applications (Liu et al., 2019). The authors reported that the magnetic heating efficiency and transverse relaxivity were superior to that of commercial superparamagnetic iron oxides (Liu et al., 2019). In addition, Hong et al. (2018) prepared protease- and light-sensitive Pickering emulsions, and later microcapsules, comprised of gold nanoparticles and α-synuclein protein, which may be further modified through the addition of magnetic particles such as iron oxide (Hong et al., 2018). α-synuclein protein has been implicated in the pathology of Parkinson's disease. These studies, along with an additional Pickering emulsion system

Table 3.3 Research studies involving the use of solid particles in Pickering emulsions for theranostic purposes.

Solid particle of Pickering emulsion	Emulsion type; oil	Therapeutic (if applicable)	Diagnostic application	References
Fe_3O_4	O/W; dodecane	N/A	Magnetic resonance imaging	Zhou et al. (2011)
s-TiO_2 (7-carbon chain silane) and t-Fe_3O_4 (3-carbon chain silane)	W/O; hexane	N/A	UV- and magnetic-responsive for imaging purposes	Xie et al. (2017)
Fe_3O_4 and cellulose nanocrystal (MCNC)	O/W; beta-carotene rich red palm olein (30 v/v%)	Curcumin as cancer therapeutic	Magnetic resonance imaging	Low et al. (2019)
Fe_3O_4 and graphene oxide Janus nanoparticles (octadecylamine as linker)	O/W; chloroform	N/A	Magnetic resonance imaging	Liu et al. (2019)
Gold nanoparticles and α-synuclein protein	O/W; chloroform	N/A	Protease- and light-responsive for imaging purposes	Hong et al. (2018)
Magnetic reduced graphene oxide (MRGO) containing $Fe_2O_3$and Fe_3O_4	W/O; dodecane	N/A	Magnetic resonance imaging	Lin et al. (2015)

prepared by Lin, Yang, Petit, and Lee (2015) (Lin et al., 2015), are detailed in Table 3.3.

3.7.1.2 Non-Pickering nano- and microemulsions

In contrast to Pickering emulsions that consist of solid particles at the interface of oil-water droplets, typical nano-, and microemulsions or non-Pickering emulsions, consist of metal particles that concentrate within the droplet core. The magnetic particles may be held in the droplet core alone or may share the space with therapeutic molecules. Scaffold systems such as crosslinked polymers may also be used to hold the magnetic particles in place within the core.

The formation of O/W and W/O nano- and microemulsions containing magnetic particles for theranostic purposes is well documented in the literature. Core-shell structures containing gel-like or polymer-based scaffolds for holding the

magnetic particles in place within the core have also been reported. Lima-Tenório et al. (2016) prepared nanosized emulsions comprised of sodium dodecyl sulfate (SDS) surfactant, organic ferrofluid as the oil phase, iron oxide particles for imaging, and amino dextran polymer to increase biocompatibility and particle stability (Lima-Tenório et al., 2016). The SDS surfactant was later removed but the nanosized colloids were 180−200 nm in diameter and demonstrated suitability for MRI as well as a cancer treatment in the form of hyperthermia (Lima-Tenório et al., 2016). Khizar et al. (2020) also prepared W/O/W (water-in-O/W) double emulsions for MRI purposes and cancer treatment using Polysorbate 80 surfactant, iron oxide nanoparticles, and doxorubicin (Khizar, Ahmad, Ahmed, Manzoor, & Elaissari, 2020). The authors used a polymer called Eudragit E100 (a cationic copolymer comprised of dimethyl amino ethyl methacrylate, butyl methacrylate, and methyl methacrylate), to stabilize these nanoemulsions (Khizar, Ahmad, Ahmed, et al., 2020). Lorton et al. (2018) also reported the use of O/W nano- and microemulsions comprised of perfluorooctyl bromide organic phase and biocompatible fluorinated surfactants suitable for the delivery of antiinflammatory therapeutics and oxygen (Lorton et al., 2018). The authors loaded ^{19}F for MRI detection (Lorton et al., 2018). Interestingly, Dehvari, Lin, and Chang (2018) prepared O/W droplets consisting of an iron oxide core, coupled with hyaluronic acid and chlorin e6, a photosensitizer for tumor destruction that is also fluorescent-sensitive (Dehvari et al., 2018). This is a prime example of dual-functionality with respect to diagnostic imaging. The hyaluronic acid was also able to target tumor cells exhibiting C44 surface receptors (Dehvari et al., 2018). Another study by Kim et al. (2017) involved the preparation of an O/W nanoemulsion for iodine and docetaxel delivery for computed tomography (CT) imaging and cancer treatment, respectively (Kim et al., 2017). These studies, as well as an additional nanoemulsion preparation by Khizar, Ahmad, Saleem, et al. (2020), are summarized in Table 3.4.

3.8 Fluorescent materials

In addition to metal particles, fluorescent materials are commonly used in theranostics for diagnostic imaging. They have afforded the advancement of diagnostic imaging tools such as fluorescence resonance energy transfer, photoinduced electron transfer, and near-infrared (NIR) imaging. In contrast to metal particles, fluorescent materials are generally easier to incorporate into emulsion systems. This is because fluorescent materials tend to be hydrophobic in nature and can easily be dissolved in a carrier oil and then combined with water and surfactant in order to form an O/W nano- or microemulsion system. They may also be coupled to the surface or interface instead of being localized within the hydrophobic core of O/W droplets. When combined with targeting molecules, the result is a nano- or microemulsion that is well equipped for targeted imaging purposes.

Table 3.4 Research studies involving the use of solid particles in nano- and microemulsions for theranostic purposes.

Solid particle	Emulsion type; surfactant; oil	Therapeutic (if applicable)	Diagnostic application	References
Fe_3O_4(amino dextran-coated)	O/W; SDS; organic ferrofluid	Fe_3O_4 for cancer treatment via hyperthermia	Magnetic resonance imaging	Lima-Tenório et al. (2016)
Fe_3O_4	W/O/W; Polysorbate 80 & Eudragit E100; not specified	Fe_3O_4 for cancer treatment via hyperthermia Doxorubicin for cancer treatment	Magnetic resonance imaging	Khizar, Ahmad, Ahmed, et al. (2020)
^{19}F	O/W; perfluorinated surfactant; perfluorooctyl bromide	Oxygen delivery	Magnetic resonance imaging	Lorton et al. (2018)
Fe3O4	O/W; oleic acid; chloroform	Fe_3O_4 for cancer treatment via hyperthermia	Magnetic resonance imaging, fluorescent imaging via chlorin e6	Dehvari et al. (2018)
Iodine	O/W; Polysorbate 80 (Span 85 as cosurfactant); lipiodol	Fe_3O_4 for cancer treatment via hyperthermia	Iodine/lipiodol for computed tomography imaging	Kim et al. (2017)
Fe_3O_4(polydimethyl diallyl ammonium chloride (PDAC)-coated)	O/W; SDS; organic ferrofluid	Fe_3O_4 for cancer treatment via hyperthermia	Magnetic resonance imaging	Khizar, Ahmad, Saleem et al. (2020)

Given the general hydrophobic nature of most fluorescent compounds, oil selection is a critical step in the development of fluorescent nano- and microemulsions for diagnostic imaging or tagging. As stated earlier, microemulsions tend to be comprised of weakly polar oils, such as medium chain triglycerides. The solvent capacity of these weakly polar oils for highly hydrophobic fluorescent compounds may be poor, resulting in insufficient loading capacity of these compounds for theranostic purposes. This can be resolved using a strictly nonpolar oil such as a hydrocarbon; however, this can present challenges with respect to surfactant selection. Such scenarios highlight the importance of solvent compatibility tests between the organic phase and fluorophore prior to nano- and microemulsion formation.

In the past few decades, research into the use of nano- and microemulsions as fluorescent molecule carriers for theranostic applications has increased. Patel, Beaino, Anderson, and Janjic (2015) prepared oil in water nanoemulsions using a medium chain triglyceride, Miglyol 810N, and surfactants Cremophor EL and Pluronic P105 for delivering fluorescent perfluorocarbon and antiinflammatory drug, celecoxib (Patel et al., 2015). Zhang et al. (2019) recently synthesized a dual-purpose diagnostic via a fluorescent and ^{19}F-containing amphiphile to support fluorescence and ^{19}F MRI (Zhang et al., 2019). Interestingly, the boron dipyrromethene (BODIPY) fluorescent molecule served as both a therapeutic and cancer theranostic via photodynamic therapy (Zhang et al., 2019). Therefore, not only did this nanoemulsion preparation possess dual-diagnostic capabilities, but it also possessed dual-treatment capabilities.

Leandro, Martins, Fontes, and Tedesco (2017) also formulated a nanoemulsion comprised of a medium chain triglyceride, Miglyol 812N, a triblock copolymer surfactant Poloxamer 188, and a photosensitive, fluorescent chloroaluminum phthalocyanine for photodynamic therapy as a prostate cancer theranostic (Leandro et al., 2017). Gianella et al. (2011) developed O/W nanoemulsions containing amphiphilic lipid, soybean oil, iron oxide nanoparticles for MRI purposes, and cy7 fluorescent dye for NIR fluorescent imaging (Gianella et al., 2011). Again, these nanoemulsions possessed dual-diagnostic capabilities. The authors also functionalized it for targeting with PEG-linked arginine-glucine-aspartic acid (RGD) peptides that target angiogenesis or blood vessel growth in tumors (Gianella et al., 2011). Most recently, Teng et al. (2020) reported the formation of nanoclusters containing iron oxide particles, a fluorophore (indocyanine-green), and a photosensitizer (chlorin-e6) for NIR imaging and photodynamic therapy, respectively (Teng et al., 2020). The authors created the nanoclusters via an O/W microemulsion system, whose oil phase was later evaporated (Teng et al., 2020). These studies, along with other nano- and microemulsion preparations, are summarized in Table 3.5.

3.9 Sonic-sensitive materials

In contrast to magnetic resonance and fluorescent imaging, ultrasound imaging is a welcome alternative given its nonionizing nature. In the human body, the difference in density between lesions and background organs and tissues can often be undistinguishable (Fu et al., 2019). As a result, clear contrast ultrasound images are not always possible (Fu et al., 2019). To solve this, ultrasound contrast images that are able to enhance echo signals are often employed in order to increase the contrast between these anatomical structures in the body and improve visualization (Fu et al., 2019). Three properties that are critical to determining the contrast performance of ultrasound contrast agents are size, density, and compressibility (Fu et al., 2019).

Table 3.5 Research studies involving the use of fluorescent material in nano- and microemulsions for theranostic purposes.

Fluorescent material	Emulsion type; surfactant; oil	Therapeutic (if applicable)	Diagnostic application	References
Perfluoropolyethylene glycol ether (PFPE) and 1,1′-Dioctadecyl-3,3,3′,3′-Tetramethylindodicarbocyanine (DiD) dye	O/W; Cremophor EL and Pluronic 105; Miglyol 810N	Celecoxib as antiinflammatory for bowel disease, cancer, arthritis and neuroinflammation treatment	Fluorescent imaging (of macrophages)	Patel et al. (2015)
Boron dipyrromethene (BODIPY)	O/W; poly(ethylene glycol)-boron dipyrromethene (PEG-F_{54}-BODIPY) amphiphile; perfluorohexane	BODIPY for photodynamic therapy in cancer	Fluorescence/ photoacoustic/^{19}F magnetic resonance multimodal imaging	Zhang et al. (2019)
Chloroaluminum phthalocyanine ($C_{32}H_{16}AlClN_8$)	O/W; Poloxamer 188; Miglyol 812N	$C_{32}H_{16}AlClN_8$ for photodynamic therapy in prostate cancer	Fluorescence/near-infrared imaging	Leandro et al. (2017)
Cy7 dye (C7-cyanine) Arginine-glycine-aspartic acid (RGD) as a targeting molecule	O/W; 1,2-Distearoyl-sn-glycero-3-phosphocholine (DSPC); soybean oil	Prednisolone acetate valerate (PAV) as cancer therapeutic	Iron oxide nanoparticles for MRI Cy7 for near-infrared fluorescent imaging	Gianella et al. (2011)
Indocyanine-green	O/W; indocyanine-green and chlorin-e6; toluene	Fe_3O_4 for cancer treatment via hyperthermia Chlorin-e6 for photodynamic therapy	Near-infrared imaging	Teng et al. (2020)

Nano- and microemulsions are attractive carriers for ultrasound contrast agents due to their small droplet sizes and uniform oil/water dispersions. These properties make nano- and microemulsions stable to ultrasonic action (Fu et al., 2019). In addition, if the dispersed oil phase possesses high density and strong hydrophobicity, such as in the case of perfluorocarbons, that distinction as compared to the aqueous phase, can afford increased ultrasound interaction and improved contrast visualization (Fu et al., 2019). Given these criteria, one can appreciate the suitability of nano- and microemulsions for ultrasound theranostic purposes given that hydrophobic perfluorocarbon oils can be easily incorporated. Aside from the use of perfluorocarbon oils, the last few decades have seen the design and development of several unique nano- and microemulsions for theranostic purposes.

Shiraishi et al. (2011) prepared nanosized O/W emulsions using a block copolymer amphiphile comprised of polyethylene glycol (PEGs) and aspartic acid, and perfluoropentane oil (Shiraishi et al., 2011). Upon ultrasonic radiation, the authors reported that the emulsion droplets lost their integrity, causing the vaporization of the liquid perfluoropentane oil (Shiraishi et al., 2011). This vapourization produced microbubbles that not only provided strong contrasts for ultrasound images, but upon cavitation or collapse, they effectively damaged tumor cells (Shiraishi et al., 2011). Therefore, while nanosized emulsions can effectively be delivered to tumor tissues, their increase in size to a microbubble upon sonication functions as an effective contrast agent and cancer therapeutic (Shiraishi et al., 2011). In 2015, Arnal et al. (2015) explored a similar system, preparing perfluorohexane nanoemulsions with gold nanoparticles coupled to PEGs-thiol and butane thiol (Arnal et al., 2015). The presence of gold broadened the absorption spectrum allowing greater functionality as a contrast agent for both sonic and photoacoustic imaging (referred to by the authors as sono-photoacoustics) (Arnal et al., 2015). Yang et al. (2019) also developed a similar technology, preparing lipid-based nanoemulsions containing perfluorohexane oil, and coupled to iodide, IR-780 (Yang et al., 2019). Upon ultrasound pulsing, the nanoparticle formed microbubbles to enhance ultrasound contrast, and the iodine IR-780 dye afforded NIR fluorescence imaging as well as photothermal therapy for tumors (Yang et al., 2019). Another interesting study by Fernandes and Kolios (2019) saw the preparation of perfluorohexane nanoemulsions using a fluorosurfactant with an anionic phosphate group (Fernandes & Kolios, 2019) The authors demonstrated the ability of these nanoemulsions to carry paclitaxel and doxorubicin as cancer therapeutics (Fernandes & Kolios, 2019). Upon ultrasound pulsing, vapourization into microbubbles was observed to increase contrast imaging and act as a therapeutic by causing direct cellular damage and by delivering paclitaxel/doxorubicin (Fernandes & Kolios, 2019). Finally, Chen et al. (2018) developed cationic perfluorocarbon nanoemulsions suitable for the delivery of small interfering RNA (siRNA) for gene silencing purposes in tumors (Chen et al., 2018). The perfluorohexane afforded ultrasound imaging and visualization, while the siRNA was able to silence the Bcl2 gene, implicated in tumor apoptosis (Chen et al., 2018). Table 3.6 illustrates various examples of ultrasonic activated theranostic nano- or microemulsion systems.

Table 3.6 Research studies involving the use of ultrasonic activated nano- and microemulsions for theranostic purposes.

Ultrasonic-responsive agent	Emulsion type; surfactant; oil	Therapeutic (if applicable)	Diagnostic application	References
Perfluoropentane	O/W; Poly (ethylene glycol)-b-poly (4,4,5,5,6,6,7,7,7-nonafluoroheptyl aspartate) (PEG-P (Asp(C7F9)x)); perfluoropentane	Microbubble as cancer therapeutic; ultrasound radiation results in cavitation and death to surrounding tumor cells	Ultrasound imaging and therapy	Shiraishi et al. (2011)
Perfluorohexane	O/W; 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC); perfluorohexane	IR-780 for photothermal ablation of tumors	Ultrasound imaging and therapy IR-780 for near-infrared fluorescent imaging	Yang et al. (2019)
Perfluorohexane	O/W; Zonyl FSP fluorosurfactant; perfluorohexane	Paclitaxel and doxorubicin as cancer therapeutic	Ultrasound and near-infrared fluorescence imaging	Fernandes and Kolios (2019)
Perfluorodecalin	O/W; fluorinated polyethyleneimine; perfluorodecalin	Small interfering RNA for silencing of Bcl2 gene (tumor apoptosis suppressor) in cancer	Ultrasound imaging	Chen et al. (2018)

3.10 Targeting purposes

3.10.1 Proteins, peptides, and antibodies

Several proteins, peptides, and antibodies are implicated in disease pathologies. These proteins function as effective targeting moieties for theranostic purposes. Aptamers (peptides) such as AS1411, overexpressed in breast, colon, lung, prostate, and gastric cancers, are effective targeting molecules that can bind and release both tagging and therapeutic molecules such as quantum dots and doxorubicin, respectively, as outlined by Zavvar et al. (2020). In essence, any cell

surface marker that has been previously established or elucidated is an effective target for which targeting protein molecules may be designed.

3.10.2 Lipids

In theranostic applications, surface lipids may be conjugated to molecules for targeting, or may assemble to form lipid nanocarriers to which targeting molecules may be attached. Some of these lipid nanocarriers may include micelles, solid/lipid nanoparticles, or liposomes. Yang et al. (2019), as mentioned earlier, used lipid-conjugated nanoparticles for cancer treatment (Arnal et al., 2015). The authors prepared 1,2-dipalmitoyl-snglycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-*N*-[polyethylene glycol-2000] coupled to IR-780 iodide to not only treat cutaneous malignant melanoma but to also detect and target it (Yang et al., 2019). The authors also used perfluorohexane as a contrast agent for ultrasound (Arnal et al., 2015). In contrast, Olerile et al. (2017) described the development of lipid nanocarriers loaded with quantum dots and paclitaxel for the effective targeting of h22 tumors in liver cancer, as well for imaging via fluorescence (Olerile et al., 2017). Polymerized phospholipids have also been reported as both liposomal components to which targeting molecules may be attached or on their own as additional surface molecules to which targeting molecules may be attached (Puri & Blumenthal, 2011). pH-sensitive lipids and liposomes have also been reported for doxorubicin loading and delivery. Xu et al. (2015) designed a pH-sensitive liposome comprised of a new lipid material, Poly(2-ethyl-2-oxazoline)-cholesterol hemisuccinate (PEtOz-CHEMS), for doxorubicin treatment and fluorescent imaging (Xu et al., 2015). The pH sensitivity was important for the escape and release of the contents of the liposome (Xu et al., 2015). Though many of these lipids are discussed in the context of liposomal delivery, these responsive lipids may be easily incorporated into nano- and microemulsions.

3.10.3 Polymers

Polymers are often used in theranostic applications due to their ability to prolong circulation half-life, sustain or trigger drug release, and participate in active or passive targeting (Luk & Zhang, 2014). In many cases, they function as scaffolds to which targeting molecules are attached. They may be pH-, temperature- and/or light-sensitive to accommodate cellular environments or to facilitate the release of therapeutics and imaging molecules. Common polymers used in theranostic applications include PEGs and block copolymers. One such example can be seen in the work by Zhang et al. (2020), who prepared molecules with simultaneous targeting (PEG-linked folic acid), imaging (gadolinium), and therapeutic (copper sulfate for photothermal therapy) capabilities (Zhang et al., 2020). The authors used folic acid to target the folic acid receptors overexpressed in a number of cancer cells including epithelial, ovarian, cervical, breast, lung, kidney, colorectal,

and brain cancer cells (Zwicke, Mansoori, & Jeffery, 2012). Poly-D,L-lactide-*co*-glycolide acid terminated (PLGA) nanoparticles were also linked to folic acid in work done by Bazylińska, Kulbacka, and Chodaczek (2019) who also managed to encapsulate and deliver a hydrophobic sensitizing dye for imaging and hydrophilic cisplatin for treatment (Bazylińska et al., 2019). Zheng et al. (2020) prepared PLGA nanoparticles for imaging (superparamagnetic iron oxide and gold nanoparticles) and therapeutic (doxorubicin) purposes, in order to target the human epidermal growth factor receptor 2 (Her2) found in breast cancer cells (Zheng et al., 2020). Finally, Gu et al. (2019) used a block copolymer containing PEG and poly-L-thyroxine for both SPECT/CT imaging and radiotherapeutic purposes (Gu et al., 2019).

3.11 The future of nano- and microemulsion technology in theranostic applications

It is evident from the research studies presented in this chapter, that nano- and microemulsions have been used very innovatively for a variety of theranostic applications. From the development of nanosized, Pickering emulsions to deliver magnetic particles for MRI, to the development of nanoemulsions possessing dual-therapeutic functionalities, emulsion systems have been functionalized for theranostic applications using various diagnostic, therapeutic, and targeting molecules. The easy preparation of nano- and microemulsion systems, along with their small droplet sizes, stability, and economical starting ingredients have rendered them an obvious choice in the development of theranostics.

We have also noted the applicability of these nano- and microemulsion theranostic preparations to a wide range of disease states including cancer, inflammatory diseases, and neurological disorders, not forgetting their utility in gene therapy applications. The benefit of using nano- and microemulsions is that any functionality can be effectively built-in once compatibility of the therapeutic or diagnostic with the surfactant and oil phases is confirmed. This versatility has many implications for the future of theranostics.

In the coming years, as already seen in recent research trends, one may expect the popularity of dual-responsive and dual-treatment emulsion systems to take center stage. Many disease states are treated using combination therapies, and the potential of nano- and microemulsions to accommodate both hydrophilic and hydrophobic compounds simultaneously, as outlined by Yang et al. (2014) will be particularly advantageous in the future (Yang et al., 2014). The study mentioned here is of particular note as Yang et al. (2014) developed nanoemulsions containing d-[alpha]-tocopheryl polyethylene glycol 1000 succinate (TPGS) as a hydrophilic surfactant, and vitamin E as an oil, in order to accommodate a hydrophilic imaging molecule (sulforhodamine B) and a hydrophobic drug (paclitaxel), respectively for cancer theranostic purposes (Yang et al., 2014). In this manner,

the authors were able to use a surfactant that also served as a solvent for a hydrophilic imaging molecule. The use of dual-purpose emulsion ingredients is bound to increase in the coming years. This has already been noted in the case of ultrasound imaging where perfluorocarbon oils act as both a contrast agent and oil phase constituent. In addition to dual-purpose emulsion ingredients, dual-diagnostic and dual-therapeutic imaging is also likely to increase as a fail-safe of sorts in the field of theranostics. Given the expected trends of the future, and the potential of nano- and microemulsions to accommodate them, the future of nano- and microemulsions in theranostics is undoubtedly bright.

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CHAPTER

Self-nanoemulsifying systems for drug delivery therapeutics

4

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4.1 Introduction

Nanotechnology is the branch of science dealing with very small materials that show impressive physicochemical properties due to their very small size. The US Environmental Protection Agency defined nanotechnology as "*the creation and use of structures, devices, and systems that have novel properties and functions because of their small size*." These unique properties allow for novel applications in different fields such as medicine, agriculture, industry, defence, and so on. A massive number of preparation methods can be used for the synthesis of new nanomaterials according to the properties of the designed nanomaterials as well as the nature of the starting compounds used in the synthesis protocol. "*Nanoemulsification*" is a method commonly used for the preparation of polymeric nanoparticles. The emulsion form is defined as an isotropic heterogeneous system that contains two different immiscible phases. The first is the internal phase, which is dispersed as tiny droplets, while the other continuous phase is the external solvent system (Fig. 4.1).

The term nanoemulsion implies that the size of the dispersed colloidal particles ranges from 20 to 600 nm in diameter. By this size, the formed particles are taking advantage of the nano-sized materials that show improved chemical and physical properties and behaviors compared with the larger-sized materials. For instance, nano-sized materials have higher chemical reactivity due to their large surface area-to-volume ratio; in other words, higher material exposure to the surroundings increases the material's probability for participation in the potential reaction. For example, if there is a block of cubic material that is 1 m on each side, the calculated surface area of this block will be 6 m^2 and the block's volume is 1 m^3. In this case, the calculated surface area-to-volume ratio is 6:1. To see the effect of decreasing the material size, the block is cut into 8 pieces, each 0.5 m in side length (Fig. 4.2). The calculated surface area of the eight pieces will be

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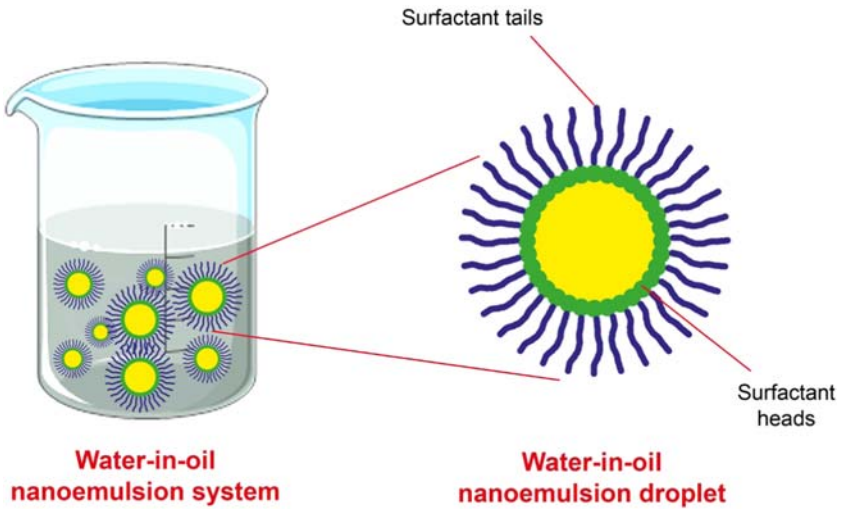

FIGURE 4.1

Water-in-oil nanoemulsion droplets.

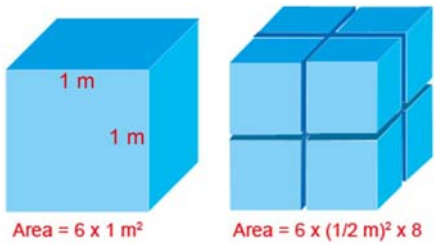

FIGURE 4.2

The difference between the surface area-to-volume ratio between two blocks with the same volume but with a different number of units.

12 m^2 while the total volume of all pieces is still the same. As a result, the calculated surface area-to-volume ratio increases significantly to 12:1, respectively.

The nanoemulsion form of material contains two different immiscible phases, one is the dispersed *colloidal* particles surrounded by the emulsifier material (called *surfactant* or *stabilizing agent*), and the other is the continuous *solvent* phase.

The first emulsion formulation was reported in 1959 by Schulman et al. It was in the micro size (i.e., microemulsion) and the system contained oil and water phases with alcohol as a surfactant. The mentioned system was kinetically and thermodynamically stable, and it was a start to developing more such emulsion formulas by using different kinds of materials alongside the same concept of preparation.

Nanoemulsion systems are classified as follows: (1) oil-in-water system (O/W) where the aqueous phase is the dominant solvent phase with the oil phase dispersed in it, (2) the reverse of the first category [i.e. water in oil (W/O)] system, where water droplets are dispersed in a continuous phase of oil containing a suitable oil-soluble surfactant. Another advanced category is (3) the double emulsion system which is prepared via two consecutive emulsion steps resulting in a system of oil in water in oil (O/W/O) or water in oil in water (W/O/W) to serve the loading of compounds with amphiphilic nature (a compound with high affinity to dissolute in both aqueous and lipid phases) and to improve their solubility, and yet facilitate their applications (Figs. 4.3 and 4.4).

The structure of the emulsion system depends on the application. For instance, for drug delivery applications, the solubility of the loaded drug determines the inner phase of the emulsion system. In the case of a lipophilic drug, it has to be

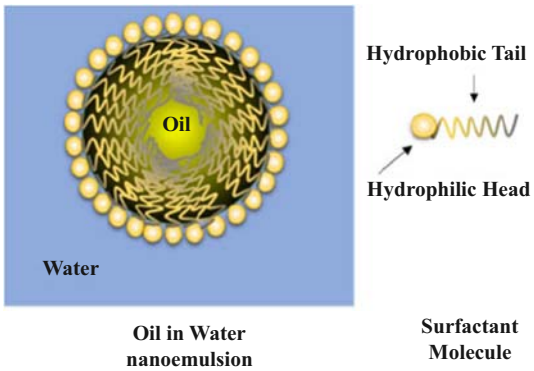

FIGURE 4.3

An amphiphilic compound serves as a spacer and a stabilizer for two different immiscible materials.

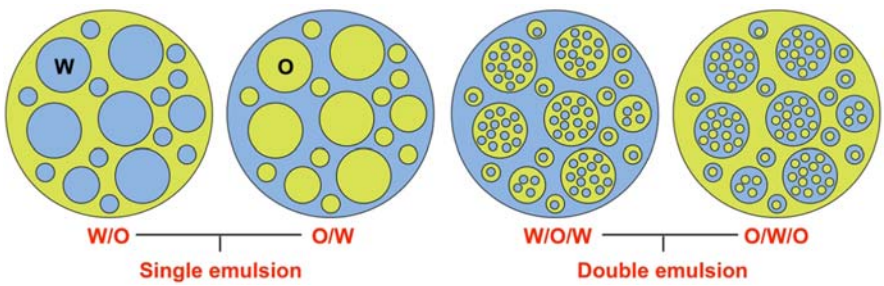

FIGURE 4.4

Classification of nanoemulsion systems.

dissolved in an oil phase first before being added to the water phase, which will act as the solvent system. On the other hand, in the case of cosmetic products used for skincare, the outer layer has to be lipophilic to be suitable for skin interaction and penetration.

The colloidal dispersion of the nanoemulsion that appears in a multiphase form of water, oil, and surfactant cannot be formed spontaneously unless some mechanical force is applied during the preparation procedure. To obtain such an isotropic nanoemulsion system, a strong mechanical force should be applied in the continuous phase so that the large added droplets can be divided into finer smaller ones. The source of this mechanical force can be obtained via ultra-high speed devices such as mechanical homogenizers, microfluidizers, and sonicators. These devices produce sufficient shear force to overcome the surface tension of the added droplets so that nano-sized droplets can be obtained.

Several factors can control the morphology of the nanoemulsion system. Some of these factors are summarized in the following points.

1. The percentage of the surfactant used is a key factor. For obtaining nano-sized droplets, the required surfactant percentage should be about 3%−10% of the continuous phase. On the other hand, using higher percentages (up to 20% or higher) will result in micro-sized droplets (microemulsion). The nano size has the advantage of higher loading (i.e. inside the droplets) due to the higher surface-area-to-volume ratio, in addition to high stability.
2. The ratio between the used polymer and the loaded ingredient is also a key factor. It is a material-dependent factor, but generally, the lower the loaded ingredient percentage, the higher the entrapment efficiency (EE) of the system. The EE is a measurement of how much ingredient is loaded inside the nanomaterial.
3. The rate of addition of the dispersed phase to the continuous phase can greatly influence the size of the obtained particles. This factor relies on the type of material used. The idea here is to avoid any probable precipitation by maintaining a relatively slow rate of addition.
4. The agitation rate and the applied mechanical forces should provide a shear force strong enough to overcome any surface tension of the added phase so that the nano-sized dispersed droplets can be obtained.

4.2 Life applications and market updates

One of the major applications of nanoemulsions is drug delivery. This field is seeking the development of pharmaceuticals with the aid of nanomaterials. Problems such as poor hydrophilicity, high volume of distribution, low specificity, and poor selectivity of the currently approved drugs are the major obstacles in the field of drug development. Nanomaterials have managed to overcome such problems efficiently, introducing a new promising era in the drug industry. The main aim of

using nanomaterials in the pharmaceutical industry can be summarized as follows: (1) increasing the drug efficacy by enhancing its solubility in water even if the drug is lipophilic in nature, (2) reducing the side effects and toxicity via limiting the drug distribution in the body and increasing the drug specificity, a process known as "*active targeting*," and (3) reduction of the required effective therapeutic dose, resulting in an essential decrease in the drug side effects as well.

It is obvious that nanoemulsion drug delivery systems represent a promising solution not only for pharmaceutical products but also for bioactive food products. Because the majority of the approved pharmaceutical products and food ingredients are lipophilic, they encounter low solubility as well as low bioavailability. Here, the role of nanoemulsion-based systems comes to increase their aqueous solubility dramatically through different types of O/W emulsion systems.

In addition to drug delivery, the cosmetics industry is another application that has been developed by the use of nanoformulations, especially those based on nanoemulsion systems. Cosmetic products interact directly with the skin, either on the skin's surface or via penetrating its outer layers. Several properties of cosmetic products must be optimized according to the desired application, such as polarity, solubility, particle size, etc. The emergence of nanoemulsion systems has significantly developed the industry of cosmetics. It solved the solubility problem of many of them since the majority of cosmetic ingredients are lipophilic. Moreover, they improved the products' stability and lifetime. Another essential point to be considered is that such nanoformulations have demonstrated great success in transdermal drug delivery because the obtained small size presents better penetration for the skin's outer layers, which allows the drug to pass easily through the pores of the skin.

Not only pharmaceutical industries but also the agricultural fertilizers industry has been developed by the emergence of nanoemulsion-based technology. The major problem of the agricultural ingredients is the poor aqueous solubility. The solubility problem has been solved by the nanoemulsion technique of preparation by dissolving the lipophilic agricultural ingredients in a suitable lipid phase followed by dispersing the resulting phase in a continuous aqueous phase. Another advantage of adding such ingredients to a coating polymeric form is the gradual release over a long period, in addition to helping the stabilization of the ingredients in the soil and limiting their dissipation.

4.3 Types of nanoemulsions

4.3.1 General structure of the emulsion systems

The chemical synthesis of nanoemulsion systems includes specific steps that result in stable, isotropic, and homogeneous forms. Firstly, the continuous solvent phase is prepared with a certain percentage of a suitable surfactant. Secondly, the dispersed phase containing the soluble ingredients is added gradually to the continuous phase

while strong stirring is applied. This main theme can be modified with some changes according to the application, as summarized in the following sections.

Emulsion systems are of several forms and components and can be classified as follows: (1) single emulsion system, for instance, O/W or W/O systems; (2) double emulsion systems such as O/W/O and W/O in water W/O/W; (3) polymeric emulsion systems where the used polymer represents the dispersed phase of the emulsion system; and (4) lipid-based emulsion systems.

4.3.2 Polymer-based emulsion systems

This type represents an advanced form of emulsion systems. It includes dissolving the polymer of interest with the ingredients to be loaded and then dispersing them in the continuous phase. The process normally ends with loading the selected ingredients into the formed polymeric particles (nano-sized particles in the case of nanoemulsion) resulting in a stable nanoemulsion system. The polymer here plays an essential role in carrying the loaded ingredients in a stable form. In addition, the polymer surface can be chemically/physically modified to serve various kinds of applications. Examples of natural polymers used to form nanoemulsion systems are sodium alginate, chitosan, gelatin, collagen, and hydroxyapatite. Whereas synthetic polymers used to form such nanoemulsion systems can include poly(lactic-*co*-glycolic acid) (PLGA) and Polyethylene glycol (PEG).

4.3.3 Solvents and cosolvents

During the preparation of polymeric nanoemulsion systems, organic solvents and cosolvents have vital roles as they represent the opposite phase of the aqueous component. In the case of an o/w emulsion, solvents and cosolvents should be volatile (either miscible or immiscible with water) so that they can leave the solution under vigorous stirring during the solidification of the dispersed polymeric particles. That is why the organic layer is usually used in a volume ratio of 1:4 or 1:5 with respect to the aqueous phase. The cosolvent's main role is to help the solvent dissolve the polymer and the other ingredients in such a small volume of solvent. The solvent and cosolvent must be miscible.

4.3.4 Surfactants and cosurfactants

Surfactants are very necessary during the preparation steps of all nanoemulsion categories. They reduce the interfacial tension of the dispersed phase so that a small-emulsified particle can be obtained in such a nonsolvent system. Surfactants also play a major role in stabilizing the formed emulsion system kinetically, chemically, and thermodynamically. According to the application, factors such as the surfactant ratio, polarity and solubility, and the use of cosurfactants need to be optimized during the synthesis of the desired emulsion system.

These factors directly affect the emulsion particle size, stability, loading efficiency, etc. The commonly used surfactants for this purpose are Tween, PEG, polyvinyl alcohol (PVA), caprypol, gelucire, cremophor, and poloxamers.

4.4 Chemical preparation methods of nanoemulsion systems

Emulsion preparation methods are classified according to the applied mechanical force into two major classes. The first is the high-energy methods, and this class includes high-pressure homogenization, microfluidization, and ultrasonication. The second class includes the *low-energy methods* and these include *phase-inversion emulsification* [e.g., transitional phase inversion (TPI), phase inversion temperature (PIT), phase inversion composition (PIC), and catastrophic phase inversion (CPI)] and *spontaneous self-nano-emulsification*.

4.4.1 High-energy methods

High-energy methods use mechanical forces strong enough to produce the needed disruptive energy during the emulsion process. With the help of the applied high kinetic energy, nano-sized droplets are obtained and the nanoemulsion gets stabilized. These strong mechanical forces are produced by high-pressure homogenizers, microfluidizers, and ultrasonication devices (Fig. 4.5). In fact, these techniques give more control over the particle size and the emulsion stability.

The high-pressure homogenizer is the most commonly used high-energy instrument in the preparation of nanoemulsions. Due to its high pressure (500−5000 psi), dispersed and stable nanoparticles can be obtained and manipulated by changing conditions such as the rpm value, time, and temperature of the homogenizer. In fact, the homogenization technique is so useful, especially in the case of polymeric emulsions. The generated high mechanical energy avoids any possible perception or aggregation of the used polymer so that a stable nanoemulsion can be obtained.

FIGURE 4.5

High-energy instruments used for the preparation of nanoemulsion systems.

In the microfluidization technique, a nanoemulsion system is obtained through two main steps. Firstly, the process includes a preemulsion step where the mixing of the emulsion ingredients (oil, water, surfactant, and any other ingredients) takes place in order to form a macroemulsion fluid. Secondly, this fluid is forced to be accelerated into micro-sized channels under high applied pressure (500−20,000 psi) and finally reaches the interaction chamber. Inside this chamber, when two or more macro fluids collide with one another with this extremely high kinetic energy, the crashing results in the formation of a nanoemulsion system. The energy released from this collision is enough to produce enough mechanical shear force for such a stable nanoemulsion to be obtained. The microfluidization technique is very useful in producing highly stable nanoemulsion systems in the case of using low surfactant concentrations. This case is widely applicable in food industries while only low surfactant concentrations are allowed.

Ultrasonication is considered the most efficient high-energy technique for nanoemulsion system preparation in terms of the ease of operation and cleaning aspects. The source of energy here is the ultrasonic waves produced by the sonicator probe with a variety of wave amplitudes. While ultrasonic waves are applied in the continuous phase, the addition of the dispersed phase takes place, resulting in a highly stable nanoemulsion system. The resulting nano-sized particles could be optimized by changing parameters such as the ultrasonic energy, temperature, and time. Due to the high energy produced in this technique, some reported studies mentioned the formation of stable nanoemulsion systems without the need for the surfactant, making this technique more promising in the food and drug industries.

4.4.2 Low-energy methods

The amount of energy consumed in low-energy methods is much lower than its counterpart in high-energy methods. The only used sources of energy in these techniques are ordinary mechanical stirring and the energy utilized from the chemical reactions occurring inside the system itself. Low-energy methods are cost-effective and largely scalable. They are categorized into two main categories, namely, the *phase inversion method* and the *self-emulsion method*.

4.4.2.1 Phase-inversion emulsification method

In the phase-inversion emulsification method, the phase of the emulsifier material is inversed from the soluble state to a dispersed colloidal solid state in the presence of a surfactant in the continuous solvent phase. During the addition of the dispersed phase under mechanical stirring, the curvature of the added surfactant changes spontaneously, leading to the formation of the emulsion system. The amount of surfactant in this method plays a very important role in determining the obtained particle size and the loading efficiency of the emulsifying ingredients. Also, the surfactant polarity and affinity to the used polymer and the other components strongly affect the stability of the obtained nanoemulsion system.

When other factors control the low-energy emulsion process such as temperature and composition, it is called TPI. However, when the temperature is the main factor, it process is called PIT. On the other hand, if the composition is the main influencing factor, the preparation method is referred to as PIC. Furthermore, the used volume of the dispersed phase can be the main player, where the dispersed phase volume is increased above its critical limit of saturation; thus inducing the emulation to take place. The method is called CPI.

4.4.2.2 The spontaneous self-nanoemulsification method

In this method, the surfactant and the cosolvent are the main influencing factors in the formation of the nanoemulsion system. The spontaneous diffusion of the surfactant and cosolvent from the dispersed phase to the continuous phase results in the nanoemulsification process. In the case of o/w emulsion systems, for instance, to accelerate the diffusion process from the organic (oil) phase to the aqueous phase it's more efficient to use more hydrophilic components (i.e. surfactants, cosolvents, drugs, etc.). This will push the spontaneous diffusion and results in stable nanoemulsion systems.

4.5 Characterization of nanoemulsion systems

4.5.1 Zeta potential

In this characterization technique, the surface charge of the formed droplets is measured, which gives an indication of the sample adsorption, electrokinetic energy, and the sample stability. A representative sample from the prepared nanoemulsion system is placed in the cuvette of the zeta potential instrument and the potential is measured in mV. The following table (Table 4.1) shows the relative stabilities of nanoemulsion systems according to their measured apparent zeta potentials.

4.5.2 Particle size and polydispersity

Measuring the particle size is the main characterization step in the assessment of the prepared nanoemulsion formulation. Knowing the obtained particle size

Table 4.1 The relation between the apparent zeta potential and system stability of nanoemulsion systems.

Apparent zeta potential range (mV)		
From	**To**	**Stability of the nanoemulsion system**
+5	−5	Unstable sample with fast aggregation rate
+20	−20	Short-term stability with a slow aggregation rate
+30	−30	Stable nanoemulsion system.
+60	−60	Perfectly stable nanoemulsion system

indicates the system properties such as homogeneity, dispersity, quality, and stability. One of the frequently used techniques for particle size determination is the dynamic light scattering technique, which gives two main parameters, namely, the mean particle diameter (Z-average) and the polydispersity index (PDI).

The general rule of thumb is "*The smaller the particle size, the higher the stability of the prepared system*." The small size has a significantly lower chance of aggregation and stands efficiently against both coalescence and flocculation. Nano-sized particles can be obtained through the previously-mentioned high-energy methods of emulsion preparation techniques.

Another essential factor to be considered is the PDI. This term represents the ratio of the standard deviation to the measured mean droplet size. PDI value lower than 0.22 represents a perfectly stable emulsion system because the suspended particles are well dispersed and are not aggregated. If the PDI value is close to zero, this means mono-dispersed droplets, meaning that the system contains only one homogenous population of particles. On the other hand, a high PDI value (i.e. close to 1) indicates that several different sized populations are present and that the system is not homogenous.

To sum up, the perfect stable homogenous nanoemulsion system should have a Z-average diameter in the nanoscale (20−200 nm) with PDI lower than 0.22.

4.5.3 Electron microscopy

One of the essential characterization techniques for the assessment of nanoemulsion systems is electron microscopy. This technique gives the most accurate indication of the particle size, shape, morphology, and homogeneity. From the most common types of electron microscopy, scanning electron microscopy (SEM) and transmission electron microscopy (TEM), differ in the operation principle and the type of the obtained images.

TEM uses a high-energy electron source (from 60 to 600 kV) so that the electrons can penetrate the sample to give a two-dimensional (2D) image with a very accurate particle size. Moreover, in the case of core-shell systems, TEM is the first-choice characterization technique that can represent the actual composition of the sample.

SEM uses a relatively low-energy electron source compared with that of TEM (from 1 to 30 kV). Using the scattered and reflected electrons, the instrument can create a three-dimensional (3D) image of the sample. This technique gives a very accurate indication of the sample shape, surface morphology, homogeneity, dispersion, and particle size as well.

4.5.4 Ultraviolet-visible spectroscopy

For the quantitative analysis of the loading efficiency of nanoemulsion systems, UV-VIS spectroscopy is used. It is useful in the determination of how much of the cargo is successfully loaded inside the emulsified dispersed particles, a quantity referred to

as EE. Another major use of the UV-VIS technique is constructing a release model for the loaded ingredients by giving quantitative measurements for the loaded ingredients over a certain period of time under constant surrounding conditions.

Constructing a release model is also an essential analytical aspect of nanoemulsion systems. By setting the suitable testing surrounding conditions (physiological conditions in the case of drug release), the emulsion system is degraded and starts to release the loaded substances. By using a previously constructed analytical plot, the release model can be plotted as a function of the incubation time.

4.6 Applications of nanoemulsion systems

4.6.1 Drug delivery

One of the principal aims of nanomedicine is to develop the pharmaceutical industry by overcoming the challenges and obstacles to the currently approved drugs. Considering the fact that most of the available drugs in the market are lipophilic, their major problem is the poor solubility in aqueous media, which significantly limits the drug efficacy. Another major challenge associated with drug design is the low selectivity, which leads to undesirable side effects. Recently, nanomedicine has managed to make amazing achievements in the field of the drug industry, making it possible to overcome the previously mentioned obstacles in a promising way. Nanoemulsion formulations represent a major part of the currently approved nanomaterial-based drugs and those drugs that are still under ongoing clinical trials. Polymeric nanoemulsion systems and lipid-based drugs showed significant results in the industrial market during the last decade, paving the way for more highly efficient drug molecules to be synthesized with maximum benefits and minimum dose as well as no side effects.

4.6.2 Polymeric nanoemulsion-modified drugs

Polymeric nanoemulsion-modified drugs are synthesized for two major purposes. The first is the chemical conjugation between polymer and drug for the sake of increasing the half-life time of the drug and improving its bioavailability. The second purpose is the physical entrapment of the drug inside the polymeric nanoparticles for the sake of developing a sustained-release system, leading to a more controlled drug release profile.

One of the most commonly used biopolymers is PEG. Due to their hydrophilicity and surface chemistry, PEGylated drugs show significantly improved aqueous solubility, biocompatibility, and bioavailability (Table 4.2). For instance, a Neulasta-approved drug is one of the PEGylated granulocyte colony-stimulating factors. The PEGylated form of this drug shows a great improvement in the plasma half-life time from 15 to 80 h in comparison with the conventional drug half-life time of 3−4 h. In fact, this significant difference is due to the low plasma

Table 4.2 A list of some FDA-approved drugs, their nanoemulsion components, and their possible applications.

Approved drugs	Dispersed phase	Surfactants/ cosurfactants	Application
Carbamazepine	Castor Oil	Tween 80 and soy lecithin	Improving the solubility over the conventional drug
Thalidomide	Olive Oil	Tween 80	Improving the solubility over the conventional drug
Docetaxel	Oleic acid	Egg lecithin	Improving the solubility and stability of the conventional drug
Insulin	Selected proteins	PVA	Stability against enzymatic degradation
Clottrimazole	Soybean oil	Pluronic F68	Improving the bioavailability
Paclitaxel	Nut oil	Lipoid 80	Increasing the cellular uptake
Sulforhodamine B	Vitamin E	TPGS	Increase the half-life time

protein binding of PEG. Another example of the PEGylated drugs is Plegridy, which is used as a treatment for relapsing multiple sclerosis. It shows a great improvement in the half-life time compared to the conventional class of drugs such as Copaxone. Plegridy should be administered once every 2−4 weeks which is far away from Copaxone's dose which is administered once daily. On the other hand, hydrophobic polymers (Table 4.2) are also used for developing some of the approved drugs to slow down their release rates and elongate the duration of action. One of the commonly used hydrophobic polymers is PLGA. Due to the slow rate of degradation of PLGA, it is considered a promising choice for synthesizing such sustained-release systems. Eligard, an FDA-approved drug used for the treatment of prostate cancer, is an example of a PLGAyated modified nanoemulsion drug delivery system. The PLGAyated formulation of this drug shows a relatively higher duration of action compared with the conventional formulation, in addition to lowering the effective dose in a significant manner.

4.6.3 Cosmetics

The cosmetics industry has been growing significantly in the last two decades. Huge amounts of funds are allocated to the development of cosmetic products, which represent a middle point between pharmaceutical products and healthcare products. The biologically active ingredients loaded in cosmetics are usually designed to be delivered to the body via the dermal route or to perform an interaction on the skin surface itself. The major challenges in the industry of cosmetic formulations are poor aqueous solubility, low stability, and a short duration of action. That is why the role of nanotechnology, especially the nanoemulsion systems, is to develop such cosmetic formulations for the sake of the following points: (1) improving the solubility of the formulation, for better skin penetration, (2) producing new nanoformulations with a

Table 4.3 Some of the cosmetic products approved by the FDA and their composition.

Brand name	Application	Active ingredient	Nano-formulation
Cosmetic Dermatology	Anti-aging	Minerals	Emulsified nanocapsules
L-Oreal/skincare	Sunscreen	Titanium dioxide and zinc oxide	Emulsion nanopegiment
L-Oreal	Anti-aging	Vitamin A	Nanosomes
Lancome Paris	Sunscreen	Multivitamins	Emulsified nanocapsules
DS Laboratories	Anti-Acne	Wheat-germ	Nanosomes
Wilma Schumann	Anti-Acne	Vitamin E	Emulsified nanocapsules

large surface area-to-volume ratio compared with conventional formulations, and thus improving the loading capacity, and (3) significantly improving the stability of cosmetic products and their lifetime. Table 4.3 summarizes some of the FDA-approved cosmetic formulations and their applications.

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CHAPTER

Polymeric micelles for therapeutics and diagnosis

5

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5.1 Introduction

Modern medicine strives to discover effective treatments and cures to combat diseases and infections that pose a threat to human life and health, reduce life expectancy and lessen the quality of life, while at the same time taking a heavy economic toll on public health care systems worldwide. Thankfully, its arsenal is constantly being replenished by new technological advances that lead to the development of novel therapeutic materials and diagnostic agents. Especially promising is the emergence of nanomedicine in recent years, which combines nanotechnological approaches with traditional medicine in order to produce agents with improved efficiency and safety (Martinelli, Pucci, & Ciofani, 2019). Among the various life-threatening diseases, cancer is beyond doubt among the primary targets since it remains one of the main causes of human death, with constantly increasing reported cases every year. Enormous research effort has been put into this battle over the last decades and significant progress has been made in regard to our understanding of cancer biology, nevertheless, we are still far from declaring victory. The most common currently available cancer treatment options apart from surgery are chemotherapy and radiotherapy, which unfortunately have adverse side effects since they utilize drugs that often kill healthy cells as well. Moreover, chemotherapy drugs are usually toxic, whilst also exhibiting low solubility, poor pharmacokinetics, undesirable biodistribution, reduced bioavailability, inefficient cellular uptake, and inability to target specific locations. Similarly, novel biological drugs such as proteins, antibodies, genes, and nucleic acids also suffer from inadequate pharmacokinetics, exhibit poor internalization by targeted cells, and are prone to rapid degradation during blood circulation (Cabral, Miyata, Osada, & Kataoka, 2018; Martinelli et al., 2019; Peer et al., 2007; Yin, Chen, Zhang, & Han, 2016; Zhou, Zhang, Yang, & Wu, 2018).

Pharmaceutical nanotechnology promises to overcome these drawbacks and limitations with the use of nanomedicines, including liposomes, polymer-drug conjugates, nanoparticles, and polymeric micelles (PMs), for selective drug delivery.

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Especially PMs have attracted extensive scientific interest since their introduction as drug delivery systems in the 1980s, owing to their numerous physicochemical and biological advantages compared to other nanoassemblies. Of course, the most important of them is the ability to solubilize hydrophobic drugs, as well as other therapeutic and diagnostic agents, through the self-assembly process of the initial block copolymer, which results in the formation of micelles with a drug-loaded hydrophobic core, surrounded by a hydrophilic corona. In this manner not only the solubility of the drug is increased, but also its biodistribution and bioavailability, which means that the pharmacokinetics are improved, while simultaneously the toxicity is reduced hence harmful side effects are minimized. Equally advantageous is the fact that polymer micelles have a tuneable size in the nanometer range (usually lower than 100 nm) and a narrow size distribution rendering them ideal for prolonged blood circulation since they do not cause blood vessel blockage and can avoid opsonization. To further achieve this goal biocompatible polymers, like the pioneer and very popular poly(ethylene glycol) (PEG), are chosen for constructing the outer shell of the micelle thus eliminating adsorption of opsonin proteins, which in turn leads to recognition and elimination from the bloodstream by the reticuloendothelial system or phagocytosis by macrophages. This prolonged circulation ability of PMs combined with their small size results in increased accumulation at tumor sites because of the enhanced permeability and retention (EPR) effect and is used as a passive targeting method (Fig. 5.1A). The EPR effect is a common trait of all abnormal tissues and is based on the high permeability of the tumor blood vessels, in combination with the impaired lymphatic drainage observed in tumor tissues, which increases the retention of the nanocarriers. Additionally, PMs possess high

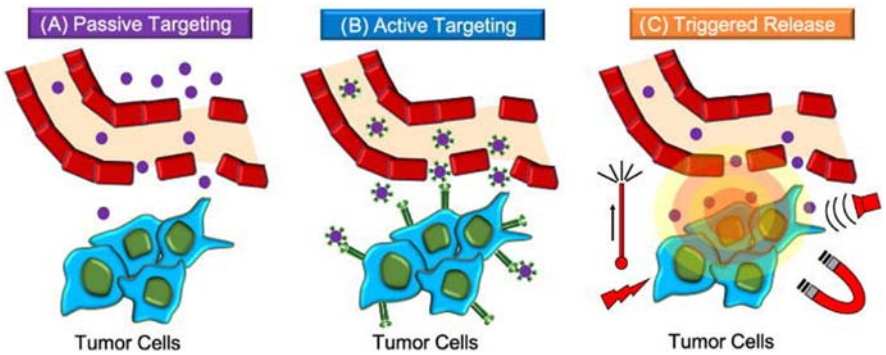

FIGURE 5.1

Targeting and release strategies. (A) Passive targeting relies on extravasation of nanoparticles through leaky tumor vasculature; (B) active targeting exploits surface-modified nanoparticles; (C) triggered release is based on stimuli-responsive nanoparticles.

Adapted from Martinelli, C, Pucci, C., & Ciofani, G. (2019). Nanostructured carriers as innovative tools for cancer diagnosis and therapy. APL Bioengineering, 3*(1), 011502.*

static and dynamic structural stability due to their low critical micelle concentration (CMC) values, thus do not dissociate under the highly diluted conditions encountered in body fluids during in vivo applications (Cabral et al., 2018; Movassaghian, Merkel, & Torchilin, 2015; Yin et al., 2016).

Another important feature of PMs is that they can be suitably modified in order to facilitate active targeting strategies (Fig. 5.1B), or in other words they can be designed to target specific cells after their extravasation from the bloodstream. This is feasible by the incorporation in their periphery of appropriate ligands (e.g., functional organic molecules, peptides, proteins, antibodies, aptamers, etc.) that recognize and bind to particular sites via their interaction with the complementary receptors which are usually overexpressed in cancer cells and tissues. The outcome of active targeting is to increase intracellular penetration and uptake, consequently improving therapeutic efficacy without affecting healthy tissues (Martinelli et al., 2019; Yin et al., 2016). Having attained the successful accumulation of the nanocarrier to the targeted site, the next step is to ensure the optimal delivery of the pharmaceutical payload. For this purpose, it is possible to precisely design and utilizes PMs that respond to localized stimuli and are subjected to triggered release (Fig. 5.1C). Nowadays it is common knowledge that tumor tissue physiology differs significantly compared to that of normal ones, displaying among others lower pH value, higher temperature, and increased concentration of certain enzymes. These characteristics can be exploited as triggers for the structural transformation or direct disassembly of the loaded micelles resulting in the controlled release of the drug. Either independently or even better synergistically to the aforementioned internal stimuli, externally applied sources of stimulus such as heat, magnetic field, ultrasound, and radiation are also being used as means for achieving responsive delivery (Martinelli et al., 2019; Yin et al., 2016; Zhou et al., 2018). The latest development in the field of nanomedicine is the construction of multifunctional nanocarriers that combine their therapeutic purpose with a diagnostic/imaging capability, giving rise to the prevalence of the accordingly termed theranostics systems. Once again PMs have a crucial role to play in the realization of such complex systems, as their compartmentalized structure allows for the encapsulation of imaging agents together with drugs within the same platform, facilitating simultaneous monitoring and treatment of diseased tissues, while maintaining the pharmacological outcome (Cabral et al., 2018; Movassaghian et al., 2015; Yin et al., 2016).

It comes as no surprise that all aspects of PMs that are deemed suitable for nanomedicine applications have been extensively studied and the vast amount of the corresponding scientific production has been thoroughly reviewed (Biswas, Kumari, Lakhani, & Ghosh, 2016; Dhara, 2020; Hwang, Ramsey, & Kabanov, 2020; Indoria, Singh, & Hsieh, 2020; Li, Wang, Kong, Zeng, & Liu, 2018; Mi, 2020; Zheng, Zhao, & Liu, 2018). Therefore, herein we turn our focus towards the most recent research results concerning exemplary cases of advanced therapeutic and diagnostic micellar systems with enhanced functionalities. Initially, an overview of the general characteristics of block copolymer micelles is given,

followed by two sections regarding micellar systems intended for therapy and bioimaging, respectively. The former section includes micellar carriers of low molecular weight hydrophobic drugs, genes, proteins, and photosensitizing agents, whilst the latter covers the delivery of analogous agents employed for magnetic resonance and optical imaging. Finally, some conclusions and future perspectives of these systems are presented in the last section.

5.2 General characteristics of block copolymer micelles

Over the recent years block copolymers and their self-assembly have been at the center of immense multidisciplinary scientific concern and research activity, ranging from the most fundamental basic understanding point of view to the vast variety of possible applications, involving nanomedicine, biotechnology, photonics, catalysis, lithography, environmental applications and so on (Agrahari & Agrahari, 2018; Brendel & Schacher, 2018; Deng & Liu, 2020). The unique properties of block copolymers are a direct consequence of their chemical composition which combines two or more distinct, usually immiscible, covalently linked macromolecular segments. The continuous evolution of synthetic chemistry has led to the development of more advanced living ionic and controlled radical polymerization techniques, which in turn resulted in an ever-growing potential of attainable well-defined block copolymers architectures, such as linear, branched, graft, comb, dendrimer, star-like, cyclic, etc. Moreover, this ability to precisely design and control the final macromolecular architecture allows for the finetuning of the interactions between the different segments of the copolymer, not only with each other but with the solvent as well, thus granting control over the self-assembly process itself (Deng & Liu, 2020; Feng, Lu, Wang, Kang, & Mays, 2017; Tritschler, Pearce, Gwyther, Whittell, & Manners, 2017).

Although it is a complicated process the self-assembly of amphiphilic copolymers in selective solvents is mainly regulated by the balance of the opposing thermodynamic forces at play, that is the attractive forces between the solvophobic blocks, the repulsive forces among the solvophilic blocks, and the surface tension of the formed interface. From the thermodynamic standpoint, copolymer chains assemble to decrease the interfacial area of the insoluble blocks with the solvent and thus minimize the free energy of the system. As the number of associating chains increases, both soluble and insoluble blocks are forced to adopt a more stretched conformation due to the increase of their density and the need to accommodate the alignment of their connection in the interface, causing a decrease in the conformational entropy. Ultimately, the gain in interfacial free energy and the conformational entropy loss compensate one another leading to thermodynamically stable assemblies of finite size. One should keep in mind that each individual set of copolymer and solvent is characterized by unique interactions between the different blocks, as well as between the blocks and the solvent, as dictated by

the Flory-Huggins parameter. Additionally, the relative sizes of the blocks, regarding their length and volume, dictate the curvature of the solvophobic/solvophilic interface and thereby the packing capability of the chains. These parameters eventually determine the morphology of the resulting micellar structure which could be either spherical or cylindrical, whereas at low curvature enclosed membrane structures are formed known as polymersomes (the macromolecular equivalent of vehicles) (Cabral et al., 2018; Jiao, Yang, Wu, Liu, & Zhang, 2020; Tritschler et al., 2017).

Owing to increased architectural complexity more unusual and intriguing morphologies like multicompartment micelles, toroids, and helices have also been observed in practice, while the secondary association of such nanostructures can lead to the formation of even more intricate hierarchical supra-structures (Brendel & Schacher, 2018; Deng & Liu, 2020). As far as linear copolymers are concerned, the simplest way to increase macromolecular complexity is by adding a third block, either of the same chemical composition as in ABA-type triblock copolymers, or a completely different polymer segment in the case of ABC triblock, where letters A, B, and C denote individual polymer blocks. When it comes to the self-assembly of triblock copolymers the miscibility of each block with the respective solvent defines to a great extent the morphology of the produced micelles. Only considering spherical micelles (for simplicity reasons), ABA triblock forms rather straightforward core-corona or star-like structures in a selective solvent for the A blocks, whereas in the opposite case (B selective solvent) flower-like micelles can assemble as a result of the looping of the B blocks. Similarly, ABC triblock copolymers dissolved in a good solvent for the extreme A and C blocks construct core-corona micelles with either a mixed or a compartmentalized corona, depending on the compatibility of the A and C blocks, with the latter commonly known as Janus micelles. Otherwise, three-layered or core-shell-corona micellar morphologies emerge with a soluble or insoluble shell if the solvent is selective for both A and B or only A blocks, respectively (Jiao et al., 2020).

Even though the possibilities are endless, most pharmaceutical applications employ linear amphiphilic block copolymers that assemble into core-corona (or shell) spherical micelles when dissolved in aqueous media. The hydrophobic core serves as a reservoir for drug loading or equivalently for the incorporation of desired bioactive agents, whereas the hydrophilic corona provides solubility, and stability, and also regulates the interaction of the micelle with its environment. In particular, the stability of the nanocarrier is of great importance, since dissociation under the dissolution encountered at in vivo conditions is always a threat. As already mentioned, PMs possess a strategic advantage against dissociation, especially in comparison to low molecular weight surfactant micelles, which is their low CMC. Nevertheless, there are also other possible ways to increase micellar stability, with one common practice being the combination of two or more amphiphilic copolymers to form mixed micelles. This type of self-assemblies offers several advantages apart from the increased thermodynamic and kinetic stability, such as enhanced drug loading capacity, more precise size control, and the

capacity to incorporate different functionalities and modifications (Cagel et al., 2017). Another method toward micelle stabilization is the crosslinking of either the core or the corona, which results in chemically fixed micelles that do not disassemble even at concentrations lower than the CMC. Therefore, crosslinking allows for increased blood circulation times and longer drug administration periods, in other words, improving temporal control. Additional benefits emerge as a consequence of shell-crosslinking, since this way it is possible to adjust the permeability of the micelle corona and like so finetune the drug release rate (Lu, Zhang, Yang, & Cao, 2018; Rösler, Vandermeulen, & Klok, 2001).

Another category of polymeric nano-assembly that has been extensively utilized in biomedicine is the micelles that form as a result of the electrostatic interaction between oppositely charged block copolymers (Cabral et al., 2018; Harada & Kataoka, 2017; Insua, Wilkinson, & Fernandez-Trillo, 2016). This type of micelles is known as polyelectrolyte or polyion complex (PIC) micelles and usually has a core-shell morphology, with the complexed ionic segments comprising the segregated core and the water-soluble blocks the stabilizing shell of the micelle. The main force for the formation of these assemblies is the release of counterions, in an analogy to the exclusion of solvent molecules from hydrophobic segments in the case of amphiphilic micelles (Cabral et al., 2018). The length and charge density of the ionic blocks, as well as the pH and ionic strength of the solution greatly affect the interactions that lead to stable block ionomer complexes. Most importantly, charged therapeutic agents can be easily incorporated into the core of such micelles via electrostatic binding, while their subsequent release can be trigger-controlled through the pH- and salt sensitivity of the micelles. A multitude of biologically active macromolecules including nucleic acids, proteins, enzymes, antibodies, polysaccharides, genes, and so more carry several charges and thus are governed by charge-charge interactions, which as a result regulate relevant biological processes. As expected, the use of PIC micelles for the development of novel platforms to stabilize and deliver drugs, proteins, nucleic acids, and genes has gained tremendous momentum over recent years as it opened a new avenue in pharmaceutical research (Harada & Kataoka, 2017; Insua et al., 2016).

From the myriad of accessible chemical compositions, when one is confronted with the selection of polymer segments intended for use in medical applications the requirement for biocompatibility, biodegradability, and low toxicity limits the possible choice (Movassaghian et al., 2015). Under these circumstances, a pool of suitable and as so commonly used hydrophilic or hydrophobic polymers have been generated (Cabral et al., 2018; Hwang et al., 2020). For the construction of the micellar nanocarrier shell the go-to option has always been poly(ethylene oxide) (PEO) (or equivalently PEG) since it provides water solubility, stability, efficient steric protection (stealth-effect), antifouling properties, and reduced immunogenicity, meanwhile being clinically approved and available in a wide range of narrowly distributed molecular weights. Less widespread but of equal relevance and applicability shell-forming polymers include poly(vinyl alcohol)

and poly(*N*-vinylpyrrolidone), both clinically approved, methacrylates and methacrylamides, like poly(methyl methacrylate) (PMMA), poly(2-hydroxyethyl methacrylate) (PHEMA), poly[*N*-(2-hydroxypropyl) methacrylamide] (PHPMA), and poly(*N*-isopropyl acrylamide) (PNIPAM), renowned thermo-responsive, as also are poly(2-oxazoline)s. Surely, besides synthetic polymers natural polysaccharides such as dextran, chitosan, cellulose, and hyaluronic acid can and have been used not only due to their intrinsic biocompatibility and biodegradability, but because they also possess inherent bioactivity, particularly mucoadhesive, antimicrobial, and antiinflammatory properties (Zhang, Wardwell, & Bader, 2013).

In parallel, the hydrophobic segments of block copolymers not only solubilize and encapsulate the poorly soluble drugs but are also responsible for their release from the core of the micelle. The segregation of the core from the aqueous environment more often than not is a consequence of hydrophobic interactions, but metal complexation, hydrogen bonding, $\pi-\pi$ stacking, and even electrostatic interactions in the case of PIC micelles, can serve the same purpose as well. In practice three main categories of regularly employed hydrophobic polymers can be identified, that is polyethers, polyesters, and poly(amino acid)s (Cabral et al., 2018; Hwang et al., 2020). Starting with polyethers the use of poly(propylene oxide) (PPO) has unambiguously prevailed, especially in the form of PEO-PPO-PEO triblock copolymers known as poloxamers or with their tradename Pluronics, whose extensive application in drug-delivery formulations from early on has rendered them a separate category of their own (Bodratti & Alexandridis, 2018; Tiwari, Kansara, & Bahadur, 2020). Polyesters are also exemplary hydrophobic polymer candidates that have been widely utilized in the formulation of drug-loaded micelles since they are biodegradable and exhibit high loading capacity. Especially, poly(ε-caprolactone) (PCL), poly(glycolic acid), poly(lactide-co-glycolide) (PLGA), and poly(D, L-lactic acid) (PDLLA or simply PLA) are among the most commonly considered polyesters and have found their way into various clinically approved formulations (Yi et al., 2018). Furthermore, investigations involving the hydrophobic poly(amino acid)s poly(β-benzyl-L-aspartate) (PBLA) and poly(γ-benzyl-α, L-glutamate) (PBLG) have also been reported. As far as charged segments are concerned for the formation of electrostatically complexed cores, hydrophilic poly(amino acid)s including amphoteric poly(L-histidine) (PHis), cationic poly(L-lysine) (PLL) or anionic poly(glutamic acid) (PGA) and poly(aspartic acid) (PASA), along with also cationic poly(ethyleneimine) (PEI) and poly(2-(*N,N*-dimethylamino)ethyl methacrylate) (PDMAEMA) polymers are some characteristic examples.

One step further in the devising of pharmaceutical carriers is the incorporation of appropriate ligand molecules in the periphery of the drug-loaded micelle that will enhance the delivery and facilitate the successful internalization of such nanomedicines into the target cells, thus intensifying their therapeutic efficacy. Possible relevant ligands include antibodies, antibody fragments, aptamers, peptides, sugars, and small functional organic molecules, while the ability of the fabricated ligand-installed nanomedicines to improve the targeting efficiency of

specific cell populations has already been confirmed. However, to avoid unfavorable pharmacokinetics and biodistribution the density of ligands on the surface of micelles and the resulting charge of the ligand-installed nanomedicines should be precisely controlled, as they govern the interaction of the carrier with its environment and especially the adsorption of serum proteins. In any case, the advantages of such systems are manifold, especially when considering the possibility of installing different ligands on the surface of the same nanocarrier, hence allowing for multivalent binding to target cells (Cabral et al., 2018; Mi, Cabral, & Kataoka, 2020). All the above in regard to the design and selection criteria for block copolymers, possible interaction routes with potential bioactive agents, and main properties of the micellar nanocarriers suitable for medicinal formulations are schematically depicted in Fig. 5.2.

Finally, a substantial advantage of block copolymer micelles in regard to their drug-delivery capacity is their potential stimuli-responsiveness, which can be attained by using polymers with the corresponding properties (Biswas et al., 2016; Dhara, 2020; Mi, 2020; Yin et al., 2016; Zhou et al., 2018). As previously mentioned, micellar carriers can be designed in such a manner to respond either to endogenous or exogenous stimuli of physical, chemical, or biochemical origins. The ability of a specific polymer to exhibit responsiveness usually involves secondary interactions, such as van der Waals forces and hydrogen bonding, hydrophobic and electrostatic interactions, as well as chemical changes like hydrolysis, oxidation-reduction, acid-base reaction, and even degradation of the polymer chains in extreme cases (Dhara, 2020). Ultimately, the stimuli-imposed reaction of the carrier facilitates on-demand or controlled drug release, enhanced tumor accumulation, ligand exposure, drug or probe activation, nanoparticle structure or size changes, charge conversion, as well as signaling in specific positions and sensing of special pathological factors, thus leading to tumor-specific diagnosis and treatment (Mi, 2020). In comparison to healthy tissues, the microenvironment of tumors presents unique properties that can be exploited as stimuli for selectively activation of drug-loaded micelles, including acidic interstitial pH values

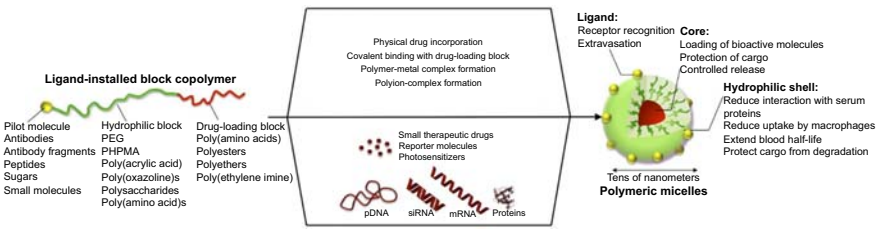

FIGURE 5.2

Block copolymer design criteria, possible interaction routes with potential bioactive agents, and main micellar properties towards the development of therapeutic nanocarriers.

Adapted from Cabral, H., Miyata, K., Osada, K., & Kataoka, K. (2018). Block copolymer micelles in nanomedicine applications. Chemical Reviews, 118*(14), 6844–6892.*

between 6.5 and 7.2, overexpression of particular biomolecules, extracellular reactive oxygen species (ROS), and altered redox potential (Cabral et al., 2018). As a consequence, a vast number of pH-, enzyme-, and redox-sensitive micellar systems have been developed and applied in the course of an internal responsive strategy for targeted delivery. Respectively, in an effort to engage external triggers like temperature, magnetic field, ultrasound, and light the equivalently responsive polymers have been employed, leading to extrinsically guided therapeutic carriers. Of course, the combination of different sensitivities can lead to sequential or simultaneous multistimuli responsive delivery systems that will facilitate more intricate drug delivery and controlled release processes in conjunction with imaging abilities.

As the demand for more and more complex, functional, and effective pharmaceutical formulations is constantly increasing, researchers are forever challenged with the development of progressively "smarter" multifunctional nanocarriers. The most recent trend is to combine therapy and diagnosis, towards a more personalized medicine approach against various diseases including, but not limited to, cancer. For this purpose, multiple interventions against a tumor should be integrated into the same platform by combining tumor-targeting ligands, triggering the release of therapeutics, and the delivery of imaging agents. PMs offer great potential when it comes to the construction of such platforms, as they can integrate different therapeutic strategies with one or more imaging functionalities. At the same time, the level of sophistication and versatility that can be achieved through appropriate design and realization allows for an unlimited supply of innovative medicinal nanoconstructs.

5.3 Micellar systems for therapy

In both developed and developing countries, cancer is still one of the serious public health concerns because it is the second major cause of death globally, following diseases of the cardiovascular and respiratory system (Bray et al., 2018; Siegel, Miller, & Jemal, 2019). The incidences of diverse new cancer cases are still on the rise and are anticipated to increase by 70% in the following two decades. Up to now, more than one hundred cancer types exist which can influence the organs and tissues in the human body by the uncontrolled and rapid growth of abnormal cells (Bor, Mat Azmi, & Yaghmur, 2019). Radiation therapy, surgery, chemotherapy, hormone, immunotherapy, or a combination of these are the conventional treatment strategies for cancer. Chemotherapy is one of the most recommended treatment modalities for cancer therapy by using drugs to kill cancerous cells or prevent them from uncontrolled dividing. However, the anticancer drugs display suboptimal features, including insufficient accessibility to tumors, wherefore higher drug dosages are required and nonselective nature, causing undesirable side effects on the healthy tissues which lead to the appearance of acute

toxicity phenomenon in patients. Furthermore, the off-target effects and the low solubility in the aqueous media of most chemotherapeutic agents result in reduced therapeutic efficacy (Liao, Wong, Yeo, & Zhao, 2020). The lack of target specificity of the current anticancer therapies and the associated challenges require the development of efficient and nontoxic systems for delivering chemotherapeutic agents to the human body.

During the last decades, the field of pharmaceutical nanotechnology has gathered increased scientific attention since it has emerged as a promising alternative technology for disease diagnosis and drug, gene, and protein delivery to minimize the side effects and maximize therapeutic effectiveness. Nanocarriers have been researched extensively in the field of cancer therapy as they have been utilized to circumvent the obstacles related to conventional anticancer drugs, including the damaging of normal cells, nonspecificity, burst release as well as other undesirable side effects. It is believed that smart nanosized carriers derived from multifunctional nanoparticles, would be an alternative treatment for cancer in the near future. Nanocarriers are formed when the therapeutic agents are incorporated into the nanoparticles by encapsulation, entrapment, surface absorption, surface attachment, or conjugation to be carried and delivered to the specific target sites in the human body. Decreased side effects, improved stability, regulated solubility, biodistribution, and pharmacokinetics, as well as specific target delivery and controlled release of drugs are some of the characteristics that nanocarriers can impart in the delivery of therapeutic compounds (Liao et al., 2020). Nanoparticles are colloidal and nanosized particles, which are utilized extensively in therapeutic agent delivery systems. Among the different types of nanoparticles, PMs have gained increased attention mainly because of their in vitro and in vivo advantages for targeting the delivery of therapeutic agents (Dutta et al., 2020). Extensive studies have shed light on the features of PMs and have evaluated them for drug, gene, and protein delivery systems, as well as for applications in the diagnostic field. PMs are the inner core/outer shell nanostructures (the sizes fluctuate from 10 to 100 nm) formed by the self-assembly of amphiphilic block copolymers in aqueous media. Copolymers as being amphiphilic consist of hydrophobic and hydrophilic segments. Therefore, after the insertion of block copolymers in an aqueous milieu and above a certain concentration (the so-called CMC), the hydrophobic segments constitute the inner part while the hydrophilic segments compose the outer part of the formed micelles (Bhawani, Ahmad, Ibrahim, & Yakout, 2019). The hydrophobic core of PMs can encapsulate hydrophobic drugs, as the hydrophilic shell contributes to the stabilization of the core, ensuring the solubility of PMs in aqueous media as well as controls in vivo pharmacokinetics. In the case of gene and protein delivery, the hydrophilic outer part of the PMs could be positively charged where nucleic acids or proteins can interact through electrostatic interactions. Apart from passive targeting of hydrophobic drugs achieved by using amphiphilic PMs, the active targeting of therapeutics agents is possible by developing stimuli-responsive PMs (responsive to environmental changes such as temperature, pH, etc.) or by surface modification of PMs with targeting ligands

(Deshmukh et al., 2017; Dhara, 2020). In addition, polyion micelles, a subcategory of PMs that are of great significance, are researched intensively for the delivery of genetic material. Polyion micelles are assembled as a consequence of hydrophobic and electrostatic interactions among charged blocks of the copolymer and other oppositely charged species (Deshmukh et al., 2017).

In general, there are a few crucial considerations for successful nanocarrier development to be used in therapeutic agent delivery systems. Initially, the nanocarrier must be biocompatible, biodegradable, and nontoxic. Moreover, the solubility of hydrophobic drugs in an aqueous milieu should be enhanced after entrapping into the nanocarrier, and the incorporated hydrophobic drugs must be released from these at a slow or rapid rate, ideally in response to intracellular stimuli. Finally, the nanocarrier should display a high-level capacity for drug loading (Majumder & Das, 2020).

Until now, numerous PMs are utilized as smart nanocarriers for therapeutic agent delivery systems and several of them have been approved by the United States Food and Drug Administration (Kapse et al., 2020). In the following section, we aim to discuss the current state of several polymeric micellar-structured formulations as nanocarriers for drug, gene as well as protein delivery and evaluate their preclinical or clinical stage. Sufficient emphasis will be given to the type and physiochemical properties of copolymers, and toxicity profiling, as well as in vitro and in vivo progress so far.

5.3.1 Micelles encapsulating low molecular weight hydrophobic drugs

5.3.1.1 Fabrication methods

Many micelle preparation techniques have been developed for the delivery of hydrophobic drugs. Nevertheless, two main methods have been established to form micelle structures of sufficient size for drug entrapment. The first method involves dissolving the amphiphilic polymer and drug in an aqueous medium. This method is used in the case of polymers that have low hydrophobicity, such as PEG, PEO, and PPO. The second approach concerns the use of an organic solvent when the polymer and the drug cannot be dissolved in water. This process can be carried out in multiple ways to prepare micelle structures. One way is to dissolve both the polymer and the drug in an organic solvent that is miscible with water. Then the mixture is subjected to vigorous stirring and eventually, the organic solvent is removed by evaporation. The gradual transition, through heating, from the organic to the aqueous phase, results in the self-organization of the polymer chains into micelles entrapping the drug in the micellar core. Another way is the dialysis method. Specifically, the polymer and the drug are dissolved in a common good solvent and placed in a dialysis bag, they are placed in a bath with the selective solvent (water or another aqueous phase). Gradually the aqueous solvent penetrates through the membrane and replaces the organic solvent.

At regular time intervals, the bath is renewed with new amounts of pure aqueous solvent, so that it is always in excess in relation to the good solvent within the membrane. Another important method of forming micelles, for drug entrapment is that of thin film hydration. The polymer and drug are dissolved in organic solvent and placed in a spherical flask, then the solvent is evaporated using a rotary evaporator and a thin film is formed on its inner surface. Then, the aqueous solvent is added and under gentle stirring, the formulation of mixed polymer/drug nanostructures is accomplished due to the self-assembly of the polymer in the aqueous solution and the subsequent drug entrapment in the core of the micelles (Tyrrell, Shen, & Radosz, 2010).

5.3.1.2 Drug encapsulation and release

As mentioned before, the ability of the amphiphilic block copolymers to self-assemble in aqueous media results in the formation of micelles. Hydrophobic drugs can be encapsulated inside the micellar core and thus their solubility in water is increased (Fig. 5.3). The entrapment of the hydrophobic drugs in the micellar nucleus occurs either by entrapment via dialysis or emulsification or by chemical conjugation. Implementation of chemical conjugation leads to the creation of covalent bonds between the drug and the hydrophobic micellar core. Subsequently, the embodiment of the insoluble drug inside the micellar core occurs. Physical entrapment is usually preferred compared to chemical conjugation mainly due to its simplicity. The embodiment of ion-bearing drugs can be accomplished via the preparation of micelles based on PICs. The congruency between drug and micelle core, the length of the block that forms the core domain, the crystallinity of the core, the concentration of the polymer and the drug solution, as well as the micelle formation methodology, are parameters that influence drug encapsulation. These factors are expressed via the terms of drug

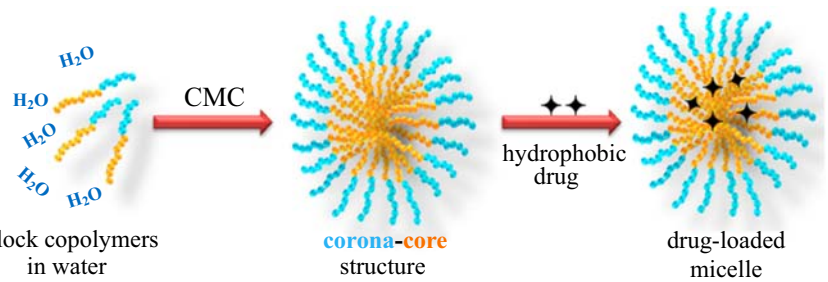

FIGURE 5.3

Schematic illustration of the micelle formation as a result of the self-assembly of amphiphilic block copolymers and drug entrapment in the micellar core.

Adapted from Hussein, Y. H. A., & Youssry, M. (2018). Polymeric micelles of biodegradable diblock copolymers: Enhanced encapsulation of hydrophobic drugs. Materials, 11*(5), 688.*

loading content (quantity of drug inside the micelles/total quantity of the micelles), drug loading efficiency (expressed in percentage, the quantity of drug in the micelle/initial addition of drug × 100%). Ideally, the highest drug loading efficiency and drug loading content are obtained. Achieving a high level of drug loading decreases the undesirable side effects from the presence of the polymer, once the drug has been distributed in the target cells, as higher drug content signifies the regulation of the corresponding drug dose but with the simultaneous utilization of a lower quantity of the polymer. The compatibility between the drug and the micelle core material indicates the capability of the drug-core miscibility, which is a critical detail for regulating the drug loading content inside the micelles (and to some extent influences subsequent drug release). In case an encapsulated drug is already soluble in a solution, an increase in the length of the core-forming block at constant hydrophilic block length, increases the core/solvent partition coefficient of hydrophobic compounds between the micelles and the solvent. Therefore, a higher level of drug encapsulation can be achieved, because the increase in the length of the hydrophobic block induces a larger hydrophobic core domain. PCL is a representative core-forming polymer whose crystallinity can increase by increasing its molecular weight. Higher core crystallinity leads to an unfavorable effect on drug loading levels because it reduces the attainable core space for drug molecules. Concentrations of both block copolymer and drug in the mixed solution have a significant effect on drug loading. In the case where polymer concentration in solution increases, drug loading attains its highest possible level. When drug concentration increases, a higher micelle aggregation number occurs in most cases. Therefore, the formation of larger micelles is induced leading to higher drug loading content. Strategies for the formulation of drug-loaded micelles have been described above (Ahmad, Shah, Siddiq, & Kraatz, 2014; Hussein & Youssry, 2018).

Drug release from the PMs can be regulated by constructing the core with specific approaches that regulate the interaction between the micellar core and the drug. There are two main options concerning in what manner the drug can be released (Ahmad et al., 2014; Kedar, Phutane, Shidhaye, & Kadam, 2010). The first one includes the dissociation/fragmentation of the micelles that is accompanied by the release of the physically encapsulated drug. The second one involves the breakage of the bond formed between the drug and the core forming polymer/block inside the micelle, followed by the diffusional release of the drug from the micelle. Moreover, drug release may be associated with chemical stimuli such as the pH of infected areas/tumors. Approaches like this are employable for controlled drug release for cancer cell treatment. The environmental pH of the tumor or the endosomes inside the tumor is acidic, so pH-responsive PMs carrying drugs are triggered by the acidic pH, and controlled release of the drug is accomplished. Gillies and Fréchet (2004), developed a strategy of controlled release which involves the formulation of a pH-responsive nanocarrier in a specific way that the hydrophobic groups are connected to the core forming block of the copolymer through an

acid-responsive covalent bond. The hydrophobic block through hydrolysis becomes hydrophilic and therefore destabilization of the micelles occurs, resulting in the release of the encapsulated drug.

5.3.1.3 Targeting strategies

The principle of passive targeting relies on the micelle accretion at the vasculature of elevated porosity around the tumor and increases the possibility that the drug-loaded micelles will reach the tumor location and will be incorporated within the cancer cells (also known as the enhanced permeation and retention effect, EPR). On the other hand, the active targeting of micelles is accomplished when functional groups are located on the surface (outer part of the corona) of the micelles and is a structural characteristic that promotes micelle accumulation in specific body areas such as tumors. The utilization of micellar surface groups specific to the membrane receptors of cells that need treatment results in the most effective active targeting. Folic acid is among the highly active targeting groups, which maintains its cancer cells receptor targeting ability after conjugation with block copolymers. Block copolymers bearing hydrophilic corona blocks such as PEG and polymerized monomers with phosphocholine functionality have been functionalized efficiently with folic acid end groups (Tyrrell et al., 2010). When folic acid is attached to the corresponding receptor at the surface of the cell, a receptor-interceded endocytosis procedure initiates and the complex formed by the combination of folic acid and the receptor inserts at the endosome and gets assimilated by the cell (Gaucher, Satturwar, Jones, Furtos, & Leroux, 2010; Tyrrell et al., 2010).

Still, the most significant barrier to effective cancer treatment is drug-resistant tumor cells. The majority of drug delivery strategies focus on approaching the cell before distributing the drug into the cytosol, yet several cancer cells have developed mechanisms to exclude drugs that approach/enter the cytosol. In order to overcome this obstacle, drug distribution should be conducted as close as possible to the area of activity. Consequently, PMs able to target not only the cancer cell itself (the cytosol) but also the nucleus of the cell are attracting attention. For nuclear drug delivery purposes, polyethyleneimine (PEI)-based nanoassemblies have been produced (Tyrrell et al., 2010).

5.3.1.4 Stimuli-responsive block copolymers micelles

Strategies for controlling the release and biodistribution of polymer micelles encapsulated drugs for the treatment of cancer, tumor and other diseases are still under development. It is known from the field of biology that the tissues of a cancerous tumor have a lower pH and a higher temperature (around 40°C) compared to normal tissues. In order to achieve a controlled and targeted release of the drug, several groups have developed block copolymer systems that are responsive to stimuli such as temperature and pH. The following sections refer to recent studies on such micelle nanosystems which are characterized by their responsiveness to the surrounding environment and have the ability to trap and release the drug depending on the stimulus they receive as an external trigger (Zhou et al., 2018).

Several studies have reported on block copolymers that are pH responsive and can be used as drug carriers. Polymers having ionizable groups can easily change their conformational and solubility properties depending on the pH, leading to targeted drug release within selected tissue and tumor areas. One such approach of DOX-loaded micelles for anticancer drug delivery has been reported by Zhang et al. (2012). They developed a series of pH-responsive poly(ethylene glycol) methyl ether-b-(poly(lactic acid)-*co*-poly(β-amino esters)) (MEG-b-(PLA-*co*-PAE)) amphiphilic block copolymers by Michael-type step polymerization with different ratios of hydrophobic PLA component. Studies in aqueous solutions have shown that MPEG-b-(PLA-*co*-PAE) self-organizes into core-shell micelles at low concentrations by the dialysis method and the encapsulation of doxorubicin (DOX) was performed by a similar procedure. The percentage of the encapsulated drugs was as high as 22% when the PLA component was increased. In vitro DOX release was controlled by the PAE layer due to protonation of amino groups at acidic pH and depended on the PLA/PAE ratio as well as on the pH changes. At the lowest examined pH value (pH = 5), DOX release of up to 95% was found. Cytotoxicity studies using HepG2 cells were also performed. The results revealed that the particular block copolymers had significantly low cytotoxicity in comparison to free-DOX. This innovative pH-sensitive block copolymer family could find application as a drug delivery carrier for antitumor targeting therapy.

A similar smart pH-responsive amphiphilic block copolymer for in vitro and in vivo cellular uptake studies was prepared by Li et al. (2014). By using atom transfer radical polymerization (ATRP) the amphiphilic diblock copolymer poly (2-diisopropylaminoethyl methacrylate)-b-poly(2-aminoethyl methacrylate hydrochloride) (PDPA-b-PAMA) was synthesized. The self-organization of the copolymers occurred by dialysis procedure against distilled water and the entrapment of the drug was accomplished similarly. Dynamic light scattering (DLS) and transmission electron microscopy (TEM) measurements showed that the diblock copolymer self-organizes in spherical micelles at pH 7.4 and 6.8, but at acidic pH (pH = 5) the micelles disassemble due to the protonation of amino groups of PDPA, thus producing molecularly dissolved extended copolymer chains in solution. The low CMC value of 0.005 mg/mL at pH 7.4 indicates that this micellar system can be stable at extreme dilution. The drug loading and drug encapsulation efficiency of DOX was 9.96% and 55.31%, respectively. The drug release profile of DOX-loaded PDPA-b-PAMA micelles at pH 5 showed rapid drug release compared to neutral pH, which was also confirmed by cellular uptake experiments in cells.

Another interesting study on the pH-responsiveness of DOX-loaded PMs was conducted by Car and coworkers (2014). A series of poly(dimethylsiloxane)-b-poly(2-(dimethylamino)ethyl methacrylate) (PDMS-b-PDMAEMA) amphiphilic diblock copolymers with a short and long length of PDMAEMA block were synthesized using ATRP. DLS and TEM measurements showed that PDMS-b-PDMAEMA self-organized into spherical micelles with dimensions from 80 to 300 nm in aqueous media by the thin film hydration technique and were stable for a couple of days. DOX was entrapped using a similar encapsulation

protocol. The copolymer with the shortest PDMAEMA block retained 80% wt. of DOX for 3 days, while the copolymer micelles in direct dissolution at acidic pH (pH = 5.5) released over 80% wt. of entrapped drug within 48 h. Cell studies were performed by the absorption of PMs through endocytosis by HELLA cells. The results revealed that in the acidic environment of the endosomes/lysosomes DOX was released within 24 h. This pH-responsive system was proven to be suitable for use as a drug delivery system.

More recently, Xiao, Huang, Moingeon, Gauthier, and Yang (2017) presented the synthesis of an amphiphilic block copolymer of PEG and PLA components with an acetal group in the connection point of the diblock chain, for encapsulation/release of an antitumor drug, namely paclitaxel (PTX). PEG-α-PLA copolymers (α stands for the middle acetal group) self-organize into spherical micelles in aqueous solutions. The blank micelles of PEG-α-PLA copolymers showed dimensions of about 100 nm in PBS solution. Moreover, by decreasing the pH value from 7.4 to 6.5 the shell started to decompose slowly, and eventually, at pH 5.5 it was quickly disorganized. A dialysis procedure was used to encapsulate the drug within the PMs. In vitro studies have shown that PTX-loaded micelles inhibited cell proliferation and enhance the apoptosis of Hella cells, while at the same they provided inhibition of tumor growth in vivo without side effects.

Finally, a multidrug pH-responsive polymeric system has been reported by Jiang, Zhou, Zhang, and Fang (2020). A polyprodrug material of poly(ethylene glycol) methyl ether-b-poly(β-amino esters) conjugated with DOX via acid-labile cis-aconityl moiety (mPEG-b-PAE-cis-DOX) was utilized to codeliver DOX and PTX. The preparation of PTX/DOX-PMs was done by dialysis process against deionized water, which resulted in self-organized micelles with sizes of about 110 nm. PTX was trapped in the nucleus of the micelles by hydrophobic interactions exhibiting high drug loading of 25.6%. The particle size increased by decreasing pH and at pH 5 the system decomposed, owning to the pH sensitivity of the copolymer. In vitro studies of PTX/DOX-coloaded micelles revealed that the rate of drug release depends on pH changes demonstrating pH-triggered drug release profiles. Cytotoxicity studies revealed that blank micelles of block copolymers have insignificant cytotoxicity in contrast with PTX/DOX-coloaded micelles which show high cytotoxicity against tumor cell lines. PTX/DOX-PMs could be potential candidates as nanocarriers for chemotherapy.

Similarly, the temperature response is one of the key features of smart nanocarrier delivery systems. There have been many studies focusing on block copolymer systems that can adapt to the temperature of their environment. Some characteristic recent studies based on thermo-responsive block copolymers for hydrophobic drug delivery will be presented. Chang et al. (2011). reported a novel thermo-responsive poly(methyl methacrylate)-b-poly(*N*-isopropyl acrylamide-*co*-*N*-acryloxysuccinimide) (PMMA-b-P(NIPAAm-*co*-NAS)) amphiphilic block copolymer, synthesized by reversible addition-fragmentation chain transfer polymerization, to encapsulate and release an antiinflammatory drug, namely prednisone. Ethylenediamine was used as a cross-linker to form shell crosslinked (SCL) micelles. Finally, two types

of noncrosslinked and SCL micelles were prepared, which were formed through dialysis against distilled water. TEM and DLS experiments have shown that both types of micelles have a spherical shape at 20°C, but the SCL micelles displayed the ability to maintain their dimensions upon temperature increase. The drug-loading and drug encapsulation efficiency were at the same levels for both types of micelles. In vitro studies have revealed that SCL micelles present a better-controlled drug release profile compared to noncrosslinked micelles, thus demonstrating that they could potentially be used as smart drug carriers for controlled drug delivery.

Thermo-responsive copolymers of the type poly(*N*-isopropyl acrylamide-co-acrylamide)-block-poly(D, L-lactide) (P(NIPAM-*co*-AM)-b-PLA) were synthesized by Xu, Zhang, and Luo (2014) By utilizing ring-opening polymerization (ROP) a series of block copolymers of varying molecular characteristics were synthesized. Using a dialysis process the copolymers formed spherical core-shell micelles in aqueous media with sizes less than 81 nm and a cloud point temperature from 43°C to 54°C. Three hydrophobic drugs that is PTX, 10-hydroxycamptothecine (HCPT), and prednisone were encapsulated within the micelles via dialysis. The experimental results on the drug-loaded micelles revealed that the PTX-loaded micelles have a greater ability to entrap the drug as well as a better thermo-induced drug release feature than those encapsulating HCPT and prednisone. In addition, drug-loaded micelles were found to have low cytotoxicity, indicating that PTX-loaded PMs can be used effectively in cancer therapy.

Hu et al. (2014) reported novel highly tuned thermo-responsive poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide)-b-poly(L-lactide)-b-poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide) (P(NIPAAm-*co*-DMAAm)-b-PLLA-b-P(NIPAAm-*co*-DMAAm)) triblock copolymers synthesized by ATRP. DMAAm was integrated into the triblock copolymer to regulate the lower critical solution temperature (LCST). LCST increased from 32.2°C to 39.1°C as the DMAAm segment increased. The formation of micelles occurred by direct dissolution of the polymeric system in the aqueous solutions. As a result, nanosized PMs (with sizes from 37 to 54 nm) were observed, which displayed the tendency to grow as the content in DMAAm segments increased. Amphotericin B (AmpB) was loaded into the micelles by dialysis. In vitro studies revealed the rapid release of AmpB above the LCST, with 90% and 75% of the encapsulated AmpB being released. Cytotoxicity assays showed that this innovative biodegradable triblock copolymer can be used safely for biomedical applications.

Sun and coworkers (2015) reported three block copolymers whose self-organization in water led to the formation of micelles with different core materials and the same thermo-responsive hydrophilic corona, composed of poly(*N*-isopropylacrylamide) (PNIPAAm) chains. The core consisted of hydrophobic poly(*N*-acryloyl-2-pyrrolidone) (PNP), poly(*N*-acryloyl-5-methoxy-2-pyrrolidone) (PMNP), or poly(*N*-acryloyl-5-butoxy-2-pyrrolidone) (PBNP), which determined the core structure of the micellar nanoparticles, the DOX-loading efficiency and the thermo-responsive DOX release. The formation of blank and DOX-loaded micelles was achieved by applying the

dialysis method. The DOX-loading results showed that the block copolymer micelles with PBNP core can load a higher amount of drug, while the micelles with PNP core loaded lower. In drug release experiments conducted above the LCST, it was shown that the drug release ratio decreases in the order of micelles with PNP core (40%), PMNP core (31%), and PNBP core (25%). In vitro cytotoxicity studies have revealed that DOX-loaded micelles exhibited comparable cytotoxicity to free DOX, confirming that these materials can be used as drug carriers for cancer therapy.

Recently Soltantabar, Calubaquib, Mostafavi, Biewer, and Stefan (2020) presented the synthesis of three thermo-responsive amphiphilic diblock copolymers of poly(γ-oligo(ethylene glycol)-ε-caprolactone)-b-poly(γ-benzyloxy-ε-caprolactone) (PME_xCL-b-PBnCL, $x = 2$, 3, 4) by ROP. The influence of different oligo (ethylene glycol) (OEG) chain lengths resulted in the adjustment of the hydrophilicity and thermo-responsiveness of the polymer. The PMs were formed by solvent evaporation, while the drug-loaded nanoparticles intended for in vitro experiments were prepared by dialysis procedure against PBS. DLS and TEM measurements showed that the empty/coloaded micelles of PME_2CL-b-PBnCL formed spherical micelles with sizes of 81.5/93.1 nm, of 29.2/39.1 nm for PME_3CL-b-PBnCL and 31.2/44 nm for PME_4CL-b-PBnCL. PME_3CL-b-PBnCL showed high LCST (41°C) but very close to the physiological temperature and therefore was used for in vitro experiments and cell studies. The best encapsulation efficiency and loading of polymer/DOX/Quercetin ratio were 10:1:5. In vitro release of DOX-coloaded micelles revealed that PME_3CL-b-PBnCL released 67% of cargo at 37°C and 100% at 42°C. Cytotoxicity studies were performed on H9c2 and HepG2 cells. H9c2 cells did not show any difference in their viability when treated with a combination of the two drugs but the HepG2 cells showed a significant reduction in cell viability when treated with DOX/QUE-coloaded micelles. It was also observed that at 42°C there was 20% more cytotoxicity on HepG2 cells due to the high release of the drug at this temperature.

5.3.1.5 Micellar nanoparticles used in clinical studies for cancer therapy

Synthetic block copolymers that are utilized in drug delivery should display specific features, which include biocompatibility, biodegradability, absolute chemical inertness, and satisfying control over the synthesis procedure (Ahmad et al., 2014). Several block copolymers that exhibit these features have already been formulated into pharmaceutical products and exist in the market or are currently under the process of clinical trials. Based on available data, one out of 5000 compounds that undergo preclinical trials, will be an approved drug. This process can take up to 10 years from conceiving the idea until the final approval for distribution in the market (Englert et al., 2018). The most established synthetic polymers for drug administration are PEG conjugates. These block copolymers self-organize into micelles, where the hydrophilic shell consisting of PEG chains protects the pharmaceutical cargo and elongates in vivo circulation, thus contributing to ameliorated retention and permeability. With the progress of precision

medicine in cancer therapy, micellar nanostructures are of great importance for cancer treatment based on passive targeting (Cho, Lai, Tomoda, & Kwon, 2015).

The group of Kabanov (Alakhova, Zhao, Li, & Kabanov, 2013) reported that Supratek Pharm Inc. developed the SP1049C product, which is a mixture of the anticancer drug DOX with micelles formed from Pluronics L61 and F127. It has been formulated to treat patients with progressed adenocarcinoma of the esophagus. Phase II clinical trials with patients treated with SP1049C have been completed and gastroesophageal junction results exhibited a significant antitumor action and propitious safety profile for SP1049C in this type of cancer. Supratek Pharm Inc. claims that SP1049C is undergoing phase III clinical trials for the therapy of cancers unaffected by DOX. SP1049C displays the ability to consume cancer stem cells and diminish tumorigenicity of cancer cells in vivo, introducing a wide spectrum of applications for SP1049C, such as therapies against leukemia and breast cancer.

Owen, Chan, and Shoichet (2012) a review article mentioned that the product named NK911 formulated by a mixture of DOX with micellar nanoparticles of poly(ethylene glycol)-b-poly(aspartic acid) (PEG-b-PAA) underwent phase I clinical studies. The obtained results showed increased circulation time compared to free DOX for solid tumor treatment. Unfortunately, toxicity measurements were close enough to the free DOX formulation, leading to restricted clinical progress.

Kato and coworkers (2012) communicated the progress of the NK105 product in phase III clinical studies. NK105 is a blend of PTX and PEG-PAA PMs. Phase I trials showed that the most appropriate dose is 150 mg PTX equivalent/m^2 delivered every three weeks. Phase II study targeted at the efficacy and safety evaluation of NK105 in patients suffering from progressed gastric cancer after first-line chemotherapy was judged as unsuccessful. Phase I and II results indicated that the delivery of PTX embodied inside the PMs is mostly limited to the plasma and partially to extracellular body liquids. The outcome of phase II showed that NK105 is very efficient without disconcerting the antitumor activity of PTX in patients who already have been treated for gastric cancer.

Endo et al. (2013) studied the efficacy and safety of the NC-6004 product on oral scabrous cancer cells. The purpose of NC-6004 is the treatment of patients with solid tumors, related to the gastrointestinal and genitourinary types of cancers. NC-6004 is composed of the anticancer drug cisplatin entrapped inside the PMs formed by the self-assembly of poly(ethylene glycol)-b-poly(glutamic acid) (PEG-PGA) copolymer. NC-6004 has been designed and developed to increase the antitumor effects and decrease the nephrotoxicity limitations. In vitro antitumor activity, investigations were conducted in four samples of oral scabrous cancer cells. The antitumor and nephrotoxic influence of NC-6004 was studied by the distribution of NC-6004 or cisplatin to nude mice fetching OSC-19. The in vitro assays showed that NC-6004 exhibited less inhibitory development compared to free cisplatin. On the other hand, the in vivo assays showed that both NC-6004 and cisplatin exhibited similar antitumor capacities. Mice treated with only cisplatin developed renal cell apoptosis while those treated with injections of

NC-6004 were not affected by renal cell damage. In addition, NC-6004 significantly reduced the metastasis rate of sentinel lymph nodes, in comparison with only cisplatin treatment. These characteristics define NC-6004 as an example of important progress in the production of platinum composite micellar nanoparticles. NC-6004 has progressed to phase I of clinical trials in the UK and to phase I/II in East Asia.

Finally, Quader et al. (2017) based on the NC-6300 product reported a different approach that includes the targeting of endothelial cells of glioblastoma multiforme (GBM) by cyclic-Arg-Gly-Asp (cRGD) peptide. Specifically, they prepared novel cRGD-inaugurated micelles loaded with epirubicin for GBM treatment purposes. The micellar nanoparticles loaded with epirubicin were developed by chemical conjugation of the drug with the PASA block of the PEG-b-PASA block copolymer via an acid-responsive hydrazine bond that permits selective epirubicin release after endocytosis. These novel cRGD peptide-functionalized micelles loaded with epirubicin (cRGD-Epi/m) exhibited low toxicity, as well as faster and greater penetration into the U87MG cell-derived spheroids in comparison to micelles without the peptide installation, via a cRGD-moderated pathway. In vivo assays showed that cRGD-functionalized micellar nanostructures successfully subdued the expansion of the orthotropic GBM tumor model by distributing elevated levels of epirubicin across the tumor tissue. These promising results demonstrate notable potential for cRGD-Epi/m as an effective and transformable therapeutics against GBM. The above-mentioned characteristic examples, along with some other well-known cases of drug-loaded micellar nanocarriers that have reached the clinical trial stage, are summarized in Table 5.1 (Li, Tan, Li, Shen, & Wang, 2017).

5.3.2 Micellar systems for gene delivery and therapy

Over the past decades, gene therapy has presented stupendous potential in the treatment of severe diseases. The specificity of gene therapy renders significant advantages compared to traditional therapeutic approaches (Song, Hart, & Du, 2021). The research of nucleic acid therapeutics mostly concentrates on deoxyribonucleic acids (DNA) such as plasmid DNA (pDNA) and ribonucleic acid (RNA) oligonucleotides such as small interfering RNA (siRNA), messenger RNA (mRNA), and micro-RNA (miRNA) that have shown attractive features as novel agents for therapeutic applications over the conventional pharmaceutical drugs. The impressive perspectives of nucleic acid therapeutic agents reside in their capability of acting as factors arbitrating cellular pathways and conferring effective properties of gene silencing or urging the expression of indispensable proteins for disease management (Pereira-Silva, Jarak, et al., 2020). However, the application of nucleic acids in systemic administration is limited because of their high instability in biological fluids. These unmodified molecules are rapidly degraded by omnipresent serum and cellular nucleases, resulting in short circulation half-time in plasma and inadequate tissue bioavailability (Gavrilov &

Table 5.1 Representative examples of micellar nanoparticles utilized in pharmaceutical products that have progressed to clinical trials (Li et al., 2017).

Product	Drug	Platform	Status	Applications
Genexol-PM	Paclitaxel	mPEG-PLA polymeric micelles	Approved	Breast cancer
Lipotecan	Camptothecin analog	Polymeric micelle	Phase I/II	Liver and renal cancer
NC-4016	Oxaliplatin	Polymeric micelle	Phase I	Solid tumors
NC-6004	Cisplatin	PEG-PGA polymeric micelle	Phase II/III	Solid tumors, gastrointestinal and genitourinary cancers
NC-6300	Epirubicin	PEG-b-PAH polymeric micelle	Phase I	Solid tumors
NK012	SN-38	PEG-PGA polymeric micelle	Phase II	Colorectal, lung, and ovarian cancers
NK105	Paclitaxel	PEG-PAA polymeric micelle	Phase II/III	Breast and gastric cancers
NK911	Doxorubicin	PEG-PAA polymeric micelle	Phase I	Solid tumors
Paclical	Paclitaxel	Polymeric micelle	Phase III	Ovarian cancer
SP1049C	Doxorubicin	Pluronic L61 and F 127 polymeric micelle	Phase II/III	Lung cancer

Saltzman, 2012; Wang, Tai, & Gao, 2019; Wittrup & Lieberman, 2015; Zhou, Zhang, & Liang, 2014). Developing strategies for stabilization and delivery of nucleic acids via encapsulation or complexation methods can overcome the aforementioned limitations (Ozcan, Ozpolat, Coleman, Sood, & Lopez-Berestein, 2015). In the beginning, the research was principally focused on the utilization of viruses as viral gene carriers because of their ability to overcome several extra and intracellular delivery obstacles and offer high efficiency of gene transfection (Ozcan et al., 2015). However, the viral vectors demonstrate significant drawbacks, specifically, integration of genome and derived oncogenesis, immunogenicity, broad tropism, obstacles to production, and inadequate capacity for carrying and delivering large nucleic acid molecules (Pereira-Silva, Jarak, et al., 2020). For the above-mentioned reasons, the scientific interest has been focused on the nonviral gene delivery strategies emerging as a safer approach, albeit they present lower efficiency of nucleic acid delivery (Yin et al., 2014). The nonviral delivery vectors can develop many functions allowing the safe accumulation of nucleic acid therapeutic agents in the specific site, enhanced intracellular delivery and rapid endosomal escape, low cytotoxicity without considerable immunogenicity, and prolonged-expression. Additionally, the nonviral vectors can provide

advantages of great importance such as high flexibility and facile quality control, allowing the development of well-defined carriers with controlled chemical and structural features and low cost of production. However, nonviral vectors also display some limitations, including low oral bioavailability, instability in blood circulation, and reduced efficiency of gene transfection (Marzbali & Khosroushahi, 2017). For the delivery of nucleic acid therapeutic agents individually or in combination with drugs and/or diagnostic agents, various nonviral vectors have been developed and applied in trials of gene therapy so as to accomplish the best formulations (Ballarín-González & Howard, 2012; Zhang, Wang, & Gemeinhart, 2013). Among the nonviral vectors, PMs have emerged as useful nanocarriers in gene therapy strategies (Asyikin Binti Abdul Aziz et al., 2017; Nishiyama, Matsumura, & Kataoka, 2016; Simões, Figueiras, Veiga, Concheiro, & Alvarez-Lorenzo, 2015). The unique ability of PMs to self-assemble into core-shell structures renders them attractive candidates as gene and/or drug delivery vectors, as illustrated in Fig. 5.4 (Pereira-Silva, Alvarez-Lorenzo et al., 2020). The PMs formed by amphiphilic block copolymers possessing one cationic block or by double hydrophilic block copolymers (DHBC) composed of a neutral hydrophilic block linked to a cationic polymer have been investigated in depth in order to transport and deliver nucleic acids in the specific therapeutic target, applying new treatment strategies focusing on gene therapy. Cationic DHBC form PIC micelles by direct ionic complexation with negatively charged nucleic acids, where the

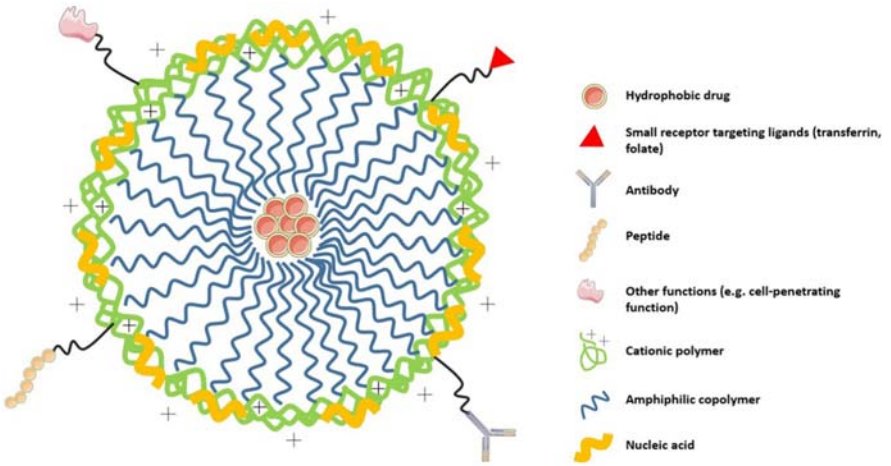

FIGURE 5.4

Illustration of a multifunctional micelleplex for dual delivery of drugs and nucleic acids.

Reprinted from Pereira-Silva, M., Alvarez-Lorenzo, C., Concheiro, A., Santos, A. C., Veiga, F., & Figueiras, A. (2020). Nanomedicine in osteosarcoma therapy: Micelleplexes for delivery of nucleic acids and drugs toward osteosarcoma-targeted therapies. European Journal of Pharmaceutics and Biopharmaceutics: Official Journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V, 148, *88–106 with permission from Elsevier.*

core is formed by the complex between the cationic block of DHBC and the nucleic acid (Cabral et al., 2018). PMs with a cationic corona can interact with nucleic acids forming micelleplexes. The micelleplexes are a complex of PMs connected to nucleic acids such as DNA and RNA molecules through the development of electrostatic interactions between the positively charged PMs and the negatively charged nucleic acid, thus improving the uptake by the cell membrane that is typically negatively charged (Fan, Li, & Loh, 2016; Kamaly, He, Ausiello, & Farokhzad, 2016; Zhang, Liu, Sen, Kral, & Gemeinhart, 2015). The efficiency of PMs as gene delivery vectors are vigorously dependent on their physiochemical features such as size, superficial charge, and shape (Navarro, Pan, & Torchilin, 2015). Different amphiphilic block copolymers can be utilized for this purpose, allowing the development of micellar structures with a variety of properties and architectures. Through an attentive design and/or a combination of amphiphilic block copolymers, the formed micelleplexes can protect the nucleic acid molecules improving the payload stability, and enhance the biological halftime due to the existence of a stealthy shell, accumulate passively in tumor tissue, and target actively via surface modification using ligands. More importantly, micelleplexes can achieve codelivery of nucleic acid and chemotherapeutic drugs for synergistic therapeutic approaches since the hydrophobic core acts as a hydrophobic drug reservoir (Asyikin Binti Abdul Aziz et al., 2017; Fernandez-Piñeiro, Badiola, & Sanchez, 2017). Amphiphilic copolymers utilized for the design of micelleplexes are constituted by polyesters, such as PLGA, poly(lactic acid) (PLA), PCL, forming the hydrophobic core. The hydrophilic/cationic part of the block copolymers is ordinarily comprised of poly(ethylenimine) (PEI), poly(L-lysine) (PLL), or poly(2-(*N*-*N*-dimethylamino)ethyl methacrylate) (PDMAEMA) (Amjad, Kesharwani, Amin, & Iyer, 2017; Pereira et al., 2017). Several therapeutic strategies utilizing micelleplexes have been used in the fight against various diseases of which cancer concentrates the main scientific interest. Some of the studies performed in the past few years using micelleplexes as smart nanocarriers with promising results are discussed below.

PIC micelles have gathered scientific interest as one of the most promising nanocarriers for systemic gene delivery due to their attractive properties, including stability in blood circulation and efficient permeability in target tissue. Concerning the efficient delivery of PIC micelles, Christie et al. (2012) synthesized functional poly(ethylene glycol)-b-poly(L-lysine) (PEG-b-PLL) block copolymers having three main characteristics: a hydrophilic nonbinding segment, a siRNA binding segment comprising thiols, and a cell-surface binding peptide. Particularly, PEG-b-PLL block copolymer containing the cyclo-Arg-Gly-Asp (cRGD) peptide on the terminal of PEG block and lysine amines modified with 2-iminothiolane (2IT) was used and self-assembled into nanosized PIC micelles for siRNA delivery to solid tumors. Improved control of micelle formation and increased stability in blood circulation was achieved by modification of PEG-b-PLL with 2IT, while improved biological activity was accomplished after installation of the cRGD peptide. The formed cRGD-PEG-b-PLL(2IT)/siRNA PICs

displayed improved cellular uptake, increased gene silencing ability, and broader subcellular distribution in vitro. In addition, they demonstrated improved accumulation in both the tumor-associated blood vessels and tumor mass after intravenous injection into mice. Moreover, the targeted and stable micelles suspended the growth of subcutaneous HeLa model tumors and showed gene silencing in the mass of tumor following the antiangiogenic siRNAs treatment. Consequently, the stable multifunctional polymeric PIC micelles can be utilized for siRNA-based cancer therapies that require intravenous injection.

A similar approach based on targeted PIC micelles composed of the cRGD-PEG-PLL block copolymer and RNA interference therapeutics was developed by Nishida et al. (2016), against human papillomavirus (HPV)-associated cancers and particularly cervical cancer. This form of cancer is highly related to E6 and E7 viral oncogenes, whose unregulated expression leads to tumorigenesis. Therefore, siRNAs were employed to silence the overexpression of E6 and E7 oncogenes, preventing cell proliferation and inhibiting tumor cell growth. In this regard, Nishida et al. utilized PIC micelles for the systematic delivery of two E6/E7 targeting siRNAs (siE6/E7) for two types of HPV, type 16 (si16E6/E7) and type 18 (si18E6/E7), to cervical cancer cells and tumors. PIC micelles were prepared using a PEG-PLL diblock copolymer, with cRGD peptide ligand installed at the PEG terminus and PLL side chains functionalized with 1-(3-mercaptopropyl)amidine (MPA) and 2-iminothiolane HCl (IT). cRGD was conjugated on micelles surface and can specifically target $\alpha_v\beta_3/\alpha_v\beta_5$ integrin receptors, which are overexpressed on cervical cancer cells. Thus, the installation of cRGD peptide facilitates cellular uptake, while the generation of disulfide cross-links leads to stabilization of the micellar core and preferable siRNA release in the cytoplasm of the targeted cells. The si16E6/E7 and si18E6/E7-loaded PIC micelles were systemically administrated to subcutaneous SiHa tumor and HeLa tumor-bearing mice, respectively. The results showed the reduction of E6 and E7 expression in both tumor types, inducing the apoptosis of the cancer cells and consequently the suppression of the tumor growth in both tumor cases. The obtained results are encouraging for the potential of these PIC micelles for siRNA delivery, as a therapeutic approach against a variety of HPV-associated cancers.

However, PMs formed from single amphiphilic block copolymers often exhibit a lack of one or more components mainly because of limitations in the number of constituting blocks. The combination of two or more different block copolymers to form mixed micelles is an efficient strategy to deliver many therapeutic agents without complicated synthetic procedures. In this proposed case, Feng et al. (2015) described the utilization of mixed cationic block copolymers consisting of poly(ethylene glycol)-b-poly(*N*-[*N*-(2-aminoethyl)-2-aminoethyl] aspartamide) [(PEG)-b-PAsp(DET)] and poly(*N*-isopropylacrylamide)-b-poly(*N*-[*N*-(2-aminoethyl)-2-aminoethyl]aspartamide) [PNIPAM-b-PAsp(DET)] as nonviral cationic vectors for gene delivery. The mixed polyplex micelles (MPMs) with thermo-responsive heterogeneous shells were used to deliver heme oxygenase-1 pDNA for nucleus pulposus regeneration. Upon heating from ambient temperature

(25°C) to body temperature (37°C), MPMs comprising heterogeneous coronas with coexisting hydrophobic and hydrophilic segments were obtained and displayed strong protein resistance as well as high nuclease tolerability. The gene transfection efficiency of polymeric mixed micelles in nucleus pulposus cells was much higher in comparison to that of micelles formed from the [(PEG)-b-PAsp (DET)] block copolymer in vitro and in vivo evaluation on account of the synergistic effect of low cytotoxicity and improved colloidal stability. High expression of heme oxygenase-1 in nucleus pulposus cells transfected by MPMs carrying heme oxygenase-1 pDNA considerably reduced the expression of matrix metalloproteinases-3 (MMP-3) and cyclooxygenase-2 (COX-2) caused by interleukin-1β (IL-1β) and concurrently raised NP phenotype-associated genes such as type II-collagen, aggrecan, and SOX-9. Furthermore, the treatment of disc degeneration caused by stab injury was tested using the MPMs carrying pDNA, evaluating the therapeutic effects. The results showed that the administration of MPMs loading heme oxygenase-1 pDNA in rat tail discs decreased inflammatory responses caused by needle stab and raised glycosaminoglycan content, ultimately accomplishing better therapeutic efficacy in comparison to PEG-b-PAsp(DET). Therefore, the mixed polyplex micelles proved to have appreciable potential as safe and high-efficiency nonviral gene delivery systems for regenerating degenerative discs or retarding disc degeneration.

As mentioned above, the properties and the architecture of the polymeric micellar systems, taking also into account their surface modifications, have a significant effect on their biologic interactions and stability. The functionalization of the PMs surface, as a strategy for active targeting, can be achieved with exogenous or endogenous targeting ligands such as antibodies and their fragments (e.g., anticancer monoclonal antibody mAb2 C5), aptamers (e.g., prostate-specific membrane antigen), carbohydrate ligands (e.g., galactose), small organic molecules (e.g., folic acid) and cell-penetrating peptides (e.g., penetratin), to acquire an effective, directed to specific cells delivery. Concerning this aspect, Liu et al. (2016). utilized micelleplexes for the delivery of siRNAs into the targeted tumor cell in a specific and efficient manner. These micelleplexes comprise the conjugate of an amphiphilic cationic copolymer with folate, that is poly(caprolactone)-b-poly(ethylene glycol) (PCL-b-PEG) grafted with poly(ethyleneimine) (PEI) and conjugated with folate as ligand. The micellar nanocarriers are based on poly (ethyleneimine)-g-poly(ε-caprolactone)-b-poly(ethylene glycol)-folate (PEI-g-PCL-b-PEG-FO) cationic copolymer were utilized for specific siRNA delivery through folate-FR recognition. This micelle-like structure had the capability of condensing the siRNAs and promoting their protection by electrostatic interaction between the positively charged PEI and negatively charged siRNAs. Moreover, the formed micelleplexes demonstrated a core-shell structure that contributes to their stability in blood circulation, resulting in efficient gene delivery. The micelleplexes exhibited an increased uptake and in vitro gene knockdown observed in an FR-overexpressing cell line (SKOV-3 cells), besides good bioavailability and accumulation in the targeted tumors in vivo. Therefore, the developed targeted

siRNA delivery nanocarrier seems to be promising for the delivery of siRNAs to ovarian cancer cells, precisely for gene knockdown in the tumor tissue.

Polymeric micelle-based nanocarriers have rendered promising outcomes concerning osteosarcoma-targeted treatments. Different studies have indicated the effect of dysregulated miRNA on osteosarcoma development. In particular, Magalhães et al. (2018) developed a micellar nanosystem consisting of the amphiphilic Pluronic L64 and the cationic polymer poly(ethyleneimine) (PEI) after their chemical conjugation. Pluronic L64/PEI micelleplexes were utilized in order to carry out trials on the delivery of unexpressed miRNA-145 invested with tumor-suppressing characteristics, thus replenishing its cellular levels. The extracted results showed that the use of L64/PEI micelleplexes for therapeutic delivery of vascular endothelial growth factor (VEGF) targeted miR-145 to osteosarcoma cells offered considerable VEGF gene silencing in MG-63 cells. The best results of L64-PEI/miR-145 were observed for the ratio of N/P = 10/1, inducing an efficient uptake of these complexes in the target cells. The obtained results by in vitro studies demonstrated reduced cell migration and proliferation capacity, as well as an increase in cell death by apoptosis and necrosis. Therefore, a novel and efficient micellar-structured nanocarrier for the delivery of therapeutic miRNAs in the future gene therapy of osteosarcoma disease was developed.

Codelivery of chemotherapeutic agents and genetic material has been recognized as a promising and effective therapeutic tool against cancer. In particular, the progress in the field of nanotechnology results in the design of novel multifunctional codelivery nanosystems displaying remarkable results because of the development of synergistic effects in one single nanoplatform, improving the overall treatment outcomes.

Based on the synergistic combination and the simultaneous delivery of chemotherapeutic drugs and miRNA therapeutics for cancer treatment, Qian and coworkers (2014) prepared a series of amphiphilic star-branched copolymers comprising PLA and poly(2-dimethylamino ethyl methacrylate) (PDMAEMA) with AB_3, $(AB_3)_2$ and $(AB_3)_3$ molecular architectures for the codelivery of the anticancer drug DOX and miR-21 inhibitor (miR-21i) to treat glioma. MiR-21inhibitor was employed to downregulate the overexpressed miR-21 and to reduce its oncogenetic and antiapoptotic activity. Star copolymers were self-assembled in nanosized micelles with low CMC, positive surface charge, and low cytotoxicity. DOX was encapsulated in the PLA hydrophobic core, while miR-21i was polyplexed to PDMAEMA on the micellar surface. The therapeutic compounds miR-21i and DOX were synergistically codelivered in vitro into LN229 glioma cells and in vivo into subcutaneous nude mouse models. The codelivering micelles exhibited high transfection efficiency in LN229 glioma cells, with a significant dependence on the copolymer molecular architecture and micellar structure. Furthermore, the micelles mediated the escape of miR-21i from lysosomal degradation and the successful release of DOX to the nucleus, leading to remarkable tumor inhibition and antiproliferative efficiency, through the regulation of expression of BCL-2 apoptosis by PI3k/AKT signal pathway. In conclusion, the

combinational delivery of miR-21i and DOX by amphiphilic PLA-PDMAEMA star copolymer micelles demonstrated enhanced therapeutic efficacy and synergistic inhibitory effect, compared with DOX or miR-21i treatment alone. The obtained results confirmed the potential of the amphiphilic star branched copolymer micelles to combine the simultaneous administration of advanced therapeutic agents in one single platform, indicating a promising codelivery system for cancer therapy and glioma treatment.

Using the combinational strategy of codelivery, Mondal, Almawash, Chaudhary, and Mahato (2017) developed a micelle-based formulation for the codelivery of Gemcitabine (GEM) and miR-205 mimic, as a therapeutic approach for the treatment of advanced pancreatic cancer. Furthermore, they utilized cetuximab (C225), an epidermal growth factor receptor (EGFR)-targeting monoclonal antibody for targeted therapy. The research group conjugated C225 to malemido-poly(ethylene glycol)-block-poly(2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol) (C225-PEG-PCD) and prepared mixed micelles with methoxy poly (ethylene glycol)-block-poly(2-methyl-2-carboxyl-propylene carbonate-graft-dodecanol-graft-tetraethylenepentamine) (mPEG-b-PCC-g-GEM-g-DC-g-TEPA) containing GEM and miR-205 for targeted codelivery. The efficacy of the micellar formulation was evaluated in vitro in GEM-resistant and EGFR expressing MIA PaCa-2^R cancer cells, and in vivo into MIA PaCa-2^R orthotopic pancreatic tumor-bearing mice. The results from the biological studies demonstrated that mixed micelles containing C225, GEM, and miR-205 enhanced cellular uptake by tumor cells, reversed GEM resistance, and suppressed the proliferation of MIA PaCa-2^R cells. Moreover, the synergistic coadministration of these therapeutic components exhibited significant tumor growth inhibition in orthotopic pancreatic tumor-bearing mice. Summing up, the combination of miR-205, GEM, and C225 in one singular micelle-based nanosystem displayed greater therapeutic potential than monotherapy with miR or anticancer drug alone, indicating a promising strategy for pancreatic cancer treatment.

Another interesting study that highlights the importance of the effective combination of hydrophobic chemotherapy drug components and RNA interference technology for cancer therapy codelivered by an intelligent micellar nanoplatform was presented by Yu and coworkers (2016). This group designed a novel triple-layered pH-responsive micelleplex composed of the triblock copolymer poly(ethylene glycol)-b-poly(aminolated glycidyl methacrylate)-b-poly(2-(diisopropylamino)ethyl methacrylate) (PEG-b-PAGA-b-PDPA), for the simultaneous delivery of alkylated cisplatin prodrug and siRNA-p65 targeted against nuclear factor kappa B (NF-Kappa B) for the treatment of metastatic breast cancer. The micelles are comprised of three separated functional domains that allow the encapsulation of the hydrophobic cisplatin prodrug in the hydrophobic PDPA core, while the cationic PAGA interlayer facilitates the siRNA complexation and protects siRNA from nuclease degradation and the hydrophilic PEG corona prevents protein absorption. Additionally, the pH-responsive behavior of PDPA core benefits the dissociation of the micelles inside acidic late endosome/lysosome and facilitates

cytosol release of cisplatin prodrug and siRNA. Using these multifunctional siRNA/drug-loaded micelleplexes in 4T1 breast cancer cells and 4T1 orthotopic tumor-bearing mice model, the researchers demonstrated the inhibition of tumor growth and the suppression of distant metastasis by downregulating NF-Kappa B expression. The successful anticancer effect by cooperatively delivery of siRNA and cisplatin prodrug confirmed the potential of this novel platform for the treatment of metastatic breast cancer.

To achieve a better-targeted treatment, Cheng, Cao, Chen, Yu, and Shuai (2012) designed a folate-targeted, multifunctional nanocarrier for simultaneous codelivery of anticancer DOX and siRNA against B-cell lymphoma 2 (BCl-2) gene, into rat C6 glioma cells overexpressing folate-receptors. The nanocarrier was prepared based on a diblock copolymer of linear PEI and poly(ε-caprolactone) (PEI-PCL). Folic acid was first conjugated to a polyanion, poly(ethylene glycol)-b-poly(glutamic acid) (FA-PEG-PGA) and then attached through electrostatic interaction onto the surface of the cationic PEI-PCL micelles preloaded with DOX and siRNA. The researchers assessed the therapeutic efficacy of the simultaneous siRNA/DOX administration from a folate-targeted nanocarrier, performing in vitro and in vivo studies in the C6 rat glioma model. The results demonstrated that the synergistic effect of the therapeutic agents resulted in downregulation of the antiapoptotic BCl-2 gene, inducing glioma C6 cell apoptosis. In addition, this folate-targeted codelivery strategy led to tumor growth inhibition and extension of rat survival time, compared to treatment with nontargeted or single-loaded formulations.

In a recent study, Tang et al. (2019) employed hybrid PMs for the simultaneous delivery of PTX and siRNA against the programmed death ligand 1 (PD-L1), aiming for effective immune-chemotherapy-based treatment of melanoma. PTX was used to induce immunogenic cell death, while siRNA could knock down PD-L1 and therefore block PD-1/PD-L1 interactions in tumors, reducing the proliferation activity of melanoma cells. The mixed micelles consisted of two amphiphilic diblock copolymers, poly(ε-caprolactone)-poly(ethylene glycol) (PCL-PEG) and poly(ε-caprolactone)-poly(ethyleneimine) (PCL-PEI). The prepared micelleplexes efficiently condensed siRNA and exhibited high PTX encapsulation efficiency. Biological studies showed high cellular uptake of the PTX/siRNA hybrid micelles from B16F10 melanoma cells. Furthermore, PTX-induced immunogenic cell death and siRNA managed effectively to knock down PD-L1, as mRNA and protein of PD-L1 levels were reduced both in vitro and in vivo models. Additionally, the synergistic combination of PTX/siRNA micelleplexes inhibited tumor growth in B16F10 melanoma tumor-bearing mice. The results enhanced the therapeutic potential of these hybrid micelles in cancer immunotherapy.

In more recent years, the utilization of micelleplexes as nanotheranostic systems has gathered ever-increasing interest from the scientific community. The outstanding characteristics of micelleplexes, include the ability to codeliver therapeutic agents of dissimilar nature, stabilize and solubilize hydrophobic molecules, prolong retention time in blood circulation and enhance targeted cellular uptake.

Nucleic acid and chemotherapeutic agent codelivery systems may provide significant information on cargo delivery in vivo. Furthermore, imaging agents and other diagnostic components may contribute to monitoring the efficacy of the therapy. Many micellar nanosystems containing diagnostic substances have been developed. Lee and coworkers (2016) utilized poly(2-(dimethylamino)ethyl methacrylate)-b-poly-(ε-caprolactone) (PDMA-b-PCL) cationic micelles as nanocarriers for drug (7-ethyl-10-hydroxycamptothecin, SN-38) and gene (siRNA, which targets human VEGF) delivery for colorectal cancer. Before complexation with the micelles, the VEGF siRNA was conjugated with PEG to improve the stability of siRNA. In order to enhance the in vivo biosafety, they prepared mixed micelles utilizing mPEG-b-PCL and PDMA-b-PCL copolymers. Moreover, they used ultra-small superparamagnetic iron oxide nanoparticles (USPIO), as diagnostic species in order to form SN-38/siVEGF/USPIO-loaded PEGylated PDMA-b-PCL multifunctional hybrid micelleplexes. The hybrid micelleplexes were able to enhance VEGF silencing, suppress the growth of tumors due to the anticancer synergistic effects of chemotherapy and RNAi therapy as well as successfully exhibit magnetic resonance image properties (MRI) during the treatment. Hence, the multifunctional features of micelleplexes render them an efficient nanosystem demonstrating the promising potential for colorectal cancer therapy.

Another multifunctional nanosystem based on triblock copolymer was developed by Endres et al. (2014). for simultaneous delivery of nucleic acids and hydrophobic fluorescent dye. Poly(ethylene glycol)-b-poly(ε-caprolactone)-b-poly (ethyleneimine) (PEG-b-PCL-b-PEI) cationic triblock copolymers having dissimilar hydrophilicity were tested separately as nanocarriers of siRNA and quantum dots (QD). The utilization of PEG-b-PCL-PEI for siRNA delivery was evaluated in vitro and in vivo, concluding that the polymeric nanocarrier displayed good knockdown efficiency. Moreover, the QDs were entrapped in the core of the micelles due to their hydrophobicity. Upon intratracheal instillation, the multifunctional nanosystems were internalized in the pulmonary epithelium and mediated gene silencing. The nanocarriers composed of hydrophobic amphiphiles (PEG500-PCL10000-PEI2500) presented better transfection efficiency than their hydrophilic counterparts (PEG5000-PCL10000-PEI2500), presumably due to their thinner shell. Moreover, entrapment of the hydrophobic QDs into the PEG500-PCL10000-PEI2500 nanocarrier and subsequent complexation with fluorescently labeled AF647-siRNA resulted in the formation of FRET-capable carriers. In this multifunctional system, the hydrophobic payload could be molecules or inorganic nanoparticles with diverse optical and photophysical properties, hydrophobic drugs, or a mixture of them, resulting in nanosystems for codelivery of drugs and nucleic acids and/or for theranostic approaches in nanomedicine.

5.3.3 Micellar systems for protein delivery

The effect of protein theranostics in combating serious life-threatening diseases is gradually increasing due to the progress made in the fields of biology,

biotechnology, and bioapplications and further comprehension of various pathogenesis (Moncalvo, Martinez Espinoza, & Cellesi, 2020). Proteins are essential elements of the human body and consist of smaller constituents, amino acids. Based on the number and order of the amino acids, proteins form a particular three-dimensional configuration that defines their biological activity (Moncalvo et al., 2020). These activities determine almost every biological procedure, such as recognizing external factors that threaten normal body functions. Recent scientific reports state that protein dysfunctions are related to the occurrence of several intimidating illnesses such as Alzheimer's, diabetes, and cancer (Chen & Stenzel, 2018). Therapeutic proteins stand out from the traditional drugs of low molecular weight due to proteins' multiple and complex activities and their high selectivity (Balasubramanian, Onaca, Enea, Hughes, & Palivan, 2010). Therefore, protein drugs possibly will not induce side effects. Due to the production of proteins in the human body, protein drugs are supposed to decrease the activation of immune responses (Liu, Wu, Ji, & Yin, 2019). As a result, protein therapeutics constitute an encouraging methodology for facing life-threatening diseases. Despite these benefits, proteins that are administrated in patients in their original form, without the incorporation of a protective factor, most likely are subjected to biodegradation, low membrane penetrability, and can be easily evicted by the blood circulation (Pachioni-Vasconcelos de et al., 2016). The inner characteristics of proteins that provoke such malfunctions which are responsible for the limited access in clinical practice are their large size, susceptible tertiary structures, surface charge, and instability to extreme pH values. Many efforts have been conducted to overcome the limitations displayed by protein therapeutics. Among all strategies, the design and development of nanocarriers have been proved successful for encapsulation and protection of proteins that would be distributed in targeted body sites in need of treatment (Kafetzi, Pispas, Bao, & Yao, 2020; Moncalvo et al., 2020; Pachioni-Vasconcelos de et al., 2016). Those nanocarriers should fulfill certain requirements to be utilized for protein administration: biocompatibility, biodegradability, lack of cytotoxicity, preparation strategies that do not influence the protein functionality, adequate endurance of the carrier-protein complex to provoke physical and chemical degradation, elevated loading efficacy, and controlled release and finally the ability of the carrier to distribute protein to the targeted body location (Balasubramanian et al., 2010). The design and development of nano delivery systems in order to be implemented in bioapplications should focus on their optimization to stabilize protein therapeutics in order to resist denaturation by enzymatic digestion (Li et al., 2017; Liu et al., 2019). PMs are a class of versatile drug nanocarriers thanks to their ameliorated properties. In distinction with liposomes that conventionally were used as drug delivery devices, block copolymers and their micelles exhibit enhanced properties such as increased stability, adjustable specificity, ameliorated blood circulation stability, and a chemical variety that entitles adjustment of the physical properties for proper drug release (stimulated, slowed, elongated) (Balasubramanian et al., 2010). Moreover, they exhibit biological-like behavior, precise targeting, bioadhesive, and cellular

uptake (Chen & Stenzel, 2018). Furthermore, they offer the possibility to be prepared according to the physicochemical characteristics of the protein and they provide stability to the polymeric micelle/ protein complex (Kamenova et al., 2018). Finally, the enhancement of the biological activity of the protein is one of the fundamental requirements of a polymeric nanocarrier to be utilized in protein delivery systems. Insulin is among the therapeutic proteins that have been most investigated for its distribution in the human body by using PMs as the nanocarrier. Insulin insufficiency is related to the occurrence of diabetes (Luo et al., 2016). This life-threatening disease is caused because either the pancreas cannot produce the necessary amount of insulin or the cells are resistant to it (Xie, Li, & Li, 2017). Therefore, patients suffering from Type-1 diabetes or Type-2 diabetes in later stages are compelled to be injected with several doses of insulin daily to maintain their blood glucose concentration to an ordinary level (Vecchio, Tornali, Bragazzi, & Martini, 2018).

Therefore, investigations based on insulin distribution should be addressed to the advancements of novel nanocarriers with the proper features that will eventually capacitate the conjunction with insulin and its release along with establishing other possible pathways of administration. A thorough study concerning the incorporation of insulin into block copolymer-based nanocarriers was reported by Pippa, Karayianni, Pispas, and Demetzos (2015). This investigation entailed the electrostatic complexation process between insulin and the poly[3,5-bis(dimethylaminomethylene) hydroxystyrene]-b-poly(ethylene oxide) (QNPHOSEO) cationic-neutral block polyelectrolyte. The outcome of this combination was studied in terms of the size and surface charge of nanocarriers in both aqueous and biological media. Circular dichroism and infrared spectroscopy were used to study the structure of the complexed insulin. Finally, Pippa and her collaborators performed cytotoxicity release assays of the encapsulated protein from the polymeric complexes under physiological circumstances. Specifically, they established the dependency of the structure and solution behavior on the ratio between the two components, and the pH and ionic strength of the solution during the complexation process. Moreover, they stated that the charge screening effect and vitiation of the electrostatic interactions, provoked by the increase in the ionic strength of the mixed solution, led to complexes of different structures. The size of complexes dispersed in fetal bovine serum showed temporal and structural stability, indicating that the QNPHOSEO copolymer provides biological stability to the complexes. Moreover, no structural degradation of the insulin was observed upon complexation. An increase in the initial concentration of insulin resulted in elevated encapsulation efficiency. Release studies showed that the release of insulin from the polymeric nanoassemblies was rather slow, especially in the case of a higher initial concentration of the protein. The study offers appreciable insight for constructing formulation strategies to develop nanocarriers for insulin that provide safety, efficiency, and tolerability (Pippa et al., 2015). An important contribution toward the delivery of insulin to targeted human sites was reported by Hu and his coworkers (2017). They designed and formulated a novel smart insulin

administration system, which offers promising results against diabetes. Their development concerns the construction of a glucose-responsive insulin distribution device, which combines the H_2O_2-responsive polymeric nanoparticles with a transdermal microneedle pathway to accomplish accelerated response, great biocompatibility, and ache-less administration. The novel polymeric nanoparticles resulted from the self-assembly of the block copolymer comprised of PEG and phenylboronic ester (PBE)-conjugated polyserine (PEG-b-P(Ser-PBE)) and stacked with glucose oxidase (GOx) and insulin. The experimental results showed that the polymeric nanoparticles operate as both GOx sensors and insulin release controllers to offer primal insulin release and trigger insulin release in response to hyperglycemic conditions. Furthermore, they declared that insulin release answers to quickly increased glucose levels and the release kinetics can be modified by regulating the concentration of GOx encapsulated into the microneedles. The in vivo assays results showed that a single patch suffices to adjust glucose levels efficiently with decreased peril of hypoglycemia occurrence (Hu et al., 2017). Another interesting study concerning the formulation of novel protein delivery nanosystems was reported by Kamenova and her collaborators (2018). This group reported on the synthesis of poly(ethylene oxide)-poly(ε-caprolactone)-b-poly(ethylene oxide) (PEO-b-PCL-b-PEO) and poly(2-(dimethylamino)ethyl methacrylate)-b-poly(ε-caprolactone)-b-poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA-b-PCL-b-PDMAEMA) amphiphilic block copolymers and their coassembly to form novel micellar systems for controlled insulin release. The two copolymers were blended at certain molar ratios to produce mixed block copolymer micelles. Afterward, the authors studied the effect of electrostatic interactions developed between the mixed micelles and insulin in terms of particulates size and morphology, surface charge, and colloidal stability in dependence on insulin concentration. Subsequently, cell viability investigations were conducted in both blank and insulin-entrapping nanocarriers indicating low cytotoxicity of the nanocarrier systems. Finally, the release of insulin from the nanocarriers in a phosphate buffer was also investigated. The results of these studies proved that the formulation of novel mixed micellar nanocarriers of insulin contributed to the accomplishment of colloidal stability and controlled insulin release. The suitable design of the shell, consisting of long PEG chains along with short PDMAEMA chains and the micellar structure that was formed by the self-organization of the block copolymers seem to be essential for the stabilization of the insulin molecules near the micellar core, formed by PCL chains. The stabilization of insulin resulted from its complexation with polycationic chains without disabling the PEO chains' function as stabilizers. The micellar composition seemed to affect the complexation procedure, the physicochemical features, and the cytotoxicity of the resulted micellar/insulin system. An interesting observation was that in the presence of excess PDMAEMA, the blank micelles were more cytotoxic and the insulin-loaded nanocarriers presented the tendency to precipitate within 60 days. Moreover, the nanocarriers prepared at a copolymer molar ratio of 1:1 displayed the most appropriate properties that would include them in the set of insulin nanocarriers (Kamenova et al., 2018).

Similarly to insulin, PIC micelle systems have been used as delivery vehicles for numerous other therapeutic proteins and antibodies as well. Of course, one should acknowledge the pioneer and extremely fruitful work of Kataoka and his collaborators over the years that have been exploring the application of PIC biopharmaceutical nanocarriers since the late-1990s (Lee & Kataoka, 2009). Some characteristic recent examples of their studies include the use of the PEG-poly(L-lysine-carboxy-dimethyl maleic anhydride) (PEG-p(Lys-CDM)) block copolymer for the formation of myoglobin-loaded micelles via concurrent ion complexation and pH-cleavable covalent bonding (Tao et al., 2020). The stability of the loaded micelles in physiological conditions, as well as the dissociation and release of functional myoglobin at pH 6.5, was successfully established, while extended half-life in blood compared to free myoglobin was confirmed. The same group has studied the delivery of various antibodies, as in the case of PIC micelles prepared by charge-converted immunoglobulin G (IgG) derivatives and PEG-poly[*N*-(*N*′-(2-aminoethyl)-2-aminoethyl)aspartamide] (PEG-PAsp(DET)) block copolymers, which facilitated endosomal escape and delivery of charge-restored IgG antibodies into the cytosol (Kim et al., 2016). Most recently, Xie et al. (2020) reported on the formation of a dual pH/redox responsive polymeric nanomicelle comprised of cationic disulfide crosslinked PEG-poly(L-lysine) (PEG-PLL) block copolymers complexed with charge-converted anionic antigen-binding fragment antibodies. This multifunctional nanocarrier system proved capable of specifically and efficiently penetrating the blood-brain barrier thus delivering the bioactive antibodies into the brain, exhibiting therapeutic potential towards inhibiting the aggregation and toxic activity of the amyloid beta peptide, the primary pathogenic mechanism leading to neurodegenerative diseases such as Alzheimer's.

Another group that has dealt among others with the use of PIC micelles for the delivery of bioactive agents in the central nervous system is the group Kabanov. In one case they prepared formulations of the antioxidant enzymes superoxide dismutase and catalase by electrostatically coupling them to PEI-PEG and poly(L-lysine)-PEG (PLL-PEG) block copolymers, followed by covalent crosslinking between polymer and enzyme. In vivo studies in mice demonstrated the increased stability of the formulations in both blood and brain and increased accumulation in brain tissues (Klyachko et al., 2012). A second example is the use of the biocompatible PEG-poly (L-glutamic acid) (PEG-PLE) block copolymer for the delivery of the brain-derived neurotrophic factor (BDNF), as reported by Jiang et al. (2018). It was shown that following intranasal administration, the nanoformulation improved BDNF delivery throughout the brain, also displaying a more preferable regional distribution pattern than the native protein. Furthermore, it resulted in superior neuroprotective effects in the mouse brain with lipopolysaccharides-induced inflammation. Both nanoformulations offer great potential as promising therapeutics for neurological diseases.

Along the same lines, a different group has constructed pH-sensitive PIC-based delivery systems targeted against cerebral ischemia. In particular, Gao et al. (2012) investigated the ability of the degradable block copolymer methoxy PEG-poly (β-amino ester) (PEG-PAE) with piperidine and imidazole rings to encapsulate

proteins, using human serum albumin as a model. The produced protein-loaded polymeric micelle exhibited a pH-tuning charge conversion from neutral to positive when pH decreased from 7.8 to 6.2. Therefore, it was possible to enhance the protein delivery efficiency and simultaneously target pH-stimuli tissue, such as cancerous tissue or ischemia. In vivo, optical imaging studies performed with suitably labeled albumin on rats with acidic pathological brain tissues confirmed the ability of these protein-containing micelles to be used as therapeutic and imaging agents in cerebral ischemia models. In an analogous study Kim, Seo, et al. (2015) used a PEG-poly(urethane amino sulfamethazine) (PEG-PUASM) copolymer for the encapsulation of stromal cell-derived factor-1α (SDF-1α) in a pH-dependent manner. Both in vitro and in vivo results showed that the SDF-1α-loaded pH-sensitive PMs can be used as pH-triggered targeting agents and can effectively modify the microenvironment to increase innate neurorestorative processes.

Other studies involving growth factors as active agents include that of Harada, Ohuchi, Hayashi, and Kato (2011) who were able to improve the pharmacokinetics of granulocyte colony-stimulating factor (G-CSF), an endogenous hematopoietic growth factor, by utilizing PEG-polyglutamate block copolymers as delivery carriers. Electrostatic interaction between G-CSF and the block copolymer resulted in the encapsulation of the protein in the resulting PIC micelles. Obtained results demonstrated a comparable in vivo efficacy of G-CSF-encapsulating micelles with PEGylated G-CSF, suggesting an alternative delivery route. Likewise, Rocker, Lee, Shandas, and Park (2020) to meet the angiogenic and antiinflammatory needs associated with myocardial infraction developed an injectable polymeric delivery system composed of platelet-derived growth factor (PDGF) encapsulating micelle nanoparticles embedded in a sulfonated reverse thermal gel, which also contained (via electrostatic binding) VEGF and the antiinflammatory cytokine interleukin-10. A PEG-poly(serinol hexamethylene urea)-PEG (PEG-PSHU-PEG) copolymer was used for the fabrication of the PDGF-loaded micelles. The ability of the delivery system to induce new blood vessel formation was analyzed in vivo using a subcutaneous injection mouse model and it was found that functional and mature vessel formation was significantly increased while inflammation was reduced.

Equally interesting is the case of the pH/sugar dual responsive core-crosslinked PIC micelles developed for protein intracellular delivery, presented by Ren and coworkers (2013). In this work PEG-b-poly(glutamic acid-*co*-glutamicamidophenylboronic acid) (PEG-b-P(Glu-*co*-GluPBA)) and PEG-b-poly(L-lysine-*co*-ε-3,4-dihydroxyphenylcarboxyl-L-lysine) (PEG-b-P(Lys-*co*-LysCA)) copolymers self-assembled into micelles with a PEG outer shell and a PGlu/PLys PIC core, capable of encapsulating either negatively or positively charged proteins. Respectively, both insulin and cytochrome C (CytC) were used as model proteins for the investigation of the loading and release capacity of the micelles, while intracellular delivery and apoptotic activity in cancer cells were confirmed for CytC. Another multiresponsive protein carrier is that reported by Wang, Wang, Yang, Zhao, and Liu (2014), who employed a well-defined hydrazide containing block copolymer poly(poly(ethylene glycol methacrylate))-b-poly(methylacryloylhydrazide) (P(PEGMA)-b-PMAH) for the bioconjugation of pyridoxal phosphate

(PLP) to the pendant hydrazide groups. The produced PLP-conjugated dynamer formed PIC micelles with lysozyme that demonstrated pH-, salt-, and enzyme-responsive features. Moreover, the enzymatic activity and lytic capacity of the protein against *Micrococcus luteus* cells were confirmed after salt-induced dissociation of the micelles and the release of lysozyme. One last example of PIC micelles for protein delivery is given in the study of Li, Chen, Wang, Stenzel, and Chapman (2020), which investigated the effect of the spacer between the polymer backbone and protein binding group on the stability of the micelles. For this reason, they utilized lysozyme as a model protein and a block copolymer comprising of a poly(ethylene glycol methyl ether acrylate) (PEGMEA) water-soluble block and four carboxylic acid monomers, which differ in the length of the carbon spacer between acid and vinyl group, as the negatively charged block. They concluded that polymers with longer spacers offer significantly higher binding affinity and stability at physiological pH and in 3D breast cancer cell spheroids, while at the same time improving protein release after cell encapsulation.

Copolymers forming other types of interactions except ion complexation have also been investigated as potential nanocarriers for the delivery of therapeutic proteins. A characteristic example is the formulation of protein delivery systems via conjugation of the polymeric nanostructure with the studied protein. Luo et al. (2017) reported on the formulation of a novel nanovaccine, which resulted from the mixture of the polymeric micelle of PEG-b-poly(2-(hexamethyleneimino)ethyl methacrylate) (PEG-b-PC7A) with the model antigen ovalbumin in order to be implemented in cancer immunotherapy. The mixture of the block copolymer with the antigen was received as a result of the chemical conjugation between the block copolymer and the antigen. The antigen/synthetic polymeric nanoparticle stimulated cytotoxic T-cell response with low systemic cytokine expression and accomplished effective cytosolic distribution of the tumor antigens to antigen-expressing cells in arduous lymph nodes, resulting in increased surface activity with simultaneous activation of type I interferon-responsive genes. They stated that the stimulator of interferon genes (STING) influences the previously mentioned effect. Moreover, they observed that the proposed nanovaccine caused influential tumor growth obstruction in melanoma and colon cancer. The coupling of the PC7A nanovaccine with an anti-PD-1 antibody exhibited great cooperation with 100% viability over 60 days in a TC-1 tumor type. Treating tumor-free mice with TC-1 cells resulted in absolute obstruction of tumor growth, indicating the production of long-term antitumor recollection. In summary, Luo et al. formulated a STING-stimulating nanovaccine that produces a safe, easy and solid methodology for enhancement of antitumor immunity in order to treat cancer immunotherapy (Luo et al., 2017).

More complicated polymeric systems have also been investigated as nanocarriers for the entrapment and delivery of proteins. A representative case was reported by Wang and his coworkers (2016). They fabricated mixed shell PMs that consist of two types of different polymeric chains in the shell and hence present the ability to regulate the surface properties of polymeric nanoassemblies. More specifically, they studied the role of thermo-responsive and hydrophilic

polymeric chains in the thermo-denaturation protection of proteins to produce artificial chaperones against denaturation. Even though the thermo-responsive character that these polymers exhibit is responsible for the formation of hydrophobic regions on the surface of the micelle, they can release unfolded protein intermediates due to their respective affinity for proteins. Wang et al. utilized three types of thermo-responsive polymers in order to produce mixed shell polymer micelles with adjustable hydrophilic/hydrophobic properties, while carbonic anhydrase B (CAB) was utilized as a model protein. The main role of the thermo-responsive polymeric chains that compose the shell is to trap the proteins during the temperature increase and obstruct the aggregation of the unfolded proteins. From the three types of thermo-responsive polymers that were investigated, poly (*N*-isopropyl acrylamide) (PNIPAM) in its shrunken condition exhibits the appropriate hydrophobic character for capturing the unfolded CAB intermediates. In the case of poly (*N*-isopropylacrylamide-co-*n*-*tert*-butyl acrylamide) (P(NIPAM-*co*-NTBA)), stronger hydrophobic interactions are developed that may provoke the denaturation of the protein. On the other hand, the lower chemical resemblance between the shrunken state of the poly(2-(2-methoxyethoxy)ethyl methacrylate) ($PMEO_2MA$) with the unfolded intermediates proved to be insufficient to trap the protein. As far as hydrophilic polymers are concerned, PEG is able to contact the partly unfolded proteins and help their refolding through interactions with the melted globule state. Unlikely PEG, poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) is unable to act as a chaperone since it could not help the protein to fold back to its native state. Moreover, they concluded that the suitable ratio of the two types of polymers should be moderated, in order to achieve the hydrophobic/hydrophilic equilibrium that is important for accomplishing the highest chaperone efficacy (Wang et al., 2016).

5.3.4 Micelles for photodynamic therapy and hyperthermia

Hyperthermia is a noninvasive cancer treatment in which body tissue is exposed to high temperatures (40°C−45°C) to kill cancer cells. At elevated temperatures, the cancer cells become more sensitive and respond to therapeutic drugs or radiation in an accelerated fashion. Hence, hyperthermia in combination with chemotherapy or radiation therapy results in better treatment efficacies (Wadajkar et al., 2013).

Magnetic-based and near-infrared (NIR) nanoparticulate systems are not only considered successful imaging agents but also ideal drug carriers, having also the ability to interact with either the magnetic field or the NIR light and release thermal energy. In magnetic hyperthermia, the magnetic nanoparticles (MNPs) generate heat as a result of the absorption of large amounts of magnetic energy by hysteresis loss (Zhao, Tang, & Feng, 2010). In photothermal hyperthermia, the plasmonic nanomaterials can convert the optical radiation into heat (Park et al., 2010).

In recent years, a few studies contemplated the development of magnetically triggered drug delivery systems for cancer therapy via the inclusion of MNPs into block copolymer micelles. For example, Glover et al. (2013) described the

synthesis of magnetic micelles using a poly(ethylene glycol-b-caprolactone) (PEG-b-PCL) block copolymer encapsulating DOX and iron oxide nanoparticles, and verified experimentally the magnetic heating of the nanoparticles When heating the MNPs, the semicrystalline micellar core melts at temperatures just above physiological conditions, indicating their potential to release a chemotherapy agent from a thermo-responsive polymer system. The magnetic heating experiments revealed excellent heating properties of the magnetic micelles reaching hyperthermia conditions after 5 min. However, a time lag was observed during the magnetic measurements, probably due to the heat of fusion of melting of PCL micelle cores before bulk solution temperatures increased. The heating mechanism of DOX-loaded magnetic micelles above the core melting point proved beneficial for drug release after administration, as DOX was released faster at elevated temperatures.

Ferrimagnetic poly(ethylene glycol)-poly(2-hexoxy-2-oxo-1,3,2-dioxaphospholane) (mPEG-b-PHEP) copolymer micelles loaded with iron oxide nanocubes and emodin were developed by Song et al. for enhanced magnetic hyperthermia and chemotherapy (Song et al., 2020). The results indicated an excellent magnetic resonance contrast ability of the ferrimagnetic therapeutic nanocarrier due to the high magnetization. The incorporation of emodin into the PMs led to dozens of times higher absorption rate and intrinsic loss power compared to the clinically available iron oxides agents, such as Feridex and Resovist, indicating the high heating conversion efficiency. The PMs with a fluid core highlighted a rapid response to magnetic hyperthermia, releasing emodin effectively under the application of alternating magnetic fields (AMF). The synergistic effect of hyperthermia and chemotherapy-induced the complete killing of cancer cells at an extremely low dosage of iron oxide nanocrystals and antitumor drugs, diminishing the risk of side effects in cancer treatment.

Recently, Kim, Kim, et al. (2015) designed magnetic-based polymeric nanocarriers for combined hyperthermia and chemotherapy, encapsulating DOX in poly(ethylene glycol)-poly(lactide) (PEG-PLA) micelles conjugated with iron oxide nanoparticles. The heating of DOX-loaded magnetic micelles under AMF elicited an enhanced and faster drug release than without AMF, followed by a change in the mobility of PLA chains. Lastly, in vitro cytotoxicity and hyperthermia experiments showed that the DOX-loaded magnetic micelles effectively killed cancer cells, by the combined effects of heat and anticancer drug.

Hybrid gold/iron oxide loaded thermosensitive micelles based on poly(*N*-isopropylacrylamide-*co*-acrylamide)-b-poly(ε-caprolactone) (P(NIPAAm-*co*-AAm)-b-PCL) amphiphilic copolymer, were developed by D.-H. Kim and coworkers for combined hyperthermia, chemotherapy, and optical imaging (Kim, Rozhkova, Rajh, Bader, & Novosad, 2009). Thermal sensitivity along with the magnetic and optical properties of the synthesized hybrid micelles could trigger an effective drug release and contribute to successful contrast agents-based optical detection/monitoring.

The fabrication of magnetic PMs that combines magnetic hyperthermia and conventional radiotherapy has been scarcely explored in the literature. Very

recently, Nguyen, De Pauw-Gillet, Gauthier, and Sandre (2018) reported the synthesis of water-dispersible magnetic PIC micelles using a double-hydrophilic block copolymer, namely the poly(acrylic acid)-block-poly(2-hydroxyethyl acrylate) (PAA-b-PHEA) embedded with MNPs, which were synthesized with the help of a polystyrene-graft-poly(2-vinylpyridine) (G0PS-g-P2VP) copolymer to control their size and improve their size distribution. The resulting core-shell micelles exhibited good colloidal stability in aqueous environments over a wide pH and ionic strength range. In vitro assays evidenced that the PIC micelles were noncytotoxic up to the highest concentration tested after 48 h of incubation. Cell internalization, presented by confocal laser scanning microscopy and TEM, displayed strong dependence on the incubation time and micelle concentration. Lastly, the dual dose-dependent effect of the synthesized complex micelles on the viability of L929 mouse fibroblasts and U87 human glioblastoma epithelial cells verified the effectiveness of the micelles for cellular radiofrequency magnetic field hyperthermia.

The acquisition of multidrug resistance in theranostics is crucial for the effective magnetic hyperthermia and chemotherapy of tumors. A few studies focus on the development of pH and NIR light-responsive PMs to meet the above-mentioned medical demands by circumventing drug resistance in cancer treatment and inducing hyperthermia damage in cancer cells. For example, Li and coworkers presented an effective approach to reverse drug resistance by synthesizing IR-780 loaded pH-responsive polymeric prodrug micelles with NIR photothermal effect to hamper drug resistance in cancer therapy (Li et al., 2016). The dual-responsive PMPC-b-P(MEMA-hydrazide-DOX) micelles were stable in the physiological environments and exhibited a fast DOX release in acidic conditions, highlighting a temperature increase under NIR laser irradiation. DOX-loaded dual-responsive micelles displayed long-term blood circulation along with a reduced premature drug release, resulting in enhanced DOX and IR-780 accumulation in tumor mass. In vivo assays, showed that both the tumor site-specific chemotherapy and hyperthermia effect contributed to significant inhibition of MCF-7/ADR tumor growth in tumor-bearing mice.

A novel hybrid micelle with pH and NIR light dual-responsive property with hyperthermia-triggered tumor penetration and cytoplasm drug release intended to reverse DOX resistance in breast cancer was reported by Yu et al. (2015). The hybrid micelle prepared from a Pluronic P123 conjugated with DOX and cypate-conjugated poly(ethylene glycol)-b-poly(diisopropylamino ethyl methacrylate) (PEG-b-PDPA) copolymer. The resulting micelles formed compact nanostructure at the physiological conditions with decreased size facilitating blood circulation and passive tumor targeting. At acidic pH, the hybrid micelles rapidly dissociated to release DOX. Additionally, NIR laser irradiation assisted micelles to trigger notable tumor penetration and cytosol release of DOX payload by inducing a tuneable hyperthermia effect. According to the results, the dual-responsive hybrid micelles successfully inhibited the growth of DOX-resistant MCF-7/ADR breast cancer in an orthotopic tumor-bearing mouse model.

Photodynamic therapy refers to a noninvasive cancer treatment that involves the utilization of oxygen, light, and a photosensitizer. When the latter is exposed to light, it generates ROS that lead cells into apoptosis or necrosis. Up until now, photodynamic therapy has been practiced on specific malignancies, but nevertheless, it faces two serious problems with the first being the photosensitizer's lack of solubility and the second being active targeting (Henderson & Dougherty, 1992; Nowis et al., 2005). Thus, many scientists have made great use of stimuli-responsive PMs to address the latter issues. Chen, Wang, and Hung (2016) reports the synthesis of pH and thermo-responsive poly(ε-caprolactone)-b-poly(*N*-isopropyl acrylamide-*co*-*N*-methacryloyl β-alanine) (PCL-b-P(NIPAAm-*co*-βA)) copolymers, along with the coencapsulation of DOX and meso-tetraphenylchlorin (m-TPC) photosensitizer. Self-assembly of the system leads to the formation of micelles with hydrodynamic diameters ranging from 231 to 269 nm, able to carry the coencapsulated substances deep further into tissues, presenting the ability of fast drug release in an acidic environment. In vivo cytotoxicity studies show that the plain block copolymer micelles and the m-TPC loaded ones present excellent cell viability. On the other hand, the m-TPC and DOX loaded system under acidic pH and light conditions prove to be efficient against HeLa cancer cells.

Shi et al. (2016) designed a theranostics polymeric system in order to combine photoacoustic imaging and chemo-photothermal therapy for cancer treatment. In this manner, the group reported the synthesis of PEGylated phenyl isocyanide (-rhodamine)-b-poly(ε-caprolactone) (PPI(-RHB)-PCL) helical amphiphilic block copolymers and the encapsulation of CPT anticancer drug and IR780, a NIR fluorescent dye. Physicochemical characterization showed the formation of spherical micelles of 90 nm diameter, with great stability that was measured for at least 10 days. In vivo studies show excellent cell permeability, transferring both the dye and the anticancer drug straight into the tumors, also enabling biodistribution studies of the PMs. The therapeutic efficiency was also evaluated by in vivo studies, showing synergistic action of the photothermal effect and the anticancer drug release due to the laser light.

Zhong and coworkers have created a pH-responsive PMs system with encapsulated porphyrin, that when inserted in an acidic environment creates a diol that favors the insertion of oxygen in the copolymers core. This leads to the enhanced production of singlet oxygen species after laser irradiation, thus increasing the efficiency of the photothermal treatment by creating the optimal microenvironment for the photosensitizers (Zhong et al., 2019).

Since hypoxia is a characteristic of the interior of cancer tumors, photodynamic therapy cannot operate efficiently. Wang et al. (2018) have reported a fluorinated PMs system that has the ability to transfer oxygen into the core of tumors. In this manner, the group has synthesized poly(perfluorocarbon)-poly(ethyleneimine) copolymer, with the chlorin e6 (Ce6) photosensitizer that leads to the formation of 122 nm diameter PMs, with a rather good size polydispersity (PDI = 0.1). In vivo and in vitro studies showed success in transferring oxygen, while laser irradiation increased the ROS content in the core of the tumors, resulting in high cytotoxicity for cancer cells.

5.4 Micellar systems for bioimaging

A recent development in the broader field of medicine is bioimaging which utilizes digital technology to display structural or functional images of living objects in real-time. Bioimaging uses electromagnetic and ultrasound waves as sources of imaging. Many imaging modalities include MRI, optical imaging, and others. These imaging techniques have already been used successfully in medical applications. More specifically, bioimaging can be utilized to create visual representations of the whole body, as well as the function of some organs or tissues (He et al., 2014). This can be done by using bioimaging probes which may be applied and modify the contrast signal targeting specific cells. Nowadays most bioimaging probes that find applications in clinical trials are low molecular weight molecules (Mandal, Darragh, Wang, & Heyes, 2013; Palmal & Jana, 2014). These bioimaging probes have a wide range of chemical components for imaging techniques, such as MRI and optical imaging. However, many times the low molecular weight of these molecules, as well as the inability to modify them, result in the instability of these molecules in in vivo environments and thus increase their toxicity in some cases. The science of polymers successfully paired with the science of imaging enabled the creation of bioimaging probes based on polymers. For this reason, many innovative studies led to the development of numerous biocompatible/biodegradable polymers with well-defined structures and different architectures (e.g., block copolymers, stars, branched polymers, etc.) (Lee et al., 2012; Li et al., 2011; Zhu, Qiu, Zhu, & Zhu, 2013). In fact, amphiphilic block copolymers have the ability to self-organize into polymeric core-shell micelles at concentrations above the CMC and are thus able to trap imaging agents or hydrophobic drugs in their micellar core (Jelonek, Li, Wu, Kasperczyk, & Marcinkowski, 2015). Due to their small size, block copolymers micelles can be potential candidates as nano-carries for the purpose of diagnosing tumors in diseases such as cancer (Movassaghian et al., 2015). This rapid development of polymers with well-defined chemical functionalities and compositions has allowed the creation of innovative bioimaging probes for optical imaging and MRI which could not have been created by traditional concepts based on low molecular weight molecules.

5.4.1 Magnetic resonance imaging

MRI has the virtues of a noninvasive in vivo medical imaging technique endowed with the high spatial and temporal resolution, good penetration depth, and exceptional tissue contrast. The basis of the MRI signal relies on the interaction of the nuclear spin of the hydrogen nucleus in water with an external magnetic field. The injection of contrast agents during MRI examination facilitates the diagnosis of fatal diseases, such as cancer and cardiovascular diseases. The imaging agents, enhance the contrast between normal and pathological tissue by shortening the relaxation times (T1 and T2) of water.

MNPs can serve as negative contrast agents and are classified according to their effects on the relaxation rate of water protons in tissues (longitudinal relaxation time, R1 = 1/T1, and transverse relaxation time, R2 = 1/T2). MNPs are composed of ferromagnetic elements such as iron (magnetite Fe_3O_4 or maghemite Fe_2O_3), cobalt (Co), nickel (Ni), dysprosium (Dy), gadolinium (Gd), or their oxides and alloys like gold (Au), zinc (Zn), manganese (Mn), and platinum (Pt) based ones. The most commonly used MNPs are the paramagnetic (Gd^{3+}/Dy^{3+}/Mn^{2+} ion complexes) and the superparamagnetic iron oxide magnetite particles (Janib, Moses, & MacKay, 2010; Pellico, Ellis, & Davis, 2019; Xiao et al., 2016).

A broad range of approaches has been applied for the incorporation of superparamagnetic iron oxide nanoparticles (SPIONS) into multifunctional theranostic micellar drug delivery systems. In a study by Hu, Qian, Wang, Liu, and Liu (2012), hybrid micelles of amphiphilic block copolymers (AmBCs), namely poly (ε-caprolactone)-b-poly(glycerol monomethacrylate) (PCL-b-PGMA) and poly (ε-caprolactone)-b-poly[(oligo(ethylene glycol) monomethyl ether methacrylate)-*co*-folic acid] (PCL-b-P(OEGMA-*co*-FA)), encapsulating PTX within the micellar cores and SPIONs within the hydrophilic coronas were successfully fabricated. Drug-loaded and SPIONs-embedded hybrid micelles exhibited a controlled and sustained release of PTX (ca. 61%) from hydrophobic micellar cores over about 130 h. In vitro assays revealed that the clustering of SPIONs within micellar coronas led to enhanced T2-type MrI contrast. In addition, the preliminary experiments proved that the hybrid micelles are suitable contrast agents for small animal MRI scanning.

Lately, Upponi et al. (2018) described the formulation of a theranostic agent based on poly(ethylene glycol)-phosphatidylethanolamine micelles (PEG-PE) composed of SPIONs and PTX for simultaneous cancer detection and therapy. The coloaded PTX and SPIONs did not affect each other's functional properties in vitro. Histological studies revealed a good localization of SPIONs along with increased apoptosis in the tumor tissue, rendering the coloaded theranostic micelles as excellent T2-weighted contrast agents.

Very recently, Xiao and coworkers (2020) developed a pH-responsive micelle based on a biodegradable poly(amino acid) block copolymer, namely the methoxy-poly(ethylene glycol)-poly(aspartic acid)-[2-(diisopropylamino)ethylamine]-*co*-poly(L-leucine) [(mPEG-PAsp-(DIP)-*co*-PLLeu), abbreviated as PDPL], coencapsulating chemotherapeutic PTX and SPIONS for the diagnosis and treatment of hepatocellular carcinoma (Fig. 5.5). SPIONs were successfully encapsulated within the micellar core ameliorating their MRI sensitivity. In addition, the SPIONs/PTX-PDPL micelle effectively delivered PTX and SPIONs to liver cancer cells and rapidly released PTX in the acidic lysosome (DIP unit) leading to cell apoptosis. Likewise, in vitro MRI evidenced that the SPIONs/PTX-PDPL traced the liver cancer cells with high sensitivity.

A new class of magnetite/doxorubicin (Fe_3O_4/DOX) coloaded PEGylated organosilica micelles utilizing poly(ε-caprolactone)-b-poly(glutamic acid) (PCL-b-PGA) polymers was recently reported by Yang and his coworkers (2019) for

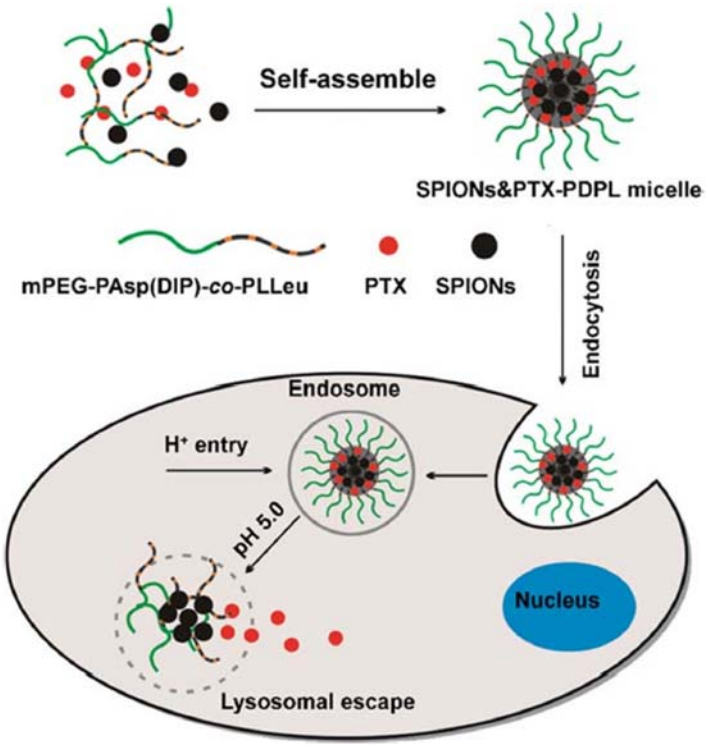

FIGURE 5.5

Schematic illustration of the preparation of SPIONs/PTX-PDPL micelles and their intracellular fate in Bel-7402 cancer cells.

Reprinted from Xiao, H., Li, X., Zheng, C., Liu, Q., Sun, C., Huang, J., et al. (2020). Intracellular pH-responsive polymeric micelle for simultaneous chemotherapy and MR imaging of hepatocellular carcinoma. Journal of Nanoparticle Research, *22(5), 105 with permission from Springer Nature.*

utilization in MRI and tumor chemotherapy (Fig. 5.6). In vitro studies indicated a unique responsive release behavior and selective cytotoxicity against tumor cells of the resultant theranostic micelles in parallel with remarkable T2-weighted MrI capability and high stability against dilution. Thanks to the strong magnetic targeting ability, under an external magnetic field, the in vivo studies revealed an enhanced antitumor efficacy of the micelle-based nanosystem accompanied by an improved Mr tumor capability.

A novel MRI-visible and pH-sensitive micellar nanodrug were constructed by Li et al. (2019). based on the labeled AmBC poly(ethylene glycol)-b-poly(β-benzyl L-aspartate) (mPEG-PBLA), coencapsulating DOX and SPIONS for hepatoma treatment. In vivo fluorescence and Mr bimodal imaging showed that the suitable size and weak positive surface charge of the pH-sensitive nanodrug

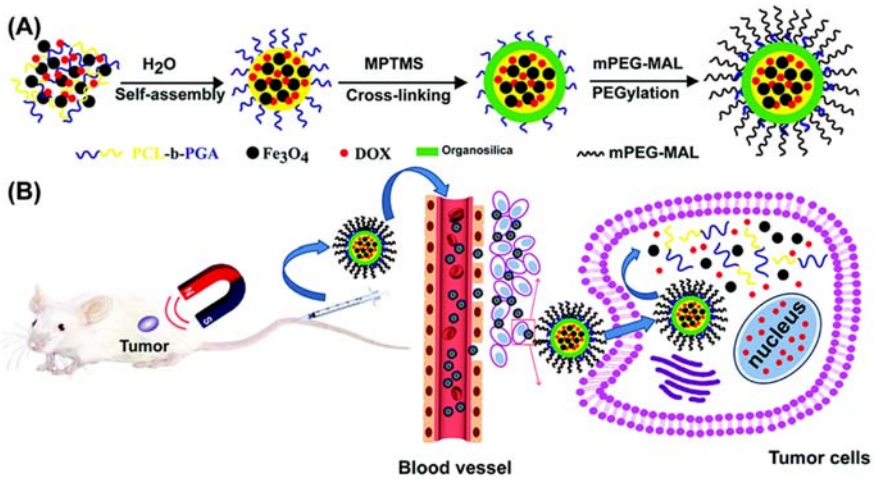

FIGURE 5.6

(A) Synthetic illustration of Fe_3O_4/DOX coloaded PEGylated organosilica micelles and (B) Schematic processes for magnetically targeted MRI and tumor chemotherapy of the SMMC-7721 tumor-bearing nude mice injected intravenously with Fe_3O_4/DOX coloaded PEGylated organosilica micelles.

Reproduced from Yang, T., Niu, D., Chen, J., He, J., Yang, S., Jia, X., et al. (2019). Biodegradable organosilica magnetic micelles for magnetically targeted MRI and GSH-triggered tumor chemotherapy. Biomaterials Science, 7*(7), 2951–2960 with permission from The Royal Society of Chemistry.*

favored its long-term blood circulation and the uptake by cancer cells. Ultimately, the effective tumor-targeting delivery along with the rapid release of intracellular DOX triggered by acidic pH, led to the remarkable anticancer activity of the micellar nano drug accompanied by low cytotoxicity.

Based on the seminal work by Xiao et al. (2019), a cyclic arginine-glycine-aspartic acid(cRGD)-poly(lactic-*co*-glycolic acid-SPIONs-DOX) (cRGD-PLGA-SPIONs-DOX) smart nanoparticle delivery system was developed including the cRGD-mediated targeting effect and SPIONs guided imaging for real-time monitoring of targeted delivery of DOX and chemotherapy in vivo. The synthesized micelles exhibited exceptional pH-responsive release properties and integrin-targeting capability. Furthermore, the in vitro and in vivo antitumor assays revealed an excellent therapeutic efficacy of cRGD-PLGA-SPIONs-DOX micelles, as well as their low toxicity. Finally, the fabricated micelles displayed prolonged plasma drug retention and a preferable pharmacodynamic profile.

A broad number of dual-modality SPIONs systems have been developed. Bifunctional nanoprobe based on a poly(ethylene glycol) methyl ether-poly(ε-caprolactone)-PEI triblock copolymer labeled with fluorescein isothiocyanate (FITC) (abbreviated as MPEG-PCL-PEI-FITC or PCIF) and loaded with SPIONs and DOX was prepared by Guo, Kuang, Cao, Li, and Wei (2015), to provide tumor therapy

and simultaneous diagnostic information via MRI and optical imaging. TEM analysis confirmed the successful loading of SPIONS into the micelles. Notably, studies on cellular uptake of the multifunctional nanoprobes evidenced that SPIONPs, DOX, and FITC labeled MPEG-PCL-PEI were simultaneously uptaken by the breast cancer cells. In vivo assays certified the simultaneous uptake of both payloads by tumoral cells with the delivery of the PCIF/SPIO/DOX micelles, resulting in efficient diagnosis and therapy of the tumors.

Qi et al. (2014) presented the synthesis of a water-dispersible bifunctional nanoprobe for MRI and fluorescence imaging in liver tumors by incorporating SPIONs into the hydrophobic core of poly(ethylene glycol)-poly(ε-caprolactone) (PEG-b-PCL) PMs, with transferrin (Tf) and NIR fluorescence molecule (Cy5.5) conjugated onto their surface. The well-tolerated multifunctional and biocompatible nanoprobes demonstrated propitious properties according to the in vitro and in vivo experiments. The magnetic/fluorescence PMs had the potential for preoperation assessment by MRI and operation navigation of tumors in the clinic.

Dual targeting micelles were produced by Gong et al. (2014) for breast tumor detection by exploiting the PEG-b-PCL copolymers as a template for the encapsulation of hydrophobic SPIONs, wherein cRGD peptide and single-chain fragment variable-EGFR (scFv-ErbB) antibody were embedded into the PEG shell of the micelle. The dual ligand targeting magnetic micelles manifested enhanced delivery efficacy to the breast cells in vivo and high MRI T2 sensitivity.

Hong, Zhou, and Yuan (2012) conducted a study where ultrasmall superparamagnetic iron oxide nanoparticles (USPIONs) were loaded into PEG-b-PCL micelles bearing folate in the distal ends of PEG chains to enhance the diagnostic efficiency and reduce toxicity. The USPIONs loaded micelles presented high MRI T2 sensitivity and long-term blood circulation largely determined by the small particle size. Moreover, folate functionalization enabled a significant raise in the cellular uptake of USPIONs-loaded micelles. In vivo assays, revealed the efficient accumulation of USPIONs loaded micelles in the tumor tissue. Particularly, a signal intensity decrease of up to 41% was detected in the T2-weighted Mr images of the tumor, 3 h after the intravenous injection into mice bearing subcutaneous xenografts of human BEL-7402 hepatoma.

Many scientific reports focus on developing micellar nanocomposites based on paramagnetic ions, such as Gd^{3+} and Mn^{2+} T1 contrast agents (Cao, Xu, Kuang, Xiong, & Pei, 2017). Shiraishi, Kawano, Maitani, and Yokoyama (2010) reported on the incorporation of Gd chelates into PEG-b-poly(L-lysine) (PEG-P(Lys)) block copolymer decorated with a chelate group, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA). The obtained diblock showed stable gadolinium chelation. Also, the PEG-P(Lys-DOTA-Gd) micelles exhibited greater T1 relaxivity time than the diethylenetriaminepentaacetic acid-gadolinium (Gd-DTPA) which is a low-molecular-weight gadolinium-chelate. Additionally, the fully DOTA-attached PEG-P (Lys-DOTA-Gd) micelles presented a 40% decrease in R1 compared to the partially DOTA-attached PEG-P(Lys-DOTA-Gd), leading to significant alterations in the accessibility of water molecules to Gd ions in the micellar core. The in vitro and

in vivo assays indicated that the PIC micelles accumulated in tumor tissues prolonged their retention time in the bloodstream, diminished toxicity, and exhibited significant enhancement of T1-weighted image in the tumor area.

A study by Sun and coworkers presented the synthesis of a biodegradable nanoprobe based on PCL-PEG micelles with T1 contrast agent DTPA-Gd^{3+} and folic acid as a tumor-targeting group on the shell, as well as a NIR dye namely 1,1-dioctadecyl-3,3,3,3-tetramethyl in dotricarbocyanine iodide (DiR) in the core for MRI and optical dual-modality imaging (Sun, Xu, Tang, Sui, & Shen, 2011). In vitro testing of PCL-b-PEGDTPA-Gd^{3+} micelle showed a threefold increase of the relaxation rate R1 compared to that of DTPA-Gd^{3+}. The strong fluorescence observed in vivo at the tumor site certified the preferential accumulation of the dual-modality micelles at the solid tumor with the mediation of folic acid and the EPR effect.

Xu et al. (2020) managed to fabricate a bimodal imaging agent using organosilica crosslinked Pluronic F127 micelles conjugated with Gd(III)-based metallosurfactant and fluorescent nanoparticles. The crosslinked micelles presented strong fluorescence intensity with great stability (20 days) and extremely high relaxivities, exhibiting also low cytotoxicity for both in vitro and in vivo imaging. Likewise, in vivo MRI testing, presented long-term blood circulation and targeted accumulation of the imaging agents in the tumor region.

Very recently, Zhou, Ling, and Li (2020) designed a star-shaped supramolecular copolymer based on inclusion complexes between the host polymer polycaprolactone-β-cyclodextrin (4sPCL-CD) and the guest polymer adamantylamine-poly-L-lactic-b-poly(N-isopropyl acrylamide-*co*-2-hydroxyethylmethacrylate diethylenetriamine penta acetic acid) (AD-PLLA-b-P(NIPAM-*co*-HEMA-DTPA)) loaded with Gd^{3+} ion and DOX for diagnosis and treatment. The Gd^{3+} ions endowed the spherical micelles with high T1 relaxivities. However, due to the noncovalent connection, the spherical PMs could disassemble to the host and guest polymer by adding 2-adamantylamine hydrochloride (AD-NH_2). Thus, the reported Gd^{3+} MRI agent could maintain a large molecular size to stay in vivo but also reduce its size rapidly and metabolize quickly from the body by disassembling. Moreover, the polymeric nanocarriers exhibited drug burst and controlled release mechanisms, due to their disassembly and thermal-response of the PNIPAM segment.

Extensive research has also been carried out on Mn^{2+} T1 contrast agents that exhibited reduced toxicity compared to Gd^{3+} based ones but suffer from relatively low R1 relaxivities (Li, Wu, Hou, Zhang, & Xu, 2018). A notable example is reported by Lu and colleagues where the mPEG-b-PCL micelles were used as a platform to encapsulate manganese doped SPIONs (Mn-SPIONs) and form ultrasensitive MRI contrast agents for liver imaging (Lu et al., 2009). TEM analysis revealed that the Mn-SPIONs were self-assembled into small clusters inside the micelles. Furthermore, the clustered nanocomposites presented very high T2 relaxivities compared to single Mn-SPION. In vivo assay showed decreased T2-weighted signal intensity in mouse liver, 5 min after the intravenous administration.

The concept of star-block copolymer micellar nanocomposites based on 4sPCL-b-P(MEO2MA-*co*-OEGMA) containing hydrophobic $Mn_{0.6}Zn_{0.4}Fe_2O_4$

(MZF) nanocrystals was proposed by Leng, Li, Ren, Deng, and Lin (2015). The MZF nanocrystals are self-assembled into small clusters inside the copolymer micelles. The superparamagnetic MZF micelles proved ideal candidates for MRI contrast agents in tumor theranostic applications presenting high R2 relaxivity value along with low cytotoxicity in the human hepatoma cell line.

The high biocompatibility and versatile properties of gold nanoparticles (AuNP) have rendered them ideal for many biomedical fields including MRI (Cole, Ross, Tilley, Vargo-Gogola, & Roeder, 2015). Recently, Zhang et al. (2018) conceived the design of multifunctional hybrid block copolymers based on the temperature-stimuli and oxidation-stress responsiveness of the poly(N-isopropylacrylamide) containing poly(2-methacryloyloxyethyl ferrocenecarboxylate)-b-poly(N-isopropylacrylamide) (PMAEFc-b-PNIPAM) AmBC bearing gold nanoparticles (AuNPs) to serve as potential MRI and optical imaging agents.

Gold-coated iron oxide nanoparticles have led to the renaissance of theranostic nanoparticles with a multitude of imaging modalities including MRI and optical imaging (Nassireslami & Ajdarzade, 2018). Recently, multicomponent block copolymer nanohybrids developed by Xu and coworkers consisting of Fe_3O_4 nanoparticles, poly(acryloyloxyethyloxyl 1-mercaptohemisuccinate), and poly (methacrylic acid) (PMAA) blocks, followed by subsequent complexation of AuNPs (Xu, Cui, Xu, & Luo, 2017). The highly stable and superparamagnetic nanomicelles exhibited pH, electrochemical and magnetic responsiveness, while the presence of AuNPs ameliorated the reversibility of the electrochemical response and plasmon resonance.

Co-doping is a crucial strategy that can be used for effectively tuning the magnetic and optical properties of various theranostic systems. Promising bioprobes for both in vitro and in vivo studies based on a commercial poly(ethylene oxide)-b-poly(methacrylic acid) (PEO_{68}-b-$PMAA_8$) AmBC embedded with Co-doped iron oxide nanoparticles were developed by Bronstein et al. (2008). The magnetometry results revealed that the hybrid micelles are superparamagnetic exhibiting very high saturation magnetization with remarkable solubility and stability in water.

Extensive applications of Ni-based nanoparticles in biological systems are also reported. For example, Bala et al. (2009) presented a facile method to decrease the size of water dispersible Ni nanoparticles by the incorporation of Pluronic triblock copolymer and oleic acid, extending blood circulation of the imaging agent and probably allowing penetration of tissues. The magnetic experiments certified that the Ni nanoparticles are superparamagnetic and could possibly be exploited in therapeutic heating or for directing these particles to definite tissues with the help of an external magnetic field.

Amphiphilic block copolymers poly(ε-caprolactone)-b-poly(propargyl methacrylate-click-mercaptosuccinic acid-*co*-poly(ethylene glycol) methyl ether methacrylate) (PCL-b-P(PMA-click-MSA-*co*-PEGMA)) loaded with SPIONs and Pt were designed by Huang and coworkers for diagnosis and treatment of bladder cancer (Huang, Neoh, Xu, Kang, & Chiong, 2012). The magnetic experiments disclosed the characteristic

superparamagnetic behavior of the SPIONs-loaded, Pt-conjugated polymeric nanoparticles at room temperature. The release profile of Pt from SPIONs-loaded, Pt-conjugated polymeric nanoparticles showed a burst release of Pt (30%) in the first 4 h followed by a slow and sustained release over 4 days, which can be enhanced by an increase in temperature. Furthermore, the conjugated nanoparticles elicited cytotoxicity against UMUC3 bladder cancer cells via a combination of drug release into the medium and further release of the drug inside the cells after endocytosis.

5.4.2 Optical imaging

Optical imaging has been developing rapidly for the last decade, since it can be used as is, or as a complementary diagnostics technique, to provide a more optimized result. Since traditional fluorophores are extremely hydrophobic and suffer from light scattering and diffusion phenomena, researchers turned to polymeric micelle encapsulation. Botz et al. (2015) have created poly(ethylene glycol-15-hydroxystearate) micelles with encapsulated hydrophobic pentamethine cyanine fluorescent dye IR-676 (1,1′,3,3,3′,3′-hexamethyl-4,5,4′,5′-dibenzoindodi-carbocyanine)-iodide that can detect vascular leakage in the paw, joint, ear, and lung of a rat. These low-cost, reproducible, and easy-to-manufacture nanoparticles have proven to be very effective for in vivo and ex vivo optical imaging, providing great fluorescence sensitivity to superficial and deep-tissue inflammation points of interest for more than 3 h while preserving cells viability, since they do not cause any toxicity.

Pan and coworkers reported the encapsulation of a water-soluble NIR dye (ADS832WS) and a hydrophobic antiangiogenic drug (fumagillin) into a simple system composed of poly(styrene)-b-poly(acrylic acid) copolymers stabilized with polyoxyethylene (Fan et al., 2016) sorbitan monooleate. The self-assembled nanoparticles were small and narrow-dispersed mixed micelles, under 20 nm in diameter, that traveled immediately at the lymph node when administered to laboratory rats, providing a very high signal for the photoacoustic imaging technique. The specific micellar system provides a quick, versatile, straightforward, and error-free way to visualize certain pathogenies, eliminating the need for the use of perioperative procedures (Pan et al., 2012).

Cho and colleagues have reported a promising two-step approach for the so-called neoadjuvant chemotherapy (NACT), which can lead to both apoptosis, targeted optical imaging, and intraoperative surgical guidance. The group initially proceeded with the conjugation of poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-b-PCL) copolymers with apoptosis targeting peptide (GFNFRLKAGAKIRFGS) and continued with the encapsulation of the hydrophobic NIR fluorescence imaging probe DiR (1,1′-dioctadecyltetramethyl indotricarbocyanine iodide). Self-assembly properties of the system led to the formation of 80 nm in diameter micelles of rather narrow polydispersity (PDI $\approx$ 0.1), while intravenous injection showed slow renal clearance and no serious uptake of the nanoparticles by the liver. The system presented active targeting towards tumors and a strong light absorbance in the NIR spectrum. Cho and

his partners successfully administered the aforementioned copolymers along with PTX, cyclopamine (CYP), and gossypol (GSP) incorporated PEG-b-PCL copolymer micelles in tumor tissues to combine optical imaging and NACT of the ovarian cancer cells (Cho et al., 2014).

Shi et al. (2015) have reported a way to label polymeric nanoparticles with fluorophores, in order to conduct biodistribution studies. In this manner, they address the synthesis of methoxy poly(ethylene glyc l)-b-(*N*-(2-hydroxyethyl) methacrylamide)-monolactate)-*co*-(ANHS ester)) with a crosslinked core, conjugated with fluorophores Dy-490 and Dy-676 for multimodal in vivo and ex vivo optical imaging. The system self-assembled in micelles of 100 nm diameter with a rather narrow size polydispersity. The group administered the micelle nanoparticles through intravenous injection into mice with CT36 tumors and both in vivo and ex vivo optical imaging studies showed accumulation in the liver, kidneys, and tumor site, with no apparent cytotoxicity.

The fabrication of functional gold-loaded block copolymer micelles based on poly(styrene-b-acrylate sodium) (S394-b-A58) and poly(ethylene oxide-b-acrylate sodium) (E136-b-A28) with intense phosphorescence emissions was reported by Guo et al. (2018). The UV-Vis results indicated a phosphorescence enhancement derived from the gold-containing spherical micelles in solution, rendering the phosphorescent micelle an effective bioprobe for cellular imaging.

Although optical imaging has been very promising, it finds limited application in the diagnostics field, mostly due to a lack of high spatial resolution (Cho et al., 2012; Vahrmeijer, Hutteman, van der Vorst, van de Velde, & Frangioni, 2013). Thus, many researchers have synthesized and studied PMs with both fluorescent probes and MNPs, in order to combine optical imaging with MRI and get complementary diagnostic results. For example, Xia, Yang, Duan, Gao, and Zhang (2016) presented a new potential probe for both MRI and optical imaging by synthesizing pH-responsive PEG-polyurethane polymers, conjugated with the fluorescent dye fluorescein isothiocyanate (FITC) and subsequent encapsulation of Fe_3O_4 nanoparticles. The self-assembly in an aqueous solution led to micellar formations of 167 nm with rather a narrow size polydispersity and an emission at 518 nm wavelength. In vitro cytotoxicity studies showed excellent cell viability that exceeded 90% for at least 48 h.

Yan et al. (2014) also reported the synthesis of fluorescent PMs with MNPs for MRI and optical imaging. The group synthesized poly(2,2,3,4,4,4-hexafluorobutyl methacrylate-*co*-9-(4-vinylbenzyl)-9H-carbazole)-*g*-poly(ethylene glycol) (P(HFMA-*co*-VB)-*g*-PEG) and encapsulated Fe_3O_4 nanoparticles. The system self-assembled in micelles of 146 nm hydrodynamic diameter, with a rather narrow polydispersity and great stability, while in vivo studies showed exceptional cell viability, over 90% for concentrations of 800 μg/mL. The system also presented blue fluorescence for optical imaging and excellent paramagnetic properties and T2 relaxivity.

Finally, Q. Zhou and coworkers presented a potential application regarding PMs for MRI and optical imaging of glioma in an effort to provide better

demarcation. Since this kind of cancer is extremely aggressive, surgical removal is essential, but precise boundaries must be set in order to deliver higher survival odds and better life quality. The group initially reported the synthesis of poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-b-PCL) modified with a NIR fluorescent probe (Cy5.5) conjugated with a glioma-targeting ligand, lactoferrin (Lf), and proceeded with the encapsulation of SPIONs. Self-assembly led to the formation of micelles with a hydrodynamic diameter size of approximately 390 nm, while cytotoxicity studies showed excellent cell viability over 90%, even at larger concentrations of 100 μg/mL. The system successfully targeted glioma cells, due to Lf, while the encapsulation of SPIONs increased their T2 relaxivity and protected the optical properties of the fluorescent probe at the same time (Zhou et al., 2015).

5.5 Conclusions

A large gamut of functional nanosystems based on PMs has been developed so far aiming at advancing available tools for effective disease treatment and diagnostics. This is due to the ingenuity, resourcefulness, and careful design of researchers with different backgrounds and expertise working together in this multidisciplinary field of research. Advances made in polymer chemistry, physics, materials and colloid science, and biology and medicine, mainly through the elucidation of biochemical and biological paths, assays, and processes have pushed forward research and innovation on theranostics. The crucial next step is the successful translation of all this fascinating research into efficient and viable market products, taking advantage of the collaboration with the pharmaceutical industry and medicine doctors, under the shield of regulatory authorities and appropriate legislation. Undoubtfully, many interesting and valuable contributions and advances are foreseen in the future, with the ultimate goal being the wellbeing of citizens and prosperity of the global human community.

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CHAPTER

Dendrimers for theranostic applications

6

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6.1 Introduction

Dendrimers are polymers with a globular structure with a core from which a hyperbranched dendritic skeleton that makes up the interior of the dendrimer. The surface displays multiple copies of chemical moieties creating multivalency useful for further functionalization. The hyperbranched inner structure creates voids that can be used for the encapsulation of molecules or metal nanoparticles. This encapsulation may or may not be assisted by bonding or coordination to atoms or groups within the dendrimer. Indeed, there is a very broad range of dendrimers described in the literature (Newkome & Shreiner, 2008, 2010; Tomalia, Christensen, & Boas, 2012) and the potential applications of these polymers have been the subject of many investigations, especially in the field of biological applications and within medicine (Dias, Santos, & da Silva, 2020; Lo, Kumar, Hsieh, & Sun, 2013; Lombardo, Kiselev, & Caccamo, 2019; Xiao, Li, Shi, & Shen, 2020). One of the most studied dendrimers is the poly (aminoamide) (PAMAM) dendrimer and as an example of the dendrimer and dendron structure, a PAMAM dendrimer and dendron is presented in Figs. 6.1 and 6.2.

Depending on the branch cell unit, the dendrimers can have internal cavities that can host small molecules. The PAMAM-dendrimers are an example of a family of dendrimers having internal cavities, while for example, the lysine-based dendrimers do not have cavities. The relation between dendrimer structure and the internal cavities has been investigated in a family of Poly(alkyl aryl ether) dendrimers by the groups of Natarajan, Gupta, Ramamurthy, and Jayaraman (2011) and it is an interesting design parameter to consider in dendrimer synthesis.

With the ability to incorporate a broad spectrum of functionalities both covalently and non-covalently, the broad spectrum of dendrimers that have been well described synthetically and characterized to show a low polydispersity tested in vitro and in vivo with a vast number of functionalities and showed promising applications in pharmaceuticals, dendrimers are logic candidates for polymeric platforms that will require a high degree of control regarding not only the polymer structure but also a high degree regarding functionalities and complexations and the stoichiometry thereof. In theranostics, the incorporation of multiple copies

Advanced Nanoformulations. DOI: https://doi.org/10.1016/B978-0-323-85785-7.00008-5

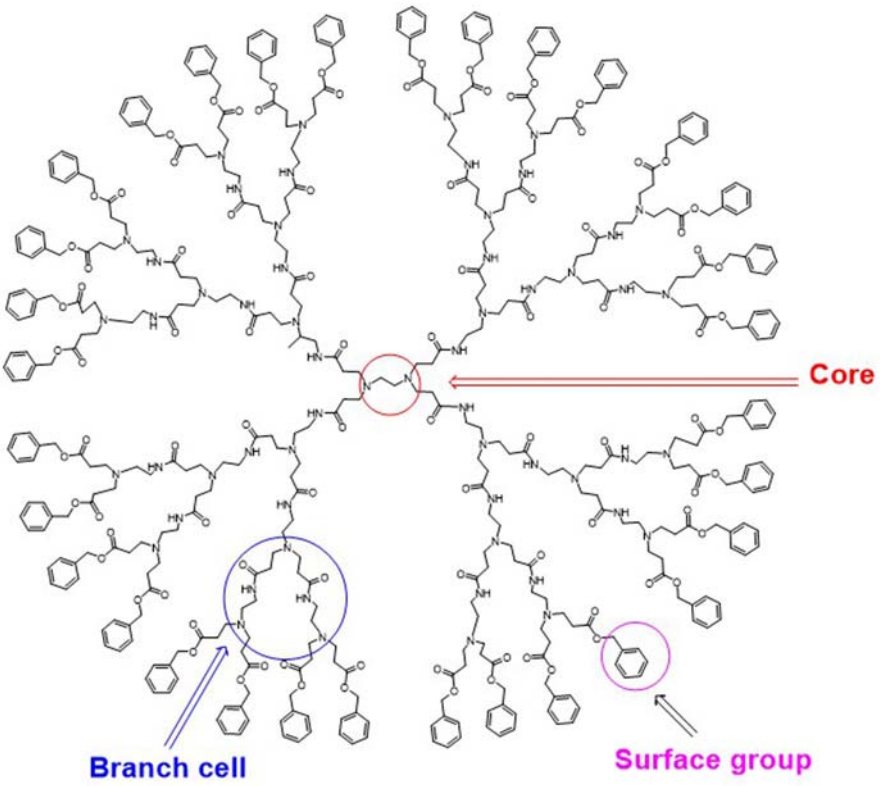

FIGURE 6.1

A generation 4 DAB-core PAMAM-dendrimer with the core marked in blue, the branch cell unit marked in red, and the surface groups marked in cyclamen.

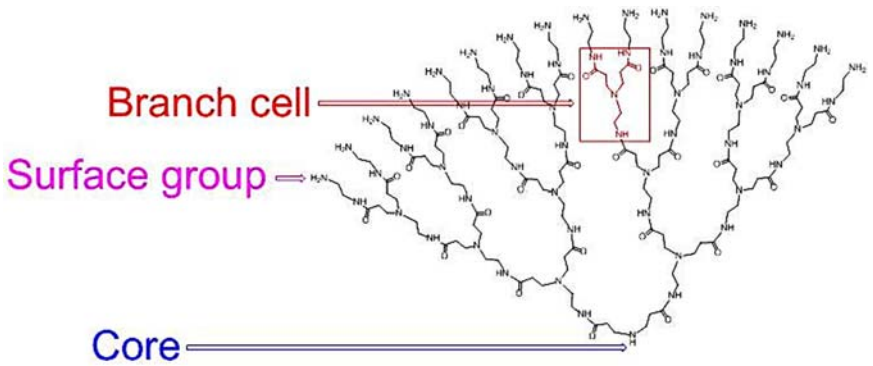

FIGURE 6.2

A PAMAM-dendron, where the focal point is marked in blue, the branch cell in red, and the surface groups in cyclamen.

of functionalities is often desired alongside the incorporation of multiple copies of different functionalities, may these be covalently or non-covalently associated with the dendrimer platform or they can be incorporated into dendrons, which can either be capable of self-assembly or can be linked to the surface of another nanoparticle forming a bigger construct.

The different approaches come both with advantages and disadvantages: Covalent binding to a dendrimer offers in principle a unique degree of control over the number and position of the conjugated groups, but that has not been realized yet, and the examples reported so far are all based on a statistical modification of the surface groups.

Non-covalent binding to a dendrimer offers a better degree of control over the number of ligands on the dendrimers, but achieving sufficiently strong binding of the non-covalent ligands to be stable in a biological environment can be an issue.

Self-assembling dendrons share many of the properties of liposomes, micelles, and similar dynamic self-assembled systems.

Dendrons linked to a preformed nanoparticle offer make it possible to solubilize and at the same time multivalently functionalize nanoparticles such as gold, iron oxide, and silica to mention a few, and also an easy way to access larger nanoparticle systems.

An overview of some of the different dendrimer-based systems is collected in Table 6.1.

Table 6.1 Different types of dendrimers and their uses as theranostic systems.

Dendrimer platform	Targeting	Imaging	Anticancer	References
PAMAM	cRGD	Au nanostar for thermic imaging and CT	siRNA, Au nanostar for PTT with NIR laser	Wei et al. (2016)
PAMAM	EPR effect	Au nanoflowers/Fe_3O_4 for Mr/CT/PA	Au nanoflowers/Fe_3O_4 for PTT and RT	Lu et al. (2018)
PAMAM	FA	Au NPs for CT	DOX (via acid-responsive *cis*-aconityl linker)	Zhu et al. (2018)
DNA Dendrimer	Aptamer scg8	Fluorescein Cy5	DOX	Le et al. (2020)
PPI	LHRH peptide	Si-phthalocyanine	Si-phthalocyanine for PDT ($^3O_2 \rightarrow {}^1O_2$)	Taratula et al. (2013)
PAMAM	Tumor penetration peptide iRGD	Cyanine dye cypate	Cyanine dye cypate for PDT and PTT. And anticancer drug DTX	Ge et al. (2019)
PAMAM	MUC-1 aptamer	Au NPs for CT and X-ray	Curcumin	Alibolandi et al. (2018)

Note that many of the individual functionalities such as Au NPs and dyes have multiple effects in terms of imaging modalities and terms of imaging and anticancer effects. In addition to the ability to incorporate a broad spectrum of targeting, imaging, and anticancer functionalities at the dendrimer platform, the dendrimer platform can be responsive to pH, UV, and GSH.

6.2 Covalent dendrimer-based theranostic systems

An ethylene-diamine core G5 PAMAM-dendrimer was used by the group of Zhao et al. (2015) as a platform for attachment of a targeting ligand (Chlorotoxin, a 36-amino acid peptide from the venom of the Scorpion *Leiurus quinquestriatus*) for matrix metalloprotease 2 (MM2), 3-(4-hydroxyphenyl)-propanoic acid (for late stage labeling with ^{131}I) and PEG-groups for Single Photon Emission Computed Tomography (SPECT) and radiotherapy of Gliomas. The synthesis of the construct is shown in Fig. 6.3.

The dendrimer without the radioactive label was tested in vitro and was selective towards Glioma cells, which was subsequently confirmed by in vivo experiments with the radioactive label in nude mice allowing localization of the compound in the tumor by SPECT.

Another ^{131}I-labeled EDA-core G5-PAMAM dendrimer conjugated to a vascular targeting peptide for targeting medullary thyroid carcinoma was reported by He et al. (2017). The activity of the dendrimer with and without the targeting ligand was tested in vitro and it turned out, that the targeting did not have significant importance for the activity. The study is, however recommendable due to the extensive and very impressive analytical work that was done to characterize the products!

Dendrimer encapsulated nanoparticles (DENs) are dendrimers having non-covalently bound nanoparticles—usually a metal nanoparticle in the interior, which is created inside the dendrimer by a two-step procedure. First, the metal ion is

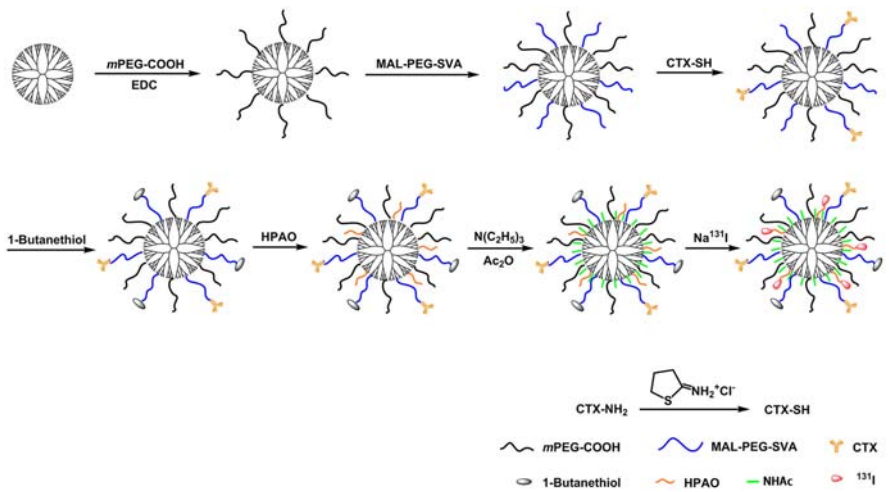

FIGURE 6.3

The schematic synthesis of the ^{131}I-G5.NHAc-HPAO-(PEG-CTX)-(mPEG) dendrimer.

Reprinted with permission by ACS from Zhao, L. Z., Zhu, J. Y., Cheng, Y. J., Xiong, Z. J., Tang, Y. Q., Guo, L. L., Shi, X. Y., & Zhao, J. H. (2015). ACS Applied Materials & Interfaces, 7, *19798–19808.*

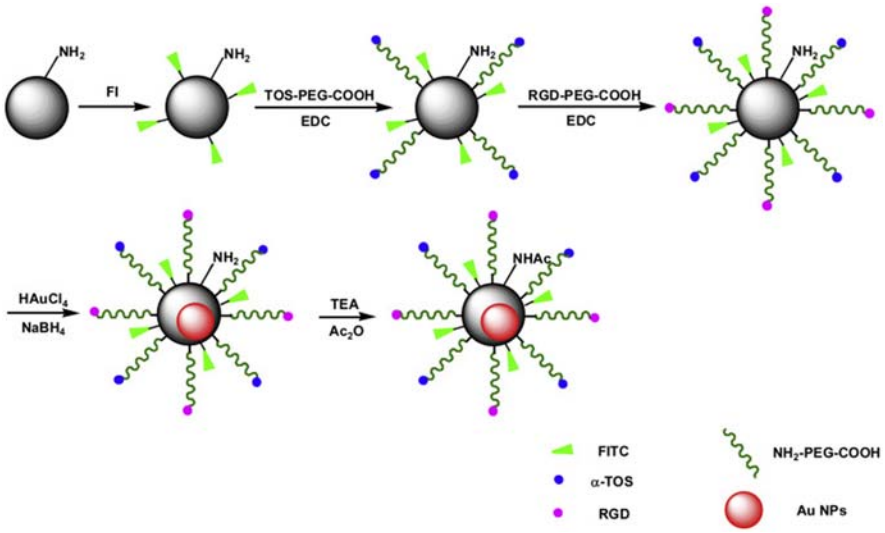

FIGURE 6.4

Schematics of the synthesis of DENs functionalized with fluoresceine, α-tocopheryl succinate- and RGD-groups on the surface.

Reprinted with permission from Elsevier from Zhu, J. Y., Fu, F. F., Xiong, Z. J., Shen, M. W., & Shi, X. Y. (2015). Colloids and Surfaces B-Biointerfaces, 133, *36–42.*

complexed into groups in the interior and secondly, this metal complex is reduced to form DENs. The group of Shi has reported theranostic generation 5 PAMAM-based DENs with a gold nanoparticle in the interior and one case tocopheryl succinate on the surface for targeting α-tocopheryl receptors and fluorescein for detection by either CT-imaging and/or fluorescence (Zhu et al., 2014). A second system had a dual targeting mode with both α-tocopheryl succinate and RGD-peptide (targeting $\alpha_v\beta_3$ integrins) covalently bound to the surface (Zhu, Fu, Xiong, Shen, & Shi, 2015a). The synthetic scheme for this system is shown in Fig. 6.4.

Anticancer activity was observed in vitro towards U87 cells and found to be due to the α-tocopheryl groups.

Another Au-DEN-based system composed of Au-nanoparticles inside an amino terminated G5-PAMAM-dendrimer with Doxorubicin attached to the surface via a cis-aconitic acid linker and Folic acid as a targeting group was described by the groups of Zhu et al. (2018) (Fig. 6.5).

The cellular uptake and the biological activity of the compound were tested in vitro in U87-cells and cellular uptake, as well as the release of Doxorubicin, was shown.

Alibolandi et al. (2018) reported a PEGylated G5 PAMAM-dendrimer hosting an Au-nanoparticle loaded with curcumin (Fig. 6.6) and finally conjugated to MUC-1 aptamer (PEG-Au-G5-PAMAM-CUR-APT) as a potential theranostic dendrimer for cancer diagnostics and treatment.

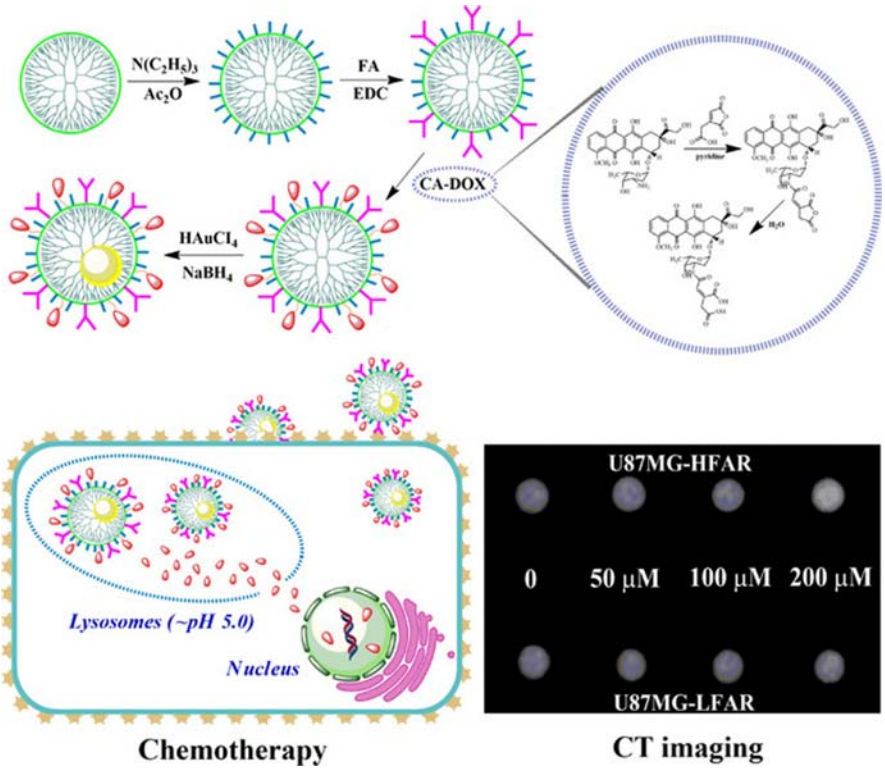

FIGURE 6.5

Upper part: Synthesis of the dendrimer-based compound. Lower left: Lysosomal release via acid-induced cleavage of the cis-aconitic acid linker. Lower right: CT images showing cellular uptake in U87-cells.

Reprinted with permission from ACS from Zhu, J. Y., Wang, G., Alves, C. S., Tomas, H., Long, Z. J., Shen, M. W., Rodrigues, J., & Shi, X. Y. (2018). Langmuir, *34, 12428–12435.*

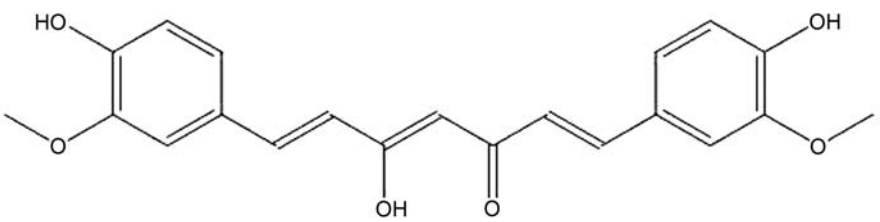

FIGURE 6.6

Structure of Curcumin, a constituent of the spice Turmeric.

Cellular uptake and cytotoxicity were demonstrated in vitro in HT29 and C26 cell lines for DENs with and without the MUC-1 aptamer with higher cytotoxicity for the construct with the aptamer due to the expression of MUC-a surface protein in the cells. The cellular uptake could be followed by the fluorescence of the curcumin and the mechanism was determined to be by endocytosis.

The in vivo activity was investigated in BALB/C mice having colorectal adenocarcinoma and the Au-DENs were visible in the CT scan (Fig. 6.7), while the results from 30 days of treatment are shown in Fig. 6.8.

The results clearly show that PEG-Au-G5-PAMAM-CUR-APT has the highest inhibiting effect of the compounds tested in the mouse model.

The covalent combination of an Au-Den with a Gd(III)-complex (DOTA) and a hypoxia-targeting ligand and potential radiosensitizer (Fig. 6.9) allowed CT/Mr imaging of hypoxic cancer cells as well as acting as a sensitizer for radiation therapy.

This system reported by Fan et al. (2020a) was based on a G5 PAMAM-dendrimer with a final acetylation step to cap the remaining amino groups, which otherwise can cause unwanted toxicity. The system showed excellent X-ray contrast, a relaxivity of 1.32 $mM^{-1}s^{-1}$ and sensitizer activity in radiation therapy.

There are many more examples of dendrimer-encapsulated gold nanoparticles in the literature and for some references (see, Kesharwani et al., 2018; Kesharwani, Choudhury, Meher, Pandey, & Gorain, 2019; Li et al., 2018; Mendoza-Nava et al., 2016; Toy & Karathanasis, 2016).

The cytotoxic properties of Copper(II)-dendrimer complexes were originally reported by El Brahmi et al. (2013), where they investigated a series of Majoral-Caminade type phosphorous dendrimers having a Cu(II)-chelating ligand on the surface. Fan et al. (2019) recently reported on the use of a G5-PAMAM dendrimer with a mixed surface of acetamides and pyridine-2-carboxamides for binding of Cu(II) as a combined MRI-contrast agent and chemotherapeutic against tumors (Fig. 6.10).

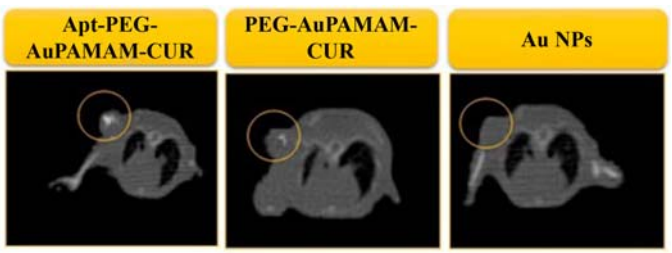

FIGURE 6.7

Clinical CT scan imaging of HC26 tumor-bearing mice 12 h post-injection of PEG-Au-G5-PAMAM-CUR-APT, PEG-Au-G5-PAMAM-CUR, and Au-G5-PAMAM nanoparticles.

Reprinted with permission from Elsevier from Alibolandi, M., Hoseini, F., Mohammadi, M., Ramezani, P., Einafshar, E., Taghdisi, S. M., Ramezani, M., & Abnous, K. (2018). International Journal of Pharmaceutics, 549, *67–75.*

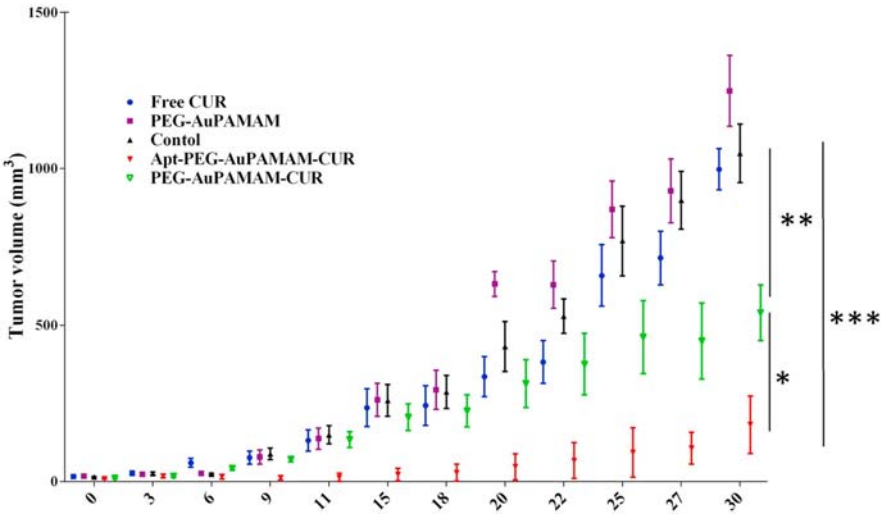

FIGURE 6.8

Tumor growth inhibitory efficacy of free CUR, PEG-AuPAMAM, PEG-AuPAMAM-CUR, and Apt-PEG-AuPAMAM-CUR in the subcutaneous mouse model of C26 (n = 5, error bars represent standard deviation). The regimen was 5 mg/kg i.v. injection twice during 30days. $P>.5$ is considered as non-significant (ns), $^{*}P<.05$, $^{**}P<.01$, $^{***}P<.001$.

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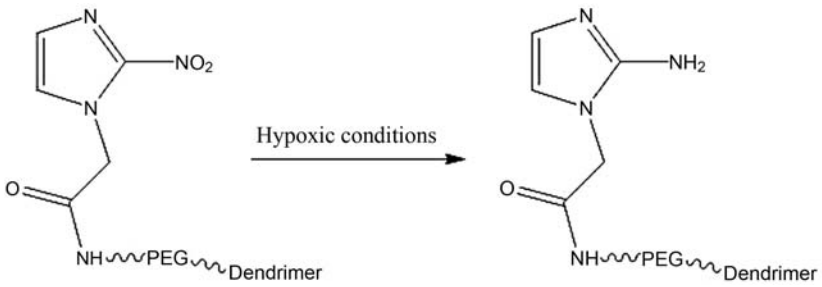

FIGURE 6.9

The hypoxia targeting ligand (left) and the transformation into the aminoimidazole, which may act as a radiosensitizer (Wardman, 2018).

The antitumor activity and effect as a T_1-Mr-contrast agent ($r_1 = 0.7024\ mM^{-1}s^{-1}$) was investigated in a subcutaneously xenografted 4T1 tumor model in mice and showed antitumor activity that could be enhanced by additional radiotherapy.

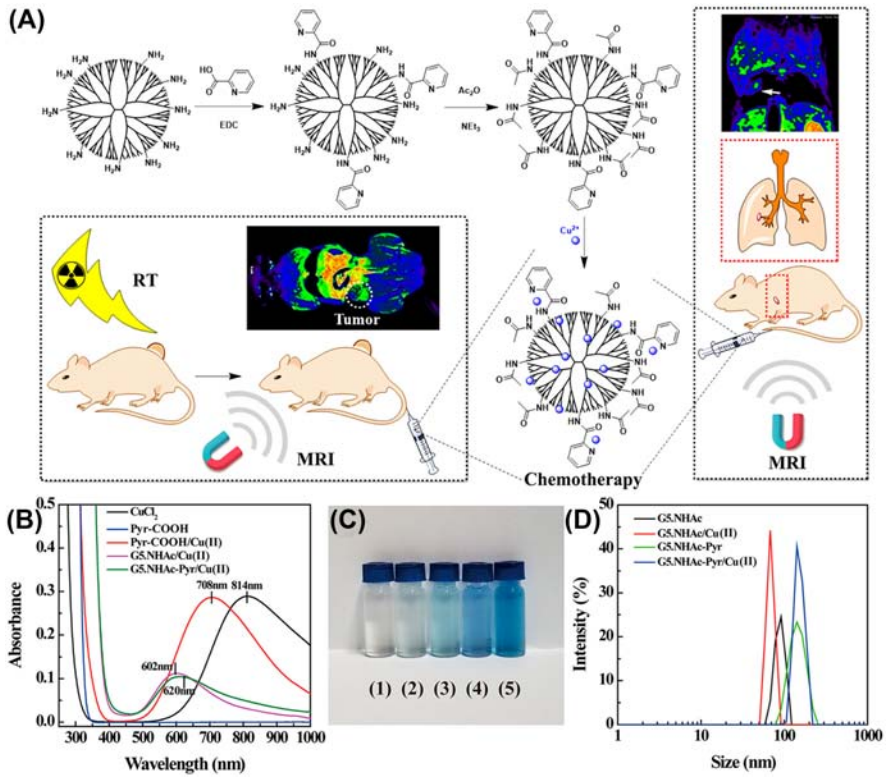

FIGURE 6.10

(A) Schematic diagram illustrating the synthesis of Cu(II) complexes with Pyr-functionalized PAMAM dendrimers. (B) UV–vis spectra of $CuCl_2$, Pyr-COOH, Pyr-COOH/Cu(II), G5NHAc/Cu(II), and G5NHAc-Pyr/Cu(II) in aqueous solution ([Cu] = 0.1 mM for Cu(II) salt and complexes; Pyr-COOH had an equivalent molar concentration to Cu(II)). (C) Digital images of the aqueous solutions of (1) water, (2) Pyr-COOH, (3) $CuCl_2$, (4) Pyr-COOH/Cu(II), and (5) G5.NHAc-Pyr/Cu(II). The Cu concentrations of samples 3–5 were set at 2 mM. (D) Hydrodynamic size distribution of different samples.

Reprinted with permission from ACS from Fan, Y., Zhang, J. L., Shi, M. H., Li, D., Lu, C. H., Cao, X. Y., Peng, C., Mignani, S., Majoral, J. P., & Shi, X. Y. (2019). Nano Letters, 19, *1216–1226.*

Citric acid-based dendrimers are interesting as biodegradable dendrimers and Alamdari et al. (2017) reported on a small citric acid/aspargine-based dendrimer capable of complexing Gd(III) and acting as a Mr-contrast agent as well as being fluorescent. Cellular uptake was shown in vitro and imaging was shown in vivo in different mice having MCF-7 xenograft tumors.

Combining cytotoxic Cu(II)-complexes with ultrasound on a Caminade-Majoral (Caminade & Majoral, 2005; Majoral & Caminade, 1998) type

phosphorous dendrimer has also been investigated as a potential theranostic agent (Fan et al., 2020b) as well as encapsulation of Doxorubicin into a dendrimer conjugated to a Gd(III)-ligand (Zhu, Xiong, Shen, & Shi, 2015b).

Photodynamic therapy is another option for the treatment of various diseases and much work has been concentrated on developing photosensitizers, that absorb light in the biological window (650−1350 nm), where the human body has minimal absorption. A recent example of combining PDT, diagnostics, and a dendrimer into a theranostic reagent comes from the group of Ge et al. (2019), a typical dye absorbing light in the NIR-region will typically be a polyene and they are highly vulnerable to light-induced oxidation. Encapsulation into a dendrimer might be one way of protecting the dye against unwanted destruction while on the other side keeping the desired optical properties. Ge et al. (2019) decorated an amino-terminated G5-PAMAM dendrimer, ethylene diamine core with covalently bound: Cypate and a targeting ligand iRGD. Additionally, Docetaxel was conjugated as a guest-host complex.

The Cypate has a dual function being a fluorophore as well as a sensitizer. The ability to generate singlet oxygen ($^1O_2$) was investigated and the quantum yields (Φ_Δ) were 0.17 for free Cypate and 0.44 for the dendrimer conjugate showing clearly that the dendrimer enhances the production of singlet oxygen. The cellular uptake and intracellular location were investigated in vitro in $\alpha\nu\beta3$-positive HepG2 cells by confocal microscopy using the fluorescence of the Cypate and showed lysosomal uptake followed by release inside the cell. Upon irradiation cell death was observed due to a combination of generation of singlet oxygen and other reactive oxygen species (ROS) as well as due to heating caused by the Cypate undergoing internal conversion from the excited state to the ground state releasing the energy as heat locally.

The theranostic platform was also tested in vitro in a mouse model (athymic nude mice) inoculated with HepG2-cells and gave promising results.

Other examples of PDT-theranostic dendrimers: G4-PPI-dendrimer with a non-covalent Silicon phthalocyanine (Taratula et al., 2013), G5-PPI-dendrimer with a non-covalent Silicon-napthocyanine (Taratula, Schumann, Duong, Taylor, & Taratula, 2015), PAMAM-dendrimer with fucoidan, manganese dioxide and the sensitizer Verteporfin (Chung et al., 2020).

6.3 Self-assembled dendrimer-based theranostic systems

^{64}Cu is a positron emitter and can be used for PET imaging provided that it is bound to a suitable ligand. Yang et al. (2018) devised a theranostic platform based on a hydroxyl-terminated G5 PAMAM-dendrimer, that was decorated statistically at the surface with hydrophobic poly(aspartate) units, PEG-groups functionalized with F3 tumor-targeting ligands, a Doxorubicin conjugate and a ^{64}Cu-chelator (Fig. 6.11).

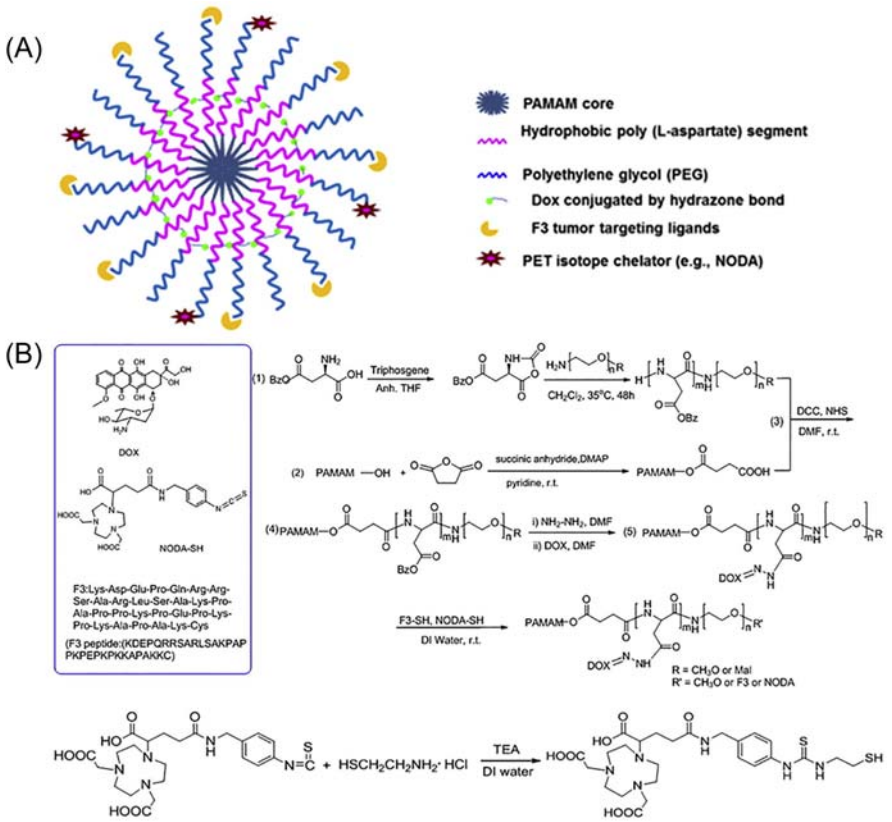

FIGURE 6.11

Self-assembling nanoparticles based on an amphiphilic hydroxyl-terminated G5-PAMAM dendrimer functionalized with targeting groups, a ^{64}Cu-chelator, a targeting ligand (F3), and a cleavable Doxorubicin conjugate.

Reprinted by permission from Elsevier from Yang, J., Lu, W. F., Xiao, J. L., Zong, Q., Xu, H. X., Yin, Y. H., Hong, H., & Xu, W. J. (2018). Acta Biomaterialia, 79, *306–316.*

This amphiphilic dendrimer was self-assembled in water to form nanoparticles with a size of around 100 nm as measured by DLS. This increases the half-life in the biological system (a mouse) as well as helps with tumor accumulation via the EPR effect. The biodistribution in the different organs was investigated with PET and is shown in Fig. 6.12 and it is clear that the targeting ligand (F3) is important for the accumulation of the nanoparticles in the tumor.

Conjugation of Doxorubicin (or another cytostatic drug) to a carrier like a dendrimer is also very interesting due to the overexpression in cancer cells of efflux pumps like glycoprotein P, which are common in all cells, where they transport unwanted molecules out of the cells. Efflux pumps are transmembrane proteins

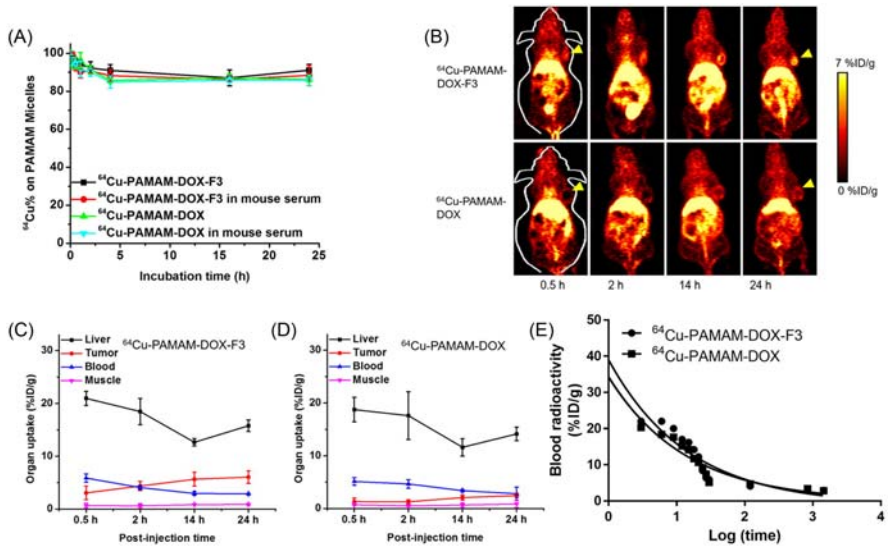

FIGURE 6.12

In vivo PET imaging of 64Cu-PAMAM-DOX-F3 and 64Cu-PAMAM-DOX in MDA-MB-231 tumor-bearing mice. (A) Serum stability of 64Cu-PAMAM-DOX-F3 and 64Cu-PAMAM-DOX. (B) Coronal PET images of MDA-MB-231 tumor-bearing mice at different time points post injection of 64Cu-PAMAM-DOX-F3 and 64Cu-PAMAM-DOX. Region of interest (ROI) analysis of tumor, liver, blood, and muscle is shown in (C) and (D). (E) The circulation time of 64Cu-PAMAM-DOX-F3 and 64Cu-PAMAM-DOX was calculated by serial blood sampling.

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that are widely found in nature and the majority are highly unselective to substrates. Teow, Zhou, Najlah, and D'Emanuele (2009) showed that it was possible to bypass glycoprotein P efflux of the drug Paclaxitel by conjugation to a dendrimer and in the study of Yang et al. (2018), they show that the nanoparticles are not effluxed leading to the higher intracellular concentration of Doxorubicin.

DNA-origami has become a discipline by itself and a recent example of a self-assembled DNA-dendrimer theranostic agent comes from Le et al. (2020) (Fig. 6.13) comprising of a DNA-dendrimer incorporating Cy5 for fluorescence imaging, a targeting aptamer (sgc8), and intercalated Doxorubicin.

The advantage of a DNA-based system is that the self-assembled structure is not formed at random, but is controlled by the sequences of the single-stranded DNA, while the stability in a biological system can be an issue due to the presence of nucleases. This problem has been solved in this case by annealing the nick-containing DNA-dendrimers at 60°C giving dendrimers with much better stability. Part of the dendrimer design was the inclusion of the aptamer sgc8 to the surface. This aptamer is specifically recognized by human protein tyrosine kinase

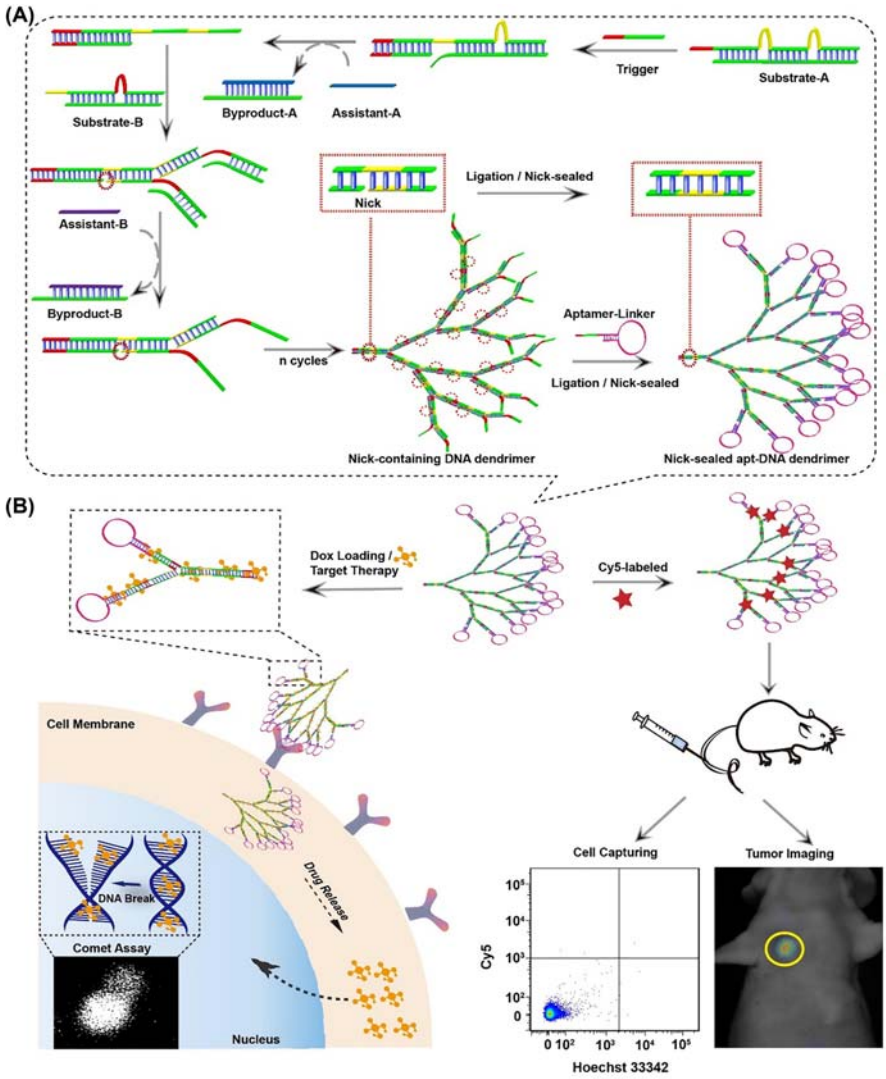

FIGURE 6.13

Schematics of self-assembly of nick-sealed aptamer-DNA dendrimers and their biological functions. (A) In the presence of Substrate-A, Substrate-B, Assistant-A, and Assistant-B, the Trigger competitively hybridizes with the exposed toehold at the 3′ ends of Substrate-A, and replaces the 5′ ends of Substrate strand-A2 as a new toehold. The resulting Substrate strand-A1/Trigger complexes combine with the exposed toeholds of two Substrate-B to form a core DNA dendrimer, which, after n cycles of self-assembly, grows to the branched DNA dendrimers with nicks that are sealed by sgc8-Linkers after ligation reaction. (B) The sturdy nick-sealed sgc8-DNA dendrimers can either carry and deliver Dox into the nucleus of the targeted cells, resulting in intercalating DNA and inhibition of macromolecular biosynthesis evidenced by the DNA comet tail, or carry fluorescent Cy5 for in vivo imaging.

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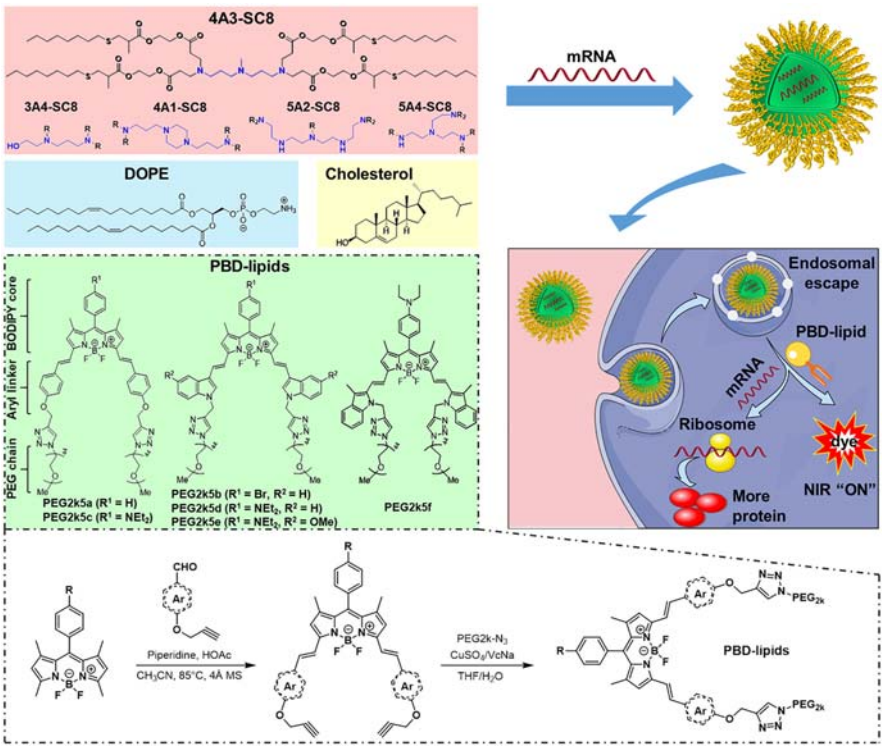

FIGURE 6.14

Schematic illustration of formulated dendrimer/DOPE/cholesterol/PBD-lipid/mRNA nanoparticles for theranostic mRNA delivery. A series of PBD-lipids was created and formulated into dendrimer lipid nanoparticles (DLNPs) that enabled intracellular mRNA delivery, expression of target proteins, and activation of pH-responsive PBD in cancer cells. All PBD-lipids except PEG2k**5a** and PEG2k**5b** are ionizable lipids.

Reproduced with permission by Elsevier from Xiong, H., Liu, S., Wei, T., Cheng, Q., & Siegwart, D. J. (2020). Journal of Controlled Release, 325, *198–205.*

(PTK7) which is overexpressed in human acute lymphoblastic leukemia cells (CCRF-CEM cells). Doxorubicin was subsequently intercalated into the DNA-dendrimer to give a non-covalent guest-host complex.

The biological activity was investigated in vivo CCRF-CEM cells and in vitro in nude mice and showed promising results for future work.

Siegwart and coworkers (Siegwart, 2016; Xiong, Liu, Wei, Cheng, & Siegwart, 2020) have introduced the concept of dendrimer-based lipid nanoparticles, that combine a small dendrimer with lipids to form nanoparticles (Fig. 6.14) and this was used for combining mRNA delivery with fluorescence NIR-imaging with a BODIPY-derivative. The nanoparticles were tested both in vitro and in vivo in a mouse model.

6.4 Theranostic systems based on dendrimers or dendrons bound to a nanoparticle

Many types of nanoparticles need a protective coating for being stable with thiolated gold nanoparticles as the most prominent example. This coating can also consist of other molecules and the bonding can be more or less covalent. Nanoparticles such as quantum dots are naturally fluorescent and the emission wavelength is adjustable via the size of a quantum dot. Carbon dots can be prepared by the hydrothermal decomposition of sodium citrate and are emitted in the blue end of the spectrum and can both be used as fluorophores and a core that can functionalize with other groups.

Ghosh, Ghosal, Mohammad, and Sarkar (2019) reported a carbon quantum dots (CDs) functionalized with EDA-core PAMAM-dendrimers (G1−G3, 8−32 surface groups), RGDS-peptide for targeting and p-DNA coding for Green Fluorescent Protein (GFP) as a demonstrator for gene therapy. The synthetic strategy is outlined in Fig. 6.15.

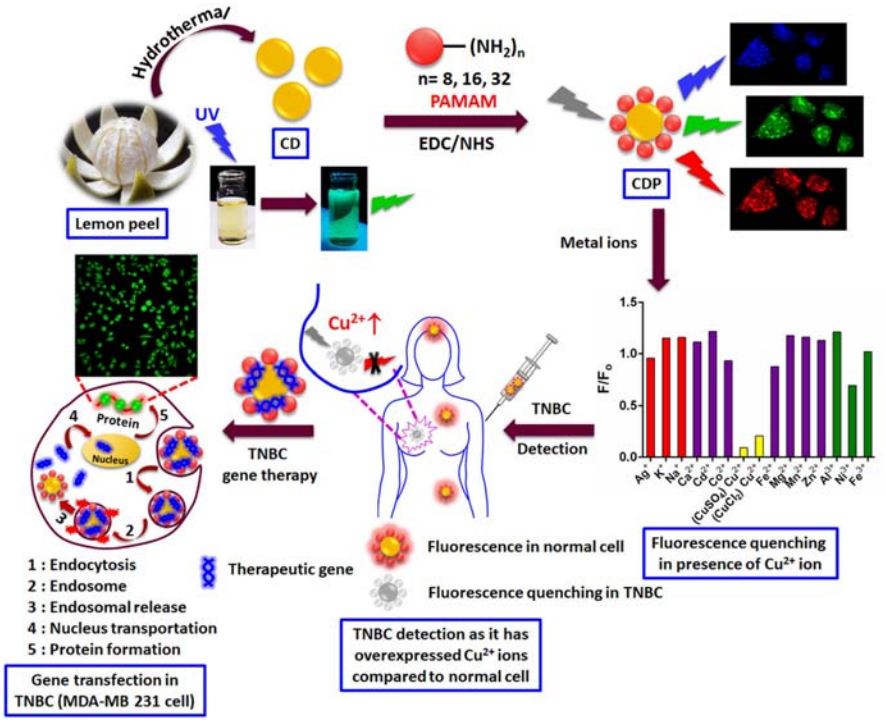

FIGURE 6.15

Schematically representation of CD and CDPs and theranostics of TNBC.

Reproduced with permission by Elsevier from Ghosh, S., Ghosal, K., Mohammad, S. A., & Sarkar, K. (2019). Chemical Engineering Journal, 373, *468–484.*

Cellular uptake in MDA-BB-231 cancer cells (Triple Negative Breast Cancer, TNBC) of the nanoparticles carrying RGDS-groups was observed by confocal microscopy, and expression of GFP was also shown. A characteristic of TNBC is a higher intracellular concentration (relative to healthy tissue) of Cu(II) ions, and this was detectable using fluorescence quenching of the CD fluorescence in vitro.

For other carbon dot-based systems see Matai, Sachdev, and Gopinath (2015).

Inorganic nanoparticles containing lanthanides are fluorescent in the NIR-region and can be solubilized by proper surface functionalization. Zhang, Zhao,

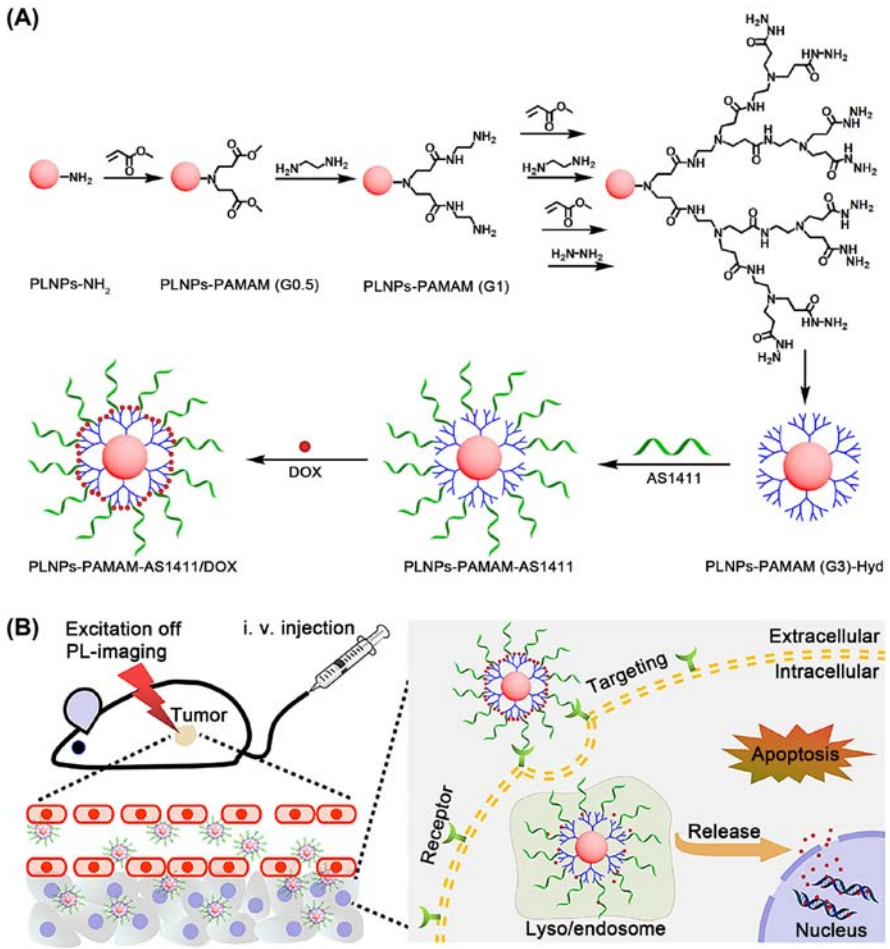

FIGURE 6.16

(A) Synthesis of Nanoparticle-PAMAM-dendron-AS1411/DOX. (B) Illustration for the utilization of Nanoparticle-PAMAM-dendron-AS1411/DOX as the nanoplatform for aptamer AS1411 guided tumor imaging and acid-responsive drug delivery.

Chen, Yang, and Yuan (2020) used zinc gallogermanate nanoparticles as a core and synthesized PAMAM-dendrons bound to the surface of the nanoparticles and conjugated aptamer AS1411 to the dendrons as a targeting group while having Doxorubicin as the drug (Fig. 6.16).

Another example of a theranostic nanoparticle containing lanthanides in the core is from Wong et al. (2015), who used $NaLF_4$-nanoparticles as a core. L was a mixture of either Ytterbium(III)/Erbium(III) or Ytterbium/Thulium(III), which gives nanoparticles capable of upconversion of light. G5-PAMAM-dendrimers were conjugated to the surface of the $NaLF_4$-nanoparticles as well as Doxorubin via a 2-nitrobenzyl linker (for photochemical release). Finally, Folic acid was conjugated as a targeting ligand. Cellular uptake and cytotoxic properties were investigated in vitro in KB cells.

Iron oxide nanoparticles in the form of superparamagnetic iron oxide nanoparticles (SPIONs) are very attractive for Mr-imaging, because they are biocompatible and highly magnetic. Luong, Sau, Kesharwani, and Iyer (2017) functionalized SPIONs with a dendrimer coating and added Folic acid to target SKOV3- and HeLa-cells overexpressing folate receptors (Fig. 6.17). The cytotoxic compound 3,4-difluorobenzylidene-curcumin was added to form a guest-host complex and the construct was tested in vitro (Fig. 6.18).

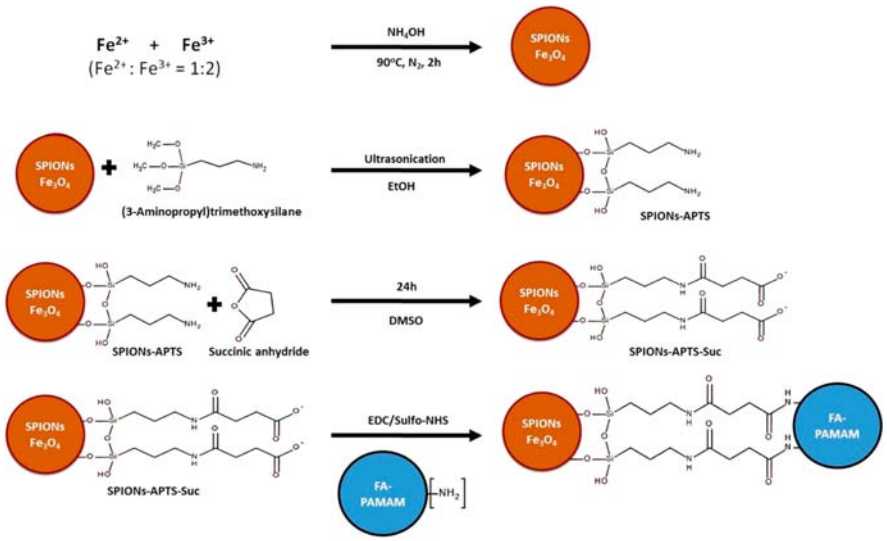

FIGURE 6.17

Synthesis of SPIONs and subsequent functionalization with a Folic acid containing PAMAM-dendrimer.

Reproduced with permission from ACS from Luong, D., Sau, S., Kesharwani, P., & Iyer, A. K. (2017). Biomacromolecules, 18, *1197–1209.*

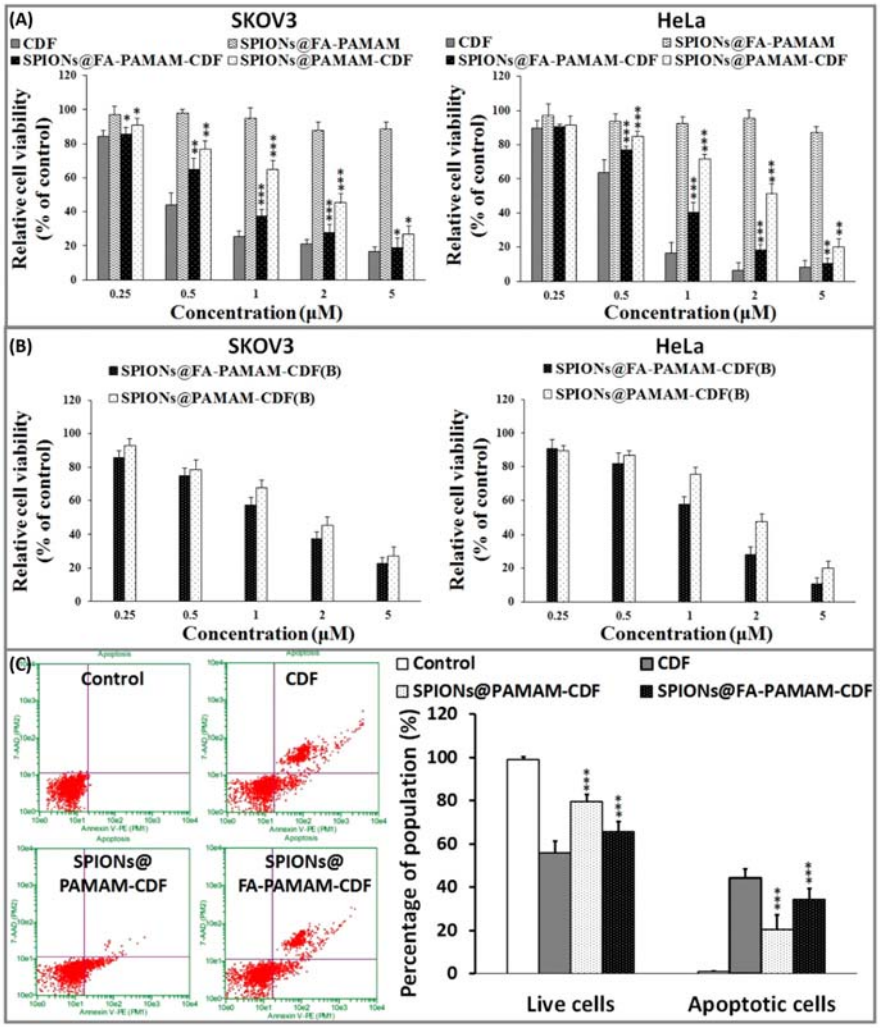

FIGURE 6.18

(A) In vitro cytotoxicity assay showing the percentage of cell viability observed at 72 h after treating SKOV3 and HeLa cells with various formulations are shown (n = 8). (B) MTT assay observed at 72 h after folate receptor blocking and treating of SKOV3 and HeLa cells with SPIONs@PAMAM-CDF and SPIONs@FA-PAMAM-CDF are shown (n = 8). (C) Induction of apoptosis in HeLa cells when treated with CDF, SPIONs@PAMAM-CDF, and SPIONs@FA-PAMAM-CDF as evaluated by Annexin V/7-AAD dual staining. An increased percentage of the apoptotic cell population was noted when cells were treated with the targeted formulation (SPIONs@FA-PAMAM-CDF) as compared to the nontargeted formulation (SPIONs@PAMAM-CDF), which suggested the better killing activity of the targeted formulation SPIONs@FA-PAMAM-CDF. $*P<.05$; $**P<.01$; $***P<.001$.

Reproduced with permission from ACS from Luong, D., Sau, S., Kesharwani, P., & Iyer, A. K. (2017). Biomacromolecules, 18, *1197–1209.*

For examples of Fe_3O_4 nanoparticles (Jedrzak et al., 2019; Lu et al., 2018; Parlanti, Boni, Signore, & Santi, 2020) and Mn_3O_4 (see, Foroushani et al., 2019).

The Boron isotope ^{10}B is very interesting in connection with neutron radiation therapy because upon absorption of a neutron, it is transformed into a ^{7}Li and a ^{4}He, which due to their kinetic energy cause tissue damage. This can be achieved by using organic boronic acids or perhaps better by using boron-containing nanoparticles. Boron nitride can form nanotubes similar to the carbon nanotubes and Guldu, Unak, and Timur (2017) described the synthesis of BN-nanotubes and coating with a small (G2) PAMAM-dendrimer to solubilize the BN-tubes and subsequent labeling with $^{125/131}I$ for diagnostics and therapy.

6.5 Outlook

The use of dendrimers and dendrons for theranostic applications is a field that is expanding with an increasing number of publications on different combinations of modalities and treatment options. However, some key issues need to be addressed before dendrimer-based theranostics can cross the abyss between bench and bedsite and one of the key questions in our opinion is: Are mice a good model system for in vivo studies at all? They are convenient and useful for many purposes, but are they suitable for testing nanomedicines? A recent paper (Moghimi & Farhangrazi, 2014) with the title "Just so stories: The random acts of anticancer nanomedicine performance" "important problem in nanotechnology" discuss some of the problems with a mouse vs human in testing cancer therapies and is highly recommendable.

The advantage of using dendrimers is that they are monodisperse materials provided that you can trust the supplier. Most groups seem to rely on commercially available dendrimers and first of all the variety of commercially available dendrimers is very limited. Secondly, there is at the present no GMP material out on the open market, which is a serious limitation for bringing dendrimers into the clinic. Hopefully, this will change as soon as more dendrimer-based drugs enter the market.

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CHAPTER

Nanogels as theranostic platforms: drug delivery, targeting, and imaging

7

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7.1 Introduction

Polymers are extensively used for encapsulation, delivery, and targeting of active molecules by creating nanosized assemblies (Saini, Bagri, Bajpai, & Mishra, 2018). In general, polymeric nanoparticles (NPs) used for the aforementioned applications may contain hydrophobic groups, hydrophilic groups, and charged groups that can interact with hydrophobic substances, can induce stability in aqueous media, and can interact with other charged molecules, respectively. To name a few realizations of polymer NPs one can refer to several examples. Polymer core-shell micelles consist of a hydrophobic core that is shielded from the aqueous environment by a hydrophilic shell and can accommodate hydrophobic compounds in their core (Cabral, Miyata, Osada, & Kataoka, 2018). A subclass of amphiphilic polymeric micelles is one of the polyelectrolyte micelles that—apart from their hydrophobic interior- have a polyelectrolyte coating which is often used for protein separation (Rosenfeldt et al., 2004; Wittemann & Ballauff, 2006) and gene transfection (Wolfert et al., 1999). Polyelectrolyte complexes, that is nanostructures originating from electrostatic interactions between oppositely charged polyelectrolytes, have a hydrophilic interior that may enclose bioactive molecules (Meka et al., 2017), although chemical modifications may induce the ability for hydrophobic interactions (Guo et al., 2013). In addition, protein-based NPs made either of pure proteins (Jain, Singh, Arya, Kundu, & Kapoor, 2018) or in combination with polyelectrolytes (e.g., polysaccharides) (Gao, Holkar, Srivastava, & Protein-Polyelectrolyte, 2019) are promising as nanocarriers because they inherit the versatile surface properties of proteins. Nanogels (NGs) are a special category of polymeric NPs that have the distinctive characteristic ability to shrink and swell in response to their environment and this way offer a multipurposed internal structure that will be extensively discussed in the following sections.

The fascinating properties of bulk hydrogels have been increasingly attracting the interest of researchers from the early studies on hydrogel capsules and hydrogel wound dressings (Hoffman, 2012). The beneficial action of hydrogels on

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human health has been proved in clinical applications (Vashist, Vashist, Gupta, & Ahmad, 2014) and it is strongly related to their ability to provide a unique stimuli-responsive spatial organization that can control the release of bioactive therapeutic molecules. Macroscopic hydrogels are formulated in thin films, patches, scaffolds, capsules, nanocomposites, etc depending on the target application. Nanoscopic hydrogels, that is NGs with hydrophilic nature open the possibility to exploit the aforementioned properties in nanotechnology applications involving nano-drug delivery and nano-bioimaging. In addition, NGs can be regarded as advantageous in comparison to conventional NPs, as the latter contains a dense impenetrable interior. The notion of a 3D polymeric crosslinked network of size in the range of 10−100 nm with the ability to accommodate large amounts of the aqueous medium has been first realized by the pioneering works of Kabanov et al., who prepared cationic NGs for delivery of oligonucleotides in cells (Vinogradov, Bronich, & Kabanov, 2002).

This chapter presents the possibilities and applications of NGs that are formed by natural and synthetic macromolecules in the field of theranostics. The discussion includes the presentation of methodologies for NG preparation that are mainly divided into two main categories; chemical and physical crosslinking. The main features of the NGs' internal structure and shape are analyzed under the prism of established characterization techniques such as small angle scattering and light scattering. The main part of the chapter focuses on the advances in the use of NGs as carriers for combined therapeutic and diagnostic actions. This part discusses the NGs that are based on natural, synthetic, or hybrid polymers separately. These materials can be used just as effectively for the early diagnosis -fluorescent or magnetic imaging- and targeted treatment of cancer. For instance, dextran (DEX) and curcumin (CCM) have been self-assembled into NGs by Nagahama, Sano, and Kumano (2015), while citrate-capped superparamagnetic iron oxide nanoparticles (SPIONs) encapsulated inside modified polyethylene glycol (PEG) networks exhibit noteworthy properties of fluorescence and magnetic hyperthermia (Vijayan, Beeran, Shenoy, Muthu, & Thomas, 2019). More such examples are discussed in detail below, in terms of synthesis, morphology, encapsulation, and bio-friendly performance. Some concern cancer theranostics and others focus on diabetes, Alzheimer's, bacterial infections, protein aggregations, and autoimmune problems. For instance, interesting work by A. Vashist et al. shows the combined therapeutic and fluorescent potential of a chitosan (CS)-hydroxyethyl cellulose composite NG targeting the central nervous system (Vashist et al., 2020). The most common drugs that are delivered are doxorubicin (DOX) for cancer and insulin for diabetes and neurodegenerative illnesses. The imaging agents that could be used for the combined diagnosis effects include carbon dots (CD), rhodamine-b or indocyanine dyes (ICD), iodixanol, graphene, and silver/gadolinium/copper sulfide/gold/iron oxide NPs. All presented works cover to a great extent the range of polymers for NG composition, types of NG performed preparation methods, and diagnosis techniques, and the relevant delivered drugs and targeted diseases.

7.2 Structure and properties of nanogels

NGs belong to the class of NPs as they have spatial dimensions in the range of 100−200 nm (Karg et al., 2019). In this sense, they share the advantages of a high surface-to-volume ratio and consequently enhanced chemical and biological reactivity (Sabir et al., 2019). NGs possess a porous internal structure that can accommodate active molecules. Their internal architecture consists of a polymeric network and therefore NGs transfer the properties of macroscopic gels at the nanoscale. They swell in a good solvent, for example, in an aqueous environment when they are based on hydrophilic polymers. The interactions with other small molecules and macromolecules may be designed by incorporating appropriate functional groups and their swelling degree, which defines both their internal mesh morphology and their overall size, and may be responsive to external stimuli (Strozyk, Carregal-Romero, Henriksen-Lacey, Brust, & Liz-Marzán, 2017). In drug delivery applications hydrogels have been extensively used (Mir et al., 2017). Inspired by these achievements, NGs are developed by utilizing biological and synthetic polymers that are physically or chemically crosslinked and contain functional groups that induce charge, hydrophobicity, specificity, and other properties to the NGs (Bencherif et al., 2009; Kabanov & Vinogradov, 2009; Soni, Desale, & Bronich, 2016). In Fig. 7.1 an outline of the basic preparation methods and characterization techniques for NGs is presented. These subjects are analyzed in the following two subsections.

7.2.1 Preparation of nanogels

Chemically crosslinked polymer nanosized networks are often synthesized by inverse emulsion polymerization. Two decades ago McAllister et al. designed NGs as gene and antisense vectors (McAllister et al., 2002). A water-in-oil emulsion containing the three acrylate hydrophilic compounds 2-acryloxyethyltrimethylammonium chloride, 2-hydroxyethylacrylate, and poly(ethylene glycol) diacrylate. These monomers have similar reactivities for free radical polymerization. The presence of ammonium offers groups for interactions with the oppositely charged DNA molecules in a pH-dependent manner. The size and swelling ratio of the NGs in water decreased with the increase of the crosslinker concentration (poly(ethylene glycol) diacrylate) and the salt content, while it increased with the increase of the cationic monomer (2-acryloxyethyltrimethylammonium). Inverse emulsion polymerization was used to prepare NGs for protein delivery to the cytosol (Raghupathi, Eron, Anson, Hardy, & Thayumanavan, 2017). The proteins were entrapped in the NG network by being present during the polymerization. A redox-responsive crosslinker was used for the release to occur in the reducing intracellular environment. Hydrogels of quaternary ammonium salt were prepared in the form of bulk hydrogels, microgels, and NGs (Sahiner, Godbey, McPherson, & John, 2006). Water-in-oil polymerization was performed for the micro and NGs. It was shown that the response to stimuli (e.g., pH,

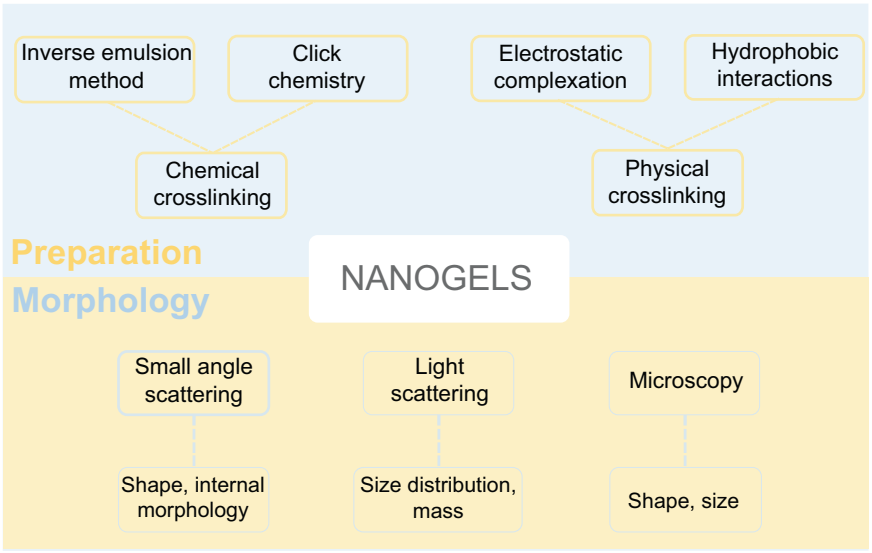

FIGURE 7.1

Preparation methods and characterization techniques in NGs.

ionic strength) of the micro/NGs was much faster than the one of the bulk hydrogels because of their small size.

The advantages of click chemistry (selectivity, yield, reactivity) have been used in the preparation of NGs that were responsive to pH. Thiolene click chemistry was applied for the construction of NGs with ortho ester linkages. Optimization of the concentrations and ratios of the reactants led to particles with monodisperse controllable size in the range of 100−200 nm. The NGs showed potential for encapsulation and release of anticancer drugs and antitumor action (Wang, Wang, Yan, Fu, & Tang, 2017). NGs may also be generated by preformed nanostructured polymeric particles. Poly(ethylene glycol)-b-poly(2-(diethylamino) ethyl methacrylate-*co*-2-cinnamoyloxyethyl acrylate) (PEG-b-P (DEAEMA/CEA)) block copolymers were synthesized by reversible addition-fragmentation chain transfer (RAFT)-controlled free radical polymerization (Yusa, Sugahara, Endo, & Morishima, 2009). These copolymers are amphiphilic in aqueous media at basic pH and self-assemble into core-shell micelles. Cinnamoyl groups are dimerized upon light irradiation leading to a photo-crosslinked micellar core. Protonation of DEAEMA causes swelling of the NG core, an effect that was confirmed to trigger the release of the hydrophobic 1-pyrenemethanol.

Polysaccharides are natural polymers, nontoxic and biocompatible in their majority and they have been extensively used in biomedical and biotechnological applications (Neamtu, Chiriac, Nita, Diaconu, & Rusu, 2019). Polysaccharides have often been used to prepare NGs by physical crosslinking. Hydroxypropyl

cellulose (HPC) substituted by cholesterol was able to self-assemble into NGs via hydrophobic interactions (Tahara et al., 2016). The size of the NGs was responsive to temperature as it could reversibly increase from sizes below 100 nm to sizes above 1000 nm above the lower critical solution temperature (LCST). Electrostatically complexed NGs were prepared by electro-spraying hyaluronic acid (HA) solutions into solutions of poly-L-lysine (Simonson, Lawanprasert, Goralski, Keiler, & Medina, 2019). With this process, different kinds of cargo (drugs, proteins) could also be encapsulated in the NGs by dispersing them in the spray or bath solution before complexation. Polysaccharide/protein electrostatic complexation has also been the basis for NPs with NG properties. Papagiannopoulos and Vlassi (2019) prepared multifunctional NPs for the encapsulation of nutraceuticals taking advantage of the thermal aggregation of bovine serum albumin within complexes with chondroitin sulfate. The NPs demonstrated NG properties as swelling-deswelling transitions were triggered by salt content and pH.

7.2.2 Morphology of nanogels

The spatial characteristics and the distribution of distinct molecular groups within the interior and at the interface of NGs are very important for their diffusion in fluids, their interactions with the environment, and their ability to encapsulate and release active molecules. These characteristics include size, shape, and internal structure. Dynamic light scattering (DLS) is traditionally used to quantify the hydrodynamic size and its distribution in dispersions of NPs. It is a very sensitive method in the presence of species of different sizes in the range of nanometers to micrometers. DLS is complemented by static light scattering (SLS) where the radius of gyration (R_g) and molar mass of scattering objects can be measured when R_g does not exceed 100−200 nm and the size distribution is monomodal. Small angle neutron and X-ray scattering techniques (SANS and SAXS) have superior resolving power at length scales from 1 to 100 nm and therefore can measure the size of not too large particles and their internal morphology. The aforementioned scattering techniques are noninvasive and provide averages of a vast number of particles in the sample as they probe macroscopic sample volumes. Microscopy methods probe a comparably much smaller number of particles, but they provide imaging of shape and size in real space.

NGs that consisted of a DEAEMA core and an a-acetal-ω-vinyl benzyl-PEG macromonomer (acetal-PEG-VB) shell were synthesized by emulsion polymerization (Tamura et al., 2012). Three different NG samples were prepared with different amounts of crosslinker ethylene glycol dimethacrylate (EGDMA). The hydrodynamic radius of the PEGylated NGs at acidic pH was 50−70 nm and decreased as a function of EGDMA molar percentage indicating the lower swelling degree of the NGs at higher crosslinking densities. At basic pH where the DEAEMA groups are deprotonated the hydrodynamic radius dropped to about 40 nm and was independent of the crosslinking density. SAXS profiles were fitted by a spherically

symmetric core-shell model. The core radii are 26−30 nm at high pH whereas at low pH they increase between 30 and 50 nm for high and low crosslinking density, respectively, due to the electrostatic repulsions between the charged DEAEMA units (Tamura et al., 2012). Interestingly, the polydispersity of the core, which was extracted from the SAXS data analysis, was found to increase more strongly at acidic pH for high crosslinking density. The authors attributed this effect to the high spatial inhomogeneity of crosslinking for high EGDMA density. This resulted in nonuniform swelling among the different NG cores.

Core-shell-shell (CSS) and hollow-shell-shell (HSS) NGs were prepared to start from spherical silica NPs (Schmid et al., 2016). The first shell consisted of poly(*N*-isopropylacrylamide) (PNIPAm) and the second shell of poly(*N*-isopropylmethacrylamide) (PNIPMAm) that was added in a second polymerization step. The two thermoresponsive polymers have different volume phase transition temperatures which are 32°C and 42°C for PNIPAm and PNIPMAm, respectively. HSS NGs were prepared from the CSS NGs by dissolving the core in hydrofluoric acid. The system was investigated by SANS and molecular dynamics simulations. In the SANS method solvent contrast variation was used to match the scattering length density contrast between the core and the solvent so that a more direct comparison between CSS and HSS could be made. Three temperatures were chosen so that the effects of the two-phase transitions were explored, that is 20°C, 40°C and 52°C. At 52°C the SANS profiles were similar in the two cases (Schmid et al., 2016). The data were optimized by two concentric layers of uniform densities and sharp interfaces. The thicknesses of the inner and outer layer were 67 and 30 nm respectively and the overall neutron scattering length radial density profiles were not much affected by the presence or absence of the core. The spherical void inside the HSS NGs was slightly larger than the core in the CSS NGs. At 40°C the inner layer (PNIPAm) was still a uniform density layer with sharp interfaces however the outer layer (PNIPMAm) was a swollen diffuse layer with gradually decreasing density as a function of radial distance. At 20°C both layers were swollen while in HSS the inner layer penetrated the initial void. These observations offered a detailed description of the dual temperature response of these NGs.

PNIPAm NGs were synthesized by emulsion polymerization in an aqueous solution (Li, Van Zee, Bates, & Lodge, 2019). NGs were investigated in phosphate buffer saline medium (PBS) with DLS, viscometry, cryogenic transmission electron microscopy (cryo-TEM), and SAXS. Hydrodynamic radius and radius measured by cryo-TEM decreased as a function of crosslinker amount while particle density increased. SAXS data were fitted by a fuzzy sphere model which represents a solid sphere with a diffuse interface. The hydrophobic drug phenytoin was found to be in the amorphous state when encapsulated in spray-dried dispersions (SDDs) of the NGs. Drug-loaded NGs were more hydrophobic and smaller than unloaded NGs. Dissolution profiles of the SDDs in vitro were greatly influenced by the crosslinking density. Phenytoin was released to roughly 100% within six hours at room temperature and the highest crosslinking density. At low crosslinking densities dissolution of the NGs resulted in slow drug release. At the

lowest crosslinking density drug release remained low. This effect was attributed to dangling chains' interpenetration and entanglement that occurred in the SDDs which led to large aggregates. Therefore, a minimum crosslinking density was necessary for the fast dissolution of drug-loaded NGs (Li et al., 2019).

The block copolymers of PEG and DEAEMA, PEG-b-P (DEAEMA/CEA), were prepared by the RAFT technique and were able to form core-shell micelles at basic pH were discussed in the previous section (Yusa et al., 2009). The radius of the crosslinked core and the thickness of the shell were determined by SAXS using a spherical core-shell model with a core and shell of uniform density. The resulting values were 9 and 3 nm respectively and they were in fair agreement with light scattering measurements. TEM images revealed spherical structures of radii between 10 and 15 nm.

Shulte et al. studied the stiffness of NGs of very low crosslinking density by using self-crosslinking of PNIPAM (Schulte et al., 2021). They also prepared PNIPAM NGs with conventional crosslinking reactions i.e with the crosslinking agent N,N'-methylenebisacrylamide. They applied the force volume mode and managed a tomographic analysis that obtained details on their shape and internal structure. The ultra-low crosslinked NGs had a very homogeneous disk-like morphology. Conventional NGs were hemi ellipsoidal in shape and had a heterogeneous and much stiffer interior. Ultra-low crosslinked NGs were more penetrable even in comparison with polymeric brushes. The volume phase transition of PNIPAm rendered the NGs stiff (Schulte et al., 2021).

Internal crosslinking of casein particles with transglutaminase was reported to result in NGs. Their internal structure was measured by three separate techniques namely SANS, SAXS, and SLS (de Kruif, Huppertz, Urban, & Petukhov, 2012). Casein NGs appeared not to swell upon the addition of urea and hence they were considered to be "frozen" NGs. The internal structure of the thermally stabilized CS/BSA NPs that were described in the previous section was resolved by SANS (Papagiannopoulos et al., 2019). The scattering profiles were modeled by a three-level hierarchical generic model of a superposition of Guinier/power law terms. The smallest structure was identified with BSA globules ($R_g \sim 4-5$ nm), which combined in intermediate clusters ($R_g \sim 15-30$ nm) and higher aggregates ($R_g > 100$ nm). Interglobular associations that are responsible for the physical crosslinking of BSA molecules upon thermal treatment were observed in SANS by an enhancement of intermediate clusters and an increase of the small structural level R_g. The swelling-deswelling transition upon addition of salt or drop of pH was observed by a transition from a two-level hydrogel-like structure to the three-level structure described above (Fig. 7.2).

7.3 Nanogels for theranostic applications

There are many reported cases of NGs being used as double agents (for therapy and diagnosis) in medical applications. Plenty of research has been focused on

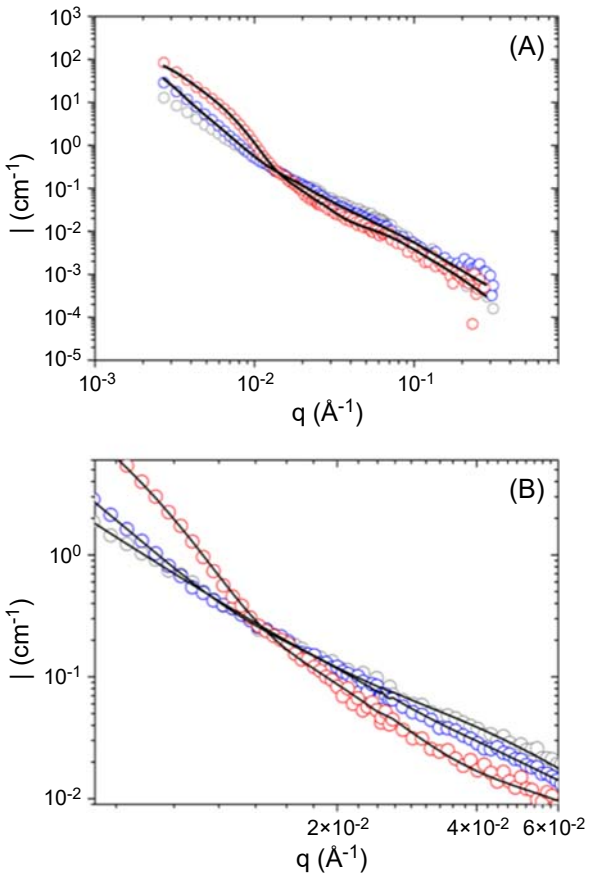

FIGURE 7.2

(A) SANS profiles from thermally stabilized CS/BSA NPs at pH 7. (B) Data of (A) in the range 6×10^{-3}–6×10^{-2} Å^{-1}. Lines are fits with hierarchical models. Color-coded distinction between 0 M (*gray*), 0.05 M (*blue*) and 0.15 M (*red*) added NaCl.

Reprinted from Papagiannopoulos, A., Vlassi, E., & Radulescu, A. (2019). Reorganizations inside thermally stabilized protein/polysaccharide nanocarriers investigated by small angle neutron scattering. Carbohydrate Polymers, 15, *218–225, Copyright 2019, with permission from Elsevier.*

cancer diagnosis and treatment, providing alternative routes to standard chemotherapy sessions. Moreover, researchers have developed NG systems for encountering neurodegenerative diseases, diabetes, encephalomyelitis, myocardial infarctions (MI), bacterial infections, protein aggregation, or even for bone regeneration purposes. The structure of an NG system, as already described, seems very promising for drugs, probes, or other biomolecules' delivery in a controlled

and efficient manner that can be monitored through magnetic, fluorescent, or photoacoustic techniques. An initial distinction among the multiple NGs that exist can be based on their composition and the chosen materials (Fig. 7.3). We distinguish between natural or synthetic polymers that constitute the basis of the NG structure. There are also more complex structures, combining a natural polysaccharide and a synthetic polymer or two synthetic polymers to obtain an NG.

7.3.1 Nanogels based on natural polymers

Polysaccharides originate from nature and are some of the most commonly used biopolymers for the preparation of nanoparticle gels (see Table 7.1). In a recent study, gadolinium NGs (GdNG) were combined with alginate via reverse microemulsion and physical crosslinking (Podgórna et al., 2017). The produced system was able to encapsulate hydrophilic drugs employed against neurodegenerative diseases. Simultaneously, the incorporated fluorescent rhodamine b (RHB) could be tracked via Magnetic Resonance Imaging (MRI). The NPs' mean diameter was 110 nm, the NG structure could remain stable for up to 2 months and no free gadolinium (III) ions were observed on its surface according to cryo-Scanning Electron Microscopy. Cytotoxicity tests like MTT and Lactate Dehydrogenase proved that the NGs were not toxic to the human neuroblastoma cell line SH-SY5Y. A different alginate-based NG is employed as an alternative to

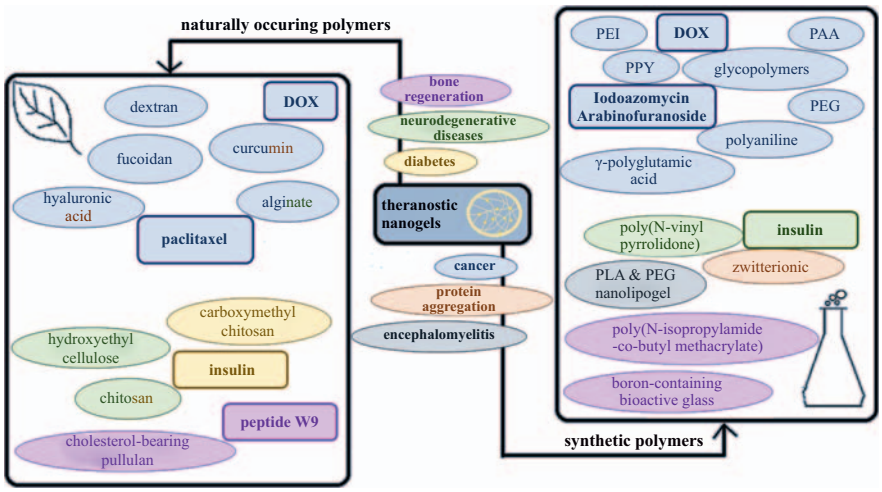

FIGURE 7.3

Natural and synthetic polymers and drugs (*rectangular bold frames*) used for theranostic applications of multiple diseases. Color-coded distinction between cancer (*blue*), diabetes (*yellow*), neurodegenerative diseases (*green*), bone regeneration (*magenta*) and other theranostic agents.

Table 7.1 Nanogels based on natural polymers.

Nanogel composition	Preparation method	Diagnosis technique	Targeted disease	Delivered drug	References
Gadolinium-alginate	Reverse microemulsion & physical crosslinking	MRI	Neurodegenerative disorders		Podgórna et al. (2017)
Disulfide-modified alginate & superparamagnetic iron oxide nanoparticles (SPIONs)	Chemical coprecipitation	MRI	Cancer	Doxorubicin (DOX)	Peng et al. (2019)
Hyaluronic acid (HA)-gold (Au)	Aqueous copolymerization	Fluorescence	Cancer	DOX	Lin et al. (2021)
Disulfide bond-linked HA & graphene		Fluorescence	Lung cancer cell line (A549)	DOX	Khatun et al. (2015)
Fucoidan	Aqueous self-assembly	Fluorescence	Cancer	Reactive oxygen species (ROS)	Cho et al. (2020)
Dendritic polyglycerol crosslinked with fluorescent peptides	Inverse nanoprecipitation without surfactants	Fluorescence	Cancer		Nagel et al. (2020)
HA-iodixanol (CT contrast agent)	Nanoprecipitation & photo-crosslinking reactions	Computer Tomography (CT scan, X-rays)	MCF-7 human breast cancer cells	Paclitaxel	Zhu et al. (2016)
Dextran—curcumin (CCM)	Self-assembly	Fluorescence	Cancer		Nagahama et al. (2015)
Epigallocatechin-3-gallate (EGCG) and curcumin modified HA	Hydrophobic-hydrophilic self-assembly		Protein aggregation		Jiang et al. (2018)

Chitosan (CS)—hydroxyethyl cellulose (HEX)	Water-in-oil emulsion polymerization	Fluorescence	Central nervous system		Vashist et al. (2020)
CS-Ag nanoclusters		Luminescent bacterial sensors	Bactericidal action		Liu et al. (2021)
Carboxymethyl (CMCS)—CS	Ionic gelation		Diabetes	Insulin	Wang et al. (2016a)
Cholesterol-bearing pullulan (CHP) crossl. pentaerythritol-tetra-polyoxyethylene			Bone regeneration	Peptide W9	Sato et al. (2015)
Carboxymethyl cellulose—bovine serum albumin (BSA)	Electrostatic complexation	Fluorescence	Cancer	Camptothecin	Liu et al. (2018)
HA—fibrinogen	Electrostatic complexation		Cancer	Curcumin	Shabbir et al. (2021)
CS—BSA			Cancer	DOX	Wang et al. (2016b)
Lysozyme—dextran—Au nps		Fluorescence	Cancer	DOX	Cai and Yao (2013)
Alginate—$CaCl_2$—glycyrrhizin			Liver cancer	DOX	Wang et al. (2019)
HA—poly-l-lysine	Electro-spraying	Fluorescence	Cancer	DOX, vancomycin	Simonson et al. (2019)

Composition, preparation method, diagnosis technique, the targeted disease, and delivered drug.

chemotherapy, carrying a drug called DOX. In this case, the NG is composed of a disulfide-modified alginate derivative and SPIONs that are magnetic and biocompatible, hence they are utilized as T_2 MRI nanoprobes for cancer diagnosis (Peng et al., 2019). The produced NG was physically characterized by various techniques, such as Nuclear Magnetic Resonance (NMR), Fourier-Transform Infrared Spectroscopy (FTIR), X-ray Photoelectron Spectroscopy, Thermo-Gravimetric Analysis, TEM and Confocal Laser Scanning Microscopy. DOX was efficiently delivered to cancer cells due to their acidic environment but did not affect the healthy nearby cells. The system was also rendered as a potential candidate for MRI diagnosis.

Another common polysaccharide employed for biomedical applications is HA due to its great colloidal stability and action in the presence of CD44 receptors, which are overexpressed in the case of malignant tumors. Ying Lin et al. have recently developed a fluorescent NG, which comprises of a derivative of HA with a vinyl group and cystamine bisacrylamide and is prepared by aqueous copolymerization, followed by an in-situ reduction of gold (Au) salt (Lin et al., 2021). The gold clusters that are created increase the fluorescence of the NG, so one could pinpoint its location through UV-Vis Spectroscopy or Photo-Luminescence Imaging. The biosystem was considered successful for its intratumoral accumulation, while its decomposition and drug release (DOX) occurred because of the glutathione (GSH) detection in cancer cells. GSH, CD44, and CD168 are all overexpressed in cancer cells and the last two are called Receptors for Hyaluronan Mediated Motility (RHAMM) and can bind to HA chains pre-existing in the extracellular matrices (ECM) and synovial fluids. Near-IR Fluorescence and Inductively Coupled Plasma Mass Spectrometry confirmed that the NGs reached the tumor sites effectively during the first 48 h, while morphological characteristics were also obtained by DLSp and TEM. A mixture of light-responsive graphene, DOX, and disulfide-bonded HA succeeded in forming a NG (120 nm in diameter), which targets and depicts tumors in a thermal and noninvasive way (Khatun et al., 2015). Once again, GSH plays an important role in activating the NG, which releases DOX to the human lung cancer cell line (A549), without affecting the noncancerous cell line (MDCK). The optical properties of graphene at 700 nm (*red*) allow deep tissue imaging and block any light-related toxicity effects. Finally, mice were injected with the produced NG (2.5 mg/kg) directly in the tail vein and both DOX and the light-sensitive graphene managed to work effectively together, killing all tumor cells in about 2 min.

A third NG consisting of HA and iodixanol (HAI-NG) has been produced as a theranostic tool against breast tumors, which can be tracked via X-Ray Computer Tomography (CT) Imaging (Zhu et al., 2016). The NGs had a diameter of about 90 nm, they produce a green fluorescent light and carry paclitaxel (PTX), an anticancer and GHS-responsive drug. PTX-loaded HAI-NGs acted against MCF-7 human breast cancer cells, releasing PTX in the blood circulation, according to MTT assays and pharmacokinetics. CT imaging was also effective in comparison to pure iodixanol, an existing radiocontrast substance. Characterization techniques

such as Gel Permeation Chromatography, High-Performance Liquid Chromatography, and fluorescence spectroscopy indicated deep tumor penetration of the biosystem. Fucoidan is another natural biopolymer example that has been used as the negatively charged polymeric base to prepare a nanogel (CFNG). More specifically, fucoidan was combined with a redox-responsive linker and a photosensitive substance, to track and simultaneously treat cancer (Cho, Li, Lo, Lee, & Choi, 2020). Additionally, hydrophobic chlorine C6 molecules provoke the self-assembly of the structure in aqueous solutions. Surface Plasmon Resonance and multiple cytotoxicity tests, such as dark toxicity, and phototoxicity, confirm that the NG produces a reactive oxygen species, which can destroy tumor cells, as a result of irradiation at a selected wavelength. Authors, however, mention that the biosystem had a hydrophobic behavior and might induce phototoxic effects in noncancer cells. Cholesterol-pullulan with modified acryloyl groups has been crosslinked with pentaerythritol-tetra-(mercaptoethyl)polyoxyethylene to form another natural NG (CHPOA) (Sato et al., 2015). This system was investigated for hindering the activity of the bone resorption peptide W9. The NGs were injected into 5-week-old mice with low calcium intake in eight daily doses (every 3 h). The studies depicted that W9 was administered much more effectively as part of the developed NGs, preventing calcium depletion in long bones, lumbar vertebras, and bone minerals. Finally, TEM images demonstrate that the shape of the NGs was compact and raspberry-like.

A dendritic polyglycerol (dPG) scaffold is crosslinked with a fluorescent peptide to produce an NG that delivers DOX to acidic environments, like those of tumor tissues (Nagel, Sousa-Herves, Wedepohl, & Calderón, 2020). The preparation process is inverse nanoprecipitation and no surfactants are necessary to produce stable conjugates of a size between 150 and 650 nm. An agarose matrix and multiple multicellular tumor spheroids were employed to study the penetration depth of the NG. Furthermore, the NG mimicked successfully the ECM and disintegrated into smaller parts of polyglycerol and DOX upon locating matrix metalloproteinase in cancer cells. DOX is finally released intra-cellularly due to the low pH of 4−5 of endosomes and lysosomes. An NG combining DEX and CCM has been prepared via self-assembly (Nagahama et al., 2015). Both components are natural and especially CCM is an excellent candidate for theranostic applications, as it is fluorescent, antioxidant, and acts against tumor cells (Hewlings & Kalman, 2017; Liu et al., 2019). Due to the presence of DEX, the system's water solubility was improved. The NG's performance was evaluated on human cervical cancer cells (HeLa), normal umbilical vein and endothelial cells (HUVEC), and normal fibroblast cells (HDF). Hydrophobic CCM has also been combined with hydrophilic epigallocatechin-3-gallate (EGCG) and HA, to achieve an antiprotein aggregation NG (Jiang et al., 2018). Protein aggregation occurs when misfolded proteins polymerize into aggregates called amyloids. Consequently, the effectiveness of protein biopharmaceuticals is decreased although these drugs tend to be well-absorbed by the human body. Protein aggregation may cause severe immune or neurodegenerative responses, such as Alzheimer's or Parkinson's disease, and

needs treatment. CCM and EGCG act as inhibitors to amyloid beta protein for curing AD. Researchers developed CCM-modified HA nanogels, EGCG-modified HA nanogels, and combined CCM-EGCG-modified HA, the latter of which could inhibit protein aggregation to a larger degree. The presence of EGCG in the final conjugate allows the amyloid beta protein to enter the structure, even at higher substitution degree values.

CS is a different polysaccharide that can be utilized as the backbone of theranostic NGs. The first presented example concerns a carboxymethyl chitosan/chitosan (CMCS/CS) NG that delivers insulin for the treatment of diabetes (Wang et al., 2016a). The negatively charged NG is produced with ionic gelation and is found to be very adhesive to intestinal parts of the human body. The biological tests that were performed in rats' jejunum show a blood glucose reduction, happening 11 h after administration. CS and Hydroxyethyl Cellulose are combined via water-in-oil emulsion polymerization to formulate auto-fluorescent micro-to-nano-gels (Vashist et al., 2020). Preparation is simple, the resulting NG is biocompatible and biodegradable. It can also be linked to drugs, DNA, RNA, and other biomolecules, according to biocompatibility tests performed on astrocytes, PBMCs, and microglia. The nanoformulation is auto-fluorescent at a wavelength range of 450−780 nm, so in-vivo imaging may also be possible, but the real challenge is for the NG to permeate the blood-brain barrier (BBB). A CS bactericidal and luminescent NG has been self-assembled and combined with thiolated silver (Ag) nanoclusters (NCs) (Liu et al., 2021). The Ag^{+} ions have antimicrobial properties, but may simultaneously be toxic to normal tissue. The biocompatible CS carrier reduces the toxicity effects and enhances the bacteria detection process. More specifically, antibacterial action has been noticed against gram-positive and gram-negative bacteria and fluorescent techniques contribute to pinpointing the bacteria in question.

Nanogel NPs were prepared by electrostatic complexation between carboxymethyl cellulose and BSA. The complexes were stabilized by thermal treatment (Liu et al., 2018). The chemotherapeutic drug camptothecin and the radionuclide 132I could be effectively encapsulated in the NGs. The NGs were spherical with a size of 120 nm, they could induce cellular uptake and they had a pH-dependent drug release profile. These NGs can be considered to have both therapeutic and diagnostic potential. Hyaluronic acid-Fibrinogen electrostatic complexes stabilized by the thermal treatment could effectively encapsulate CCM, hence be promising for theranostic applications (Shabbir, Rubab, & Tyagi, 2021). The thermally treated CS/BSA NPs with nanogel properties, reported in the previous section (Papagiannopoulos & Vlassi, 2019), were capable of β-carotene encapsulation, another nutraceutical compound. Another case of NGs derived from protein-polysaccharide complexes was the one between CS and BSA (Wang et al., 2016b). These complexes were stabilized by electrostatic attraction above BSA's isoelectric point. The NGs had a good capacity for entrapment and sustained release of DOX and were successful in fast transport of the drug to cancer cells. Lysozyme (LYS)-based NGs were prepared by complexation with DEX and

heat treatment at pH 8 (Cai & Yao, 2013). The LYS-DEX NGs were mixed with $HAuCl_4$ and converted to Au NP-containing NGs after UV irradiation. This provided good dispersion of Au NPs. Additionally, DOX was loaded to the produced NGs. Synchronous optical cell imaging and DOX release was achieved by this new system. PTX-surviving ovarian cancer cells were investigated by flow cytometry to evaluate the heterogeneity of tumor cells using polysaccharide NGs with fluorescent-tag (Wang et al., 2020). It was observed that cells had varying subtypes regarding drug resistance and capability of migration and invasion. The characteristics were found inhomogeneous between the cells while for the most malignant cell subtypes polysaccharide NGs were distributed in the cell membrane. Lipid modification of the polysaccharide NGs was proved to inhibit the viability of cells with strong drug resistance as they increased the amount of the delivered chemotherapeutic agents within the cells. This is a good example of NGs with theranostic properties combined with an evaluation of tumor drug resistance and metastasis.

Wang et al. produced alginate NGs for the delivery of anticancer drugs. These NGs were prepared by the inverse emulsion method where alginate was cross-linked by $CaCl_2$ in the presence of the drugs DOX and glycyrrhizin (GL) (Wang et al., 2019). Ca^{2+} ions create ionic bridges between the -COO- groups of alginate whereas GL may form such bridges by hydrogen bonding. Therefore, GL not only binds to the NGs nonspecifically but also may stabilize the NG structure. The release of DOX at pH 7.4 was much smaller than at pH 5 which was pointed out as an efficient way to enhance therapeutic action on lesion sites and reduce side effects. Mouse macrophage cells were used to evaluate the interaction of the NGs with the innate immune system in vitro. RBITC-labeled alginate was a probe for the cellular phagocytosis of the NGs. It was found that when GL was incorporated phagocytosis was reduced, showing an efficient way to prevent the removal of therapeutic and diagnostic agents from the bloodstream and prolong circulation. In vivo tests were performed with rats where it was confirmed that DOX circulation time was significantly increased when the drug was loaded on the NGs. The liver and hepatoma cell targeting effect of GL (Negishi, Irie, Nagata, & Ichikawa, 1991) provided NGs with efficient active targeting of the liver and tumor sites of mice. The combination of the two drugs was proposed as a strategy for using chemotherapeutic and natural anticancer drugs, especially against liver cancer. These GL-containing NGs could be effectively used for theranostic applications, provided that a diagnostic agent was also included (Wang et al., 2019).

NGs of HA and poly-l-lysine prepared by electro-spraying (discussed in the previous section) were loaded with several cargos such as green fluorescent protein (GFP), DOX, and vancomycin (VAN) (Simonson et al., 2019). They were mixed with the spray or the bath solution or mixed with the solution of the NGs. The release of the loaded drugs and protein from the NGs was monitored in physiological media, that is PBS buffer at 37°C. The hydrophilic drug, VAN, is released quickly within 4 h following first-order kinetics. The hydrophobic drug, DOX, follows zero-order kinetics and a sustained release that lasts 48 h. On the

other hand, GFP shows very little release in the first 24 h, and then it is completely released within 48 h. It was pointed out that NG swelling did not give rise to the release of the protein and it was when the NGs disrupted that full release was achieved. Fluorescent confocal microscopy was employed to evaluate the delivery of GFP to A549 cells. The NGs could penetrate the membrane and transfer the cargo to the cytoplasm of the cells which is not feasible for free GFP (Simonson et al., 2019). The authors explored the mechanism of the cargo transfer further and showed that the gel NPs are internalized by binding to the anti-CD44 antibody. It was further illustrated that the NGs, after their internalization, escape the endosome and deliver their cargo to the cell cytoplasm. These NGs are based on biocompatible and nontoxic components and have the potential for combined drug delivery and bioimaging applications.

7.3.2 Nanogels based on synthetic polymers

Various NGs in recent literature include PEG in their main structure. In a recent promising report, PEG is combined with maleic acid, glycine, N-dimethyl amino-ethyl-methacrylate, and citrate-capped SPIONs, creating a magnetic and fluorescent NG with core-shell structure (Vijayan et al., 2019). A SPION is employed for its magnetic hyperthermia properties, that is the conversion of an imposed magnetic field to thermal energy. Cancer cells can be destroyed by increasing the temperature to 42°C, as their DNA is damaged and apoptosis occurs shortly after. In this NG, the SPIONs constitute the core, whereas its skeleton comprises of the biodegradable and nontoxic PEG. TEM showed that the composed nanoparticle was about 80 nm in diameter, while MTT, living/dead cells assays, and flow cytometry gave information on the system's cytotoxicity after administration to the heart, liver, and kidneys of mice. The researchers performed biodistribution studies in mice via fluorescent microscopy, which confirmed that the NG was successfully inserted and its fluorescence signals decreased until they disappeared completely (complete biodegradation) after 48 h. PEG and maleimide in an aqueous solution self-assemble into a different NG after thermal activation (Chambre, Degirmenci, Sanyal, & Sanyal, 2018). Afterward, the formulated aggregates are crosslinked with dithiols, thiol, and hydroxyl groups and are functionalized with a thiol-containing peptide, which also includes a fluorescent ICD and DOX. The nanosystem was tested against the MDA-MB-231 breast cancer cell line and it was found that DOX was successfully released in acidic pH in a controlled way, without being further toxic to healthy cells.

PEG-based NGs have also been developed by V. M. Vijayan, Shenoy, and Muthu (2017). This NG is fluorescent because of the comacromer [PEG-maleic acid-4 amino-benzoic acid] and it combines diethylene-glycol-dimethacrylate and octreotide, too. Octreotide is injected in people suffering from the acromegaly condition, where their body produces excessive amounts of the growth hormone, causing joint pain and enlargement of parts of the body and face. In this case, however, the NG can carry DOX and release the drug in the tumor sites

during the first 5 days after administration. The structure is spherical and its mean diameter is 40 nm. FTIR and NMR confirmed that it remains stable and it exhibits fluorescence so it can be utilized as a diagnostic agent, too. A photo-cross-linkable biodegradable photoluminescent polymer (PBPLP) NG based on citric and maleic acid, L-cysteine, and PEG are suggested as a DOX carrier, targeting prostate malignant tumors (Gyawali, Kim, & Yang, 2018). The NG was functionalized by RGD peptides, but no solvents or surfactants were necessary during preparation via the polycondensation technique. Moreover, the crosslinking took place in the presence of a photo-initiator when the aqueous solution that contained PEG and the other components was exposed to UV radiation for a short period. The developed nanosystem could remain stable for two weeks, has an average size of less than 200 nm, and can be utilized as a fluorescent diagnosis agent.

More synthetic polymers are employed for the preparation of NGs, such as poly acrylic acid (PAA) and polypyrrole (PPY) or those included in Table 7.2. In a recent study, Fe_3O_4 NPs with superparamagnetic properties were enclosed inside a porous PAA skeleton (Chen et al., 2015a). The drug loading capacity was up to 98% and the release of the cyclic arginine-glycine-aspartic acid peptide with anticancer activity was achieved in a controlled way. Simultaneously, the NGs constituted a T_2-weighted imaging agent for MRI. PPY NGs of an average size of 200 nm has been proposed as an alternative route to chemotherapy because of their photothermal activity and photoacoustic imaging (PAI) ability (Theune et al., 2019). PAI is a method that converts NIR optical absorption (light energy) to local thermal expansion of tissues and this information is captured as acoustic pressure waves. The received images were characterized by excellent spatial resolution and high contrast, rendering the technique promising for diagnosis applications. It is nonionizing and can penetrate deep beneath the skin surface in comparison to other optical methods. It can even be considered a more precise and sensitive technique than MRI in cancer detection and illustration.

A different synthesis of 2-lactobion-amido-ethyl meth-acrylamide (LAEMA) with 2-amino-ethyl-methacryl-amide hydrochloride (AEMA) as the outer shell and di (ethylene glycol) methyl-ethyl-methacrylate (DEGMA) with the cross-linker N,N-methylenebis (acrylamide) as the inner core has been proposed and prepared by Quan, Wang, Zhou, Kumar, and Narain (2015). The created NG has a dense shell and a thermoresponsive core that encapsulates iodoazomycin arabinofuranoside (IAZA), chemotherapy, and radioimaging agent acting against hypoxic tumors. In hypoxia, the oxygen levels of cancer cells drop and Hepatocellular Carcinoma finds favorable conditions to grow. The loading of IAZA is dependent on how hydrophobic is the core of the NG at the LCST. Biocompatibility and cytotoxicity tests have been performed on the nanosystem on HepG2 cells and the selectivity and targeting of the defective cells have been confirmed. The novel NG is applicable to single photon emission tomography and molecular or external beam radiotherapy. Poly (ethylene imine) (PEI) NGs were proposed as antibacterial systems (Chattopadhyay et al., 2016). They were

Table 7.2 Nanogels based on synthetic polymers.

Nanogel composition	Preparation method	Diagnosis technique	Targeted disease	Delivered drug	References
[PEG-maleic acid-4 aminobenzoic acid], diethylene glycoldimethacrylate, and octreotide		Fluorescence	Cancer	DOX	Vijayan et al. (2017)
PEG, maleimide, and hydroxyl/carbonate groups	Aqueous thermoresponsive self-assembly	Fluorescence	Breast cancer cells	Doxorubicin (DOX)	Chambre et al. (2018)
[PEG-maleic acid-glycine], N,N-dimethyl aminoethyl methacrylate, and citrate-capped SPIONs		Fluorescence, magnetic hyperthermia	Cancer		Vijayan et al. (2019)
Photocrosslinkable Biodegradable Photoluminescent Polymer (PBPLP)	Polycondensation	Fluorescence	Prostate cancer cells	DOX	Gyawali et al. (2018)
Fe_3O_4-poly acrylic acid (PAA)		MRI	Cancer	Cyclic arginine-glycine-aspartic acid peptide	Chen et al. (2015a)
Polypyrrole (PPY)		Photoacoustic imaging (PAI)	PTT (cancer)		Theune et al. (2019)
Glycopolymers: LAEMA, AEMA, DEGMA, MBAm		Fluorescence	Hypoxic hepatocellular carcinoma	Iodoazomycin arabinofuranoside (IAZA)	Quan et al. (2015)
Carboxyl-functionalized poly(*N*-vinyl pyrrolidone)	Ionizing radiation	Fluorescence (potentially)	Alzheimer's	Insulin	Picone et al. (2016)
Zwitterionic	Reversible addition fragmentation chain-transfer (RAFT) polymerization		Protein aggregation		Rajan and Matsumura (2017)
Poly (ethylene-imine)	Water post-polymerization and modification		Antibacterial action		Chattopadhyay et al. (2016)

Composition, preparation method, diagnosis technique, the targeted disease, and delivered drug.

prepared using water as a solvent and by physical crosslinking. The system was characterized by NMR, DLS, and cryo-TEM and NPs about 25 nm in size were observed. The radii were dependent on the polymer's local concentration. The physically crosslinked NGs were reported to have better antimicrobial performance as compared to pure PEI due to the increased local concentration of cationic groups upon contacting the cells' membranes. Moreover, the NG showed improved bactericidal activity against gram-positive bacteria.

A carboxyl-functionalized poly(*N*-vinyl pyrrolidone) NG has been proposed as a biological nanosystem to treat AD (Picone et al., 2016). The goal of the study is to deliver insulin to the brain in a thermosensitive and hemo-biocompatible way through the BBB. The NGs are formulated by ionizing radiation, which is a sterilizing technique that can be easily upscaled in the biomedical industry. Insulin and other fluorescent molecules can be covalently attached to the system's core, while its surface remains negatively charged. The extra fluorescence property could prove beneficial for insulin detection purposes or maybe to investigate future diagnostic aspects of the NG. The release of insulin affects negatively the peptide amyloid beta which is found in the extracellular space, as well as the tau protein which is located inside the neurons' axons. Moreover, insulin is found in healthy hippocampi in comparison to hippocampi of patients with AD, confirming its usefulness against neurological symptoms associated with AD. Zwitterionic-based NGs with core-shell structures are reported to cope with protein aggregation (Rajan & Matsumura, 2017). The formulation is more successful in protecting LYS's action and cryoprotecting the cells in general, the greater its molecular weight and its hydrophobicity are. Zwitterionic polymers are positively and negatively charged and the NG preparation can happen easily through RAFT polymerization.

7.3.3 Nanogels based on synthetic copolymers or natural/synthetic polymer hybrids

In this last section, more complex structures will be discussed, combining two or more polymers of natural or synthetic origin. A DOX-bearing NG combines PEG and HA with CD (Jia et al., 2016). PEG was functionalized by folic acid (FA), whereas HA interacted with DOX electrostatically and located cancer cells due to the excess presence of glycoprotein CD44 (Fig. 7.4). On the other hand, CDs are biocompatible and fluorescent, allowing the imaging of the tumor areas. UV-Vis spectroscopy showed that controlled release of DOX was achieved, while its loading capacity was up to 32.5%. Another hybrid natural-synthetic NG was comprised of oxidized alginate and PEG. It encapsulated DOX with Schiff base bonds and RHB for the fluorescent imaging of both cancer and healthy cells (Pei, Jia, Zhao, Li, & Liu, 2018). The created theranostic NG released DOX upon entering the acidic environment and was incorporated into cancer cells via FR-mediated endocytosis. It was nontoxic and biodegradable.

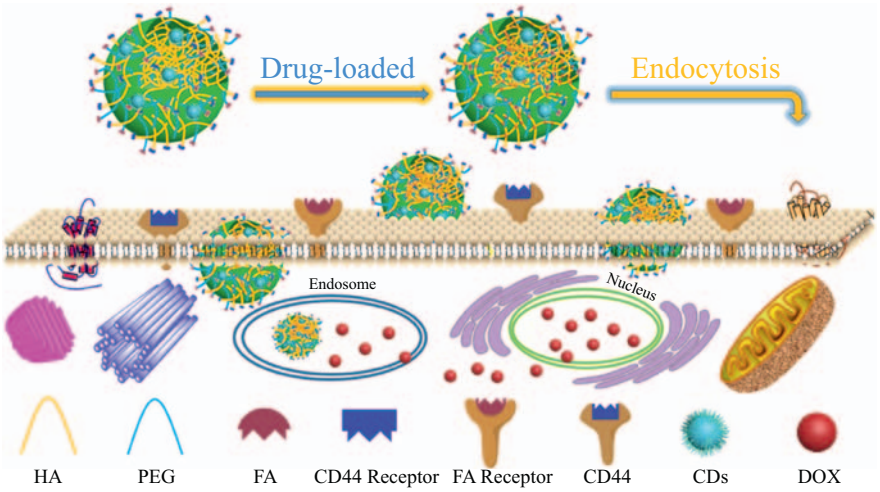

FIGURE 7.4

A characteristic example of DOX-loaded hybrid theranostic NG. Its main ingredients are PEG and HA. DOX is the encapsulated drug and CDs are the fluorescent substance that allows imaging. FA and CD44 are responsible for the interaction between the cells and the NG, stimulating the drug's release.

Reprinted from Jia, X., Han, Y., Pei, M., Zhao, X., Tian, K., Zhou, T, et al. (2016).Multi-functionalized hyaluronic acid nanogels crosslinked with carbon dots as dual receptor-mediated targeting tumor theranostics. Carbohydrarte Polymers, 5, *152:391–397, Copyright 2016, with permission from Elsevier.*

Poly (N-isopropyl acrylamide-*co*-acrylamide) (P (NIPAM-*co*-AAM)) NGs incorporated DOX and ICD in their structure for therapy and diagnosis of 4T1 murine breast cancer cells (Yu et al., 2018). The nanosystem had a liposomic polymeric shell that provided mechanical stability and its core was thermosensitive and could potentially release DOX at 40°C in a volume- and temperature-related phase transition. Interestingly, (photo)thermal activation was used in more NG cases, triggering a response in the NIR spectral range. Some examples of organic photothermal agents, displaying the desired biocompatibility and optical stability, are PPY, dopamine-melanin, and polyaniline (PANI). In the work of Yiwei Zhou et al., PANI and γ-polyglutamic acid (γ-PGA) were combined by double emulsion to create a NG with photothermal properties (Zhou et al., 2017). Cystamine was utilized for crosslinking and the average diameter of the NGs was 71.9 nm. The NG can be considered theranostic due to its PAI abilities in the NIR and it is also mechanically stable, cytocompatible, and hemocompatible. Another multifunctional NG was prepared by the copolymer N-isopropyl acrylamide-*co*-acrylic acid (Poly (NIPAM-*co*-AA)) and fluorescent BSA with incorporated gold NCs (Su, Wang, Liu, Wu, & Nie, 2013). The thermo- and pH-responsive NGs could be tracked in vitro by the red fluorescence of the Au NCs, specific targeting

was achieved by the tumor imaging N-end cysteine peptide tumor-homing peptide (iRGD) and enhanced cellular uptake with efficient cytotoxicity by DOX was observed.

Recently, a lot of light is shed on the possibility of NIR-II imaging, in the higher spectral range of 1000−1700 nm. This wavelength range is characterized by less photon scattering and deeper tissue penetration, which is more desirable for theranostic applications. A study that takes advantage of NIR-II imaging of tumor areas has proposed a PEI NG that incorporates gadolinium (Gd) and copper sulfide (CuS) and targets FA with a PEG spacer and 1,3-propane sultone (Zhang et al., 2020). The NGs are 85 nm in diameter, dispersible in water, photothermally active, and protein resistant, they absorb light in the NIR-II range and target tumors that overproduce the FA receptor. Magnetic and PA imaging are both possible. The incorporated CuS is a semiconductor with photothermal properties in the NIR-II window and low toxic effects, but it is chemically unstable and cannot be maintained long-term in the formulation. NG scaffolds intended for bone formulations with great mechanical endurance are comprised of p (N-isopropyl acrylamide-*co*-butyl methyl acrylate) (PIB) and boron-containing mesoporous bioactive glass (BMBG) (Chen et al., 2015b). Bone tissue was regenerated during the first four weeks after injection and defects of various shapes could be treated in a thermoresponsive and biocompatible way. BMBG accelerates the defective areas' healing because it triggers the release of growth factors and cytokines. The NG's differential scanning calorimetry studies concerning phase transitions indicate that there is a swollen gel, a flowable solution, and a shrunken gel state. Researchers have found that the PIB-BMBG NGs undergo a liquid-like to solid-like transition when the temperature rises from room temperature to body conditions.

PNIPAM-*co*-PAA NGs can encapsulate human cardiac stem cells in an attempt to treat MI in a safe way (Tang et al., 2017). Normal function and wound healing in the heart area have been observed in mice and pigs that were injected with the NG. Biologically speaking, the NG is porous enough to allow oxygen and other nutrients to flow, while at the same time it is compact enough to prevent T-cells from attacking the incorporated CSCs. It also promotes cardiac cell proliferation and is nontoxic to kidney or liver cells. A poly(lactic acid-*co*-ethylene glycol-*co*-lactic acid) nanolipogel that encapsulates the inhibitor KN93 is employed to treat the autoimmune disease encephalomyelitis (Otomo et al., 2015). Encephalomyelitis is an autoimmune disease triggered by T helper 17 (TH17) cells. Interestingly, the delivery of KN93 --when released from the NG-- hinders TH17 cell differentiation in the spinal cord or kidneys of mice suffering from autoimmune syndrome. It is also notable that this could also work for similar diseases, such as multiple sclerosis, systemic lupus erythematosus, psoriasis, and rheumatoid arthritis, but the investigation is still ongoing. The nlg-encapsulated KN93 is more effective than the free KN93 and the dose that is needed to achieve the same immunoreaction is significantly decreased because of the NG. The performed tests showed that KN93 targets a protein called kinase

IV, which normally regulates various functions of the cells, but was overexpressed inside the nuclei of T-cells of a patient with encephalomyelitis.

Multifunctional NGs of PAA and HPC were synthesized by polymerizing acrylic acid in aqueous solutions of HPC (Wu et al., 2010). The surfactant sodium dodecyl sulfate was used in the aqueous mixture above its critical aggregation concentration. CdSe quantum dots (QDs) were incorporated into the NGs by first adding Cd^{2+} ions with Cd $(Cl4O)_2$ solution and then Se^{-2} ions with a Na_2SeSO_3 solution. The resulting NGs were pH-responsive because of the presence of PAA. Their size increased as a function of pH. At a pH lower than the pKa of PAA, HPC and PAA are coupled by a hydrogen bond. At a higher pH, the hydrogen bonds weaken while PAA becomes negatively charged. The gradual increase of PAA association as pH increases leads to a volume phase transition where the NGs' hydrodynamic radius increases strongly (roughly doubles) between pH 4.5 and pH 6.5, although hardly exceeds 100 nm. Remarkably the fluorescence of QDs embedded in the NGs follows a similar trend with pH. At low pH where the NGs are shrunk, quenching of fluorescence is strong whereas upon pH increase the volume phase transition allows for a much stronger fluorescence signal. The ability of the NGs to sense the pH of their environment was proposed by the authors for a system to image and detect the acidified endosomes and lysosomes. The model anticancer drug temozolomide (TMZ) was loaded to the NGs by hydrogen bonding. Its release could be tuned by the solution pH as it was faster as pH increased. TMZ-loaded NGs showed high anticancer activity (Wu et al., 2010).

7.4 Conclusion and future perspective

This chapter focused on nanogel formulations that can encapsulate and release drugs and other substances in a controlled and efficient manner upon stimulation. Nanotechnology has offered a new insight into alternative routes to the treatment of pathological conditions and multiple reports employed NGs as the most effective agents that combine healing and diagnostic features. Their versatile morphology allows easier penetration across biological tissues and membranes. Nanogel action can be simultaneously therapeutic, nutritional, bactericidal, and even reduce toxicity in healthy tissues or portray the release profile of a drug. Therefore, theranostic NGs are considered smart drug delivery systems at the nanoscale and constitute a promising topic with prospects for further investigation. Their multidisciplinary aspects require collaboration between chemists, material scientists, biomedical engineers, biologists, and medical scientists.

Although NGs have already been studied extensively, clinical use is still out of reach or very limited. For instance, there are only a few reports of NGs used for diseases other than cancer in current literature. Some of the biological systems that we mentioned have been investigated with respect to their therapeutic effects

against diabetes, neurodegenerative disorders, protein aggregation, and autoimmune diseases, but did not include imaging/monitoring properties. In the future, researchers could focus more on noncancer applications and explore their dual responsiveness by incorporating the appropriate agents into the nanostructures. Moreover, some studies note that, for instance, the magnetic NPs used as imaging agents tend to exhibit toxic, nonbiofriendly behaviors. This could be encountered by either chemically altering the structures to achieve greater biocompatibility and biodegradability or by exploring the potentials of other molecules with fluorescent or magnetic capabilities and incorporating them into the NGs. Lastly, deep tissue imaging also needs development and more in vivo trials. In conclusion, despite NGs being still a work in progress, nanomedicine could greatly benefit from their potential for personalized patient-oriented therapies and monitoring.

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CHAPTER

Nanocrystals as a versatile platform for theranostic applications

8

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8.1 Introduction

A Nanocrystal (NC) is a material particle that consists of a regular arrangement of atoms around a crystal plane. NCs are generally formulated as sols that display conventional colloidal behavior and are distinguished from conventional crystals by having a mean particle diameter of < 1 μm (Rao, Thomas, & Kulkarni, 2007). The unique properties of these materials, viz. shape, particle size, surface volume ratio, crystallinity, morphology, and surface functionalization, result in a unique group of materials that can be used for the development of versatile and tailored platforms for therapeutic and diagnostic applications (Gigliobianco, Casadidio, Censi, & Di Martino, 2018; Mohammad, Hu, Yin, & He, 2019). This chapter reviews the advancements in the field of NC platform development for theranostic applications, with a focus on the most promising application pathways, viz. therapeutic drug monitoring and image-guided therapy in cancer theranostics (Chen, Wu, & Lu, 2020). This includes platforms for the delivery of sparingly water-soluble drugs (Joshi, Chandra, Jain, & Talegaonkar, 2019; Peltonen & Hirvonen, 2018), targeted drug delivery platforms (Kumar, Jha, Dr, & Mishra, 2020; Lu, Li, & Wu, 2016a), theranostic platforms for chemotherapy, photodynamic therapy (PDT), and photothermal therapy (Joseph & Singhvi, 2019); and discussion on the stabilization, functionalization, and decoration of NCs for theranostic applications.

8.2 Versatile platform development

In a pharmaceutics context, NCs can incorporate a solid nanocrystalline core of a pharmacologically active drug molecule or biomaterial, resulting in so-called drug NCs that are advantageous nanocarriers in drug delivery platforms by enhancing solubility or enabling targeted drug delivery (Jarvis, Krishnan, & Mitragotri, 2019).

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The unique properties and versatility of these materials have resulted in the development of new drug formulations (Chen et al., 2020). In addition, defects in NCs afford opportunities for the inclusion of imaging agents, while surface functionalization of the NC core can lead to further targeting or imaging applications (Siafaka, Okur, Karantas, Okur, & Gündoğdu, 2020). NCs can also be used for the efficient design of platforms that allow for both therapy and medical imaging in a single system.

8.2.1 Drug delivery platforms with enhanced solubility

Drug NC formulations that incorporate a core of pharmaceutically active material are generally characterized as having enhanced solubility due to the effects of nanosizing, as well as higher drug loading, stability, bioavailability, and dissolution rates—relative to the parent formulation (Junghanns & Müller, 2008). This has led to the development and application of drug NC formulations in novel drug delivery platforms, for example, NCs of hydrophobic drugs (Chen et al., 2020).

Drug NCs can be prepared with or without a carrier, but generally requires a stabilizer or polymeric coating to prevent NC particles from aggregating to achieve a lower energy state (Gigliobianco et al., 2018). A drug-loading of up to 100% w/w is theoretically possible when no carriers are used in the formulation, with experimental values of between 50% and 90% w/w reported in the literature (Fuhrmann et al., 2013), which allows for low-dose formulations while still sustaining therapeutic levels (Müller, Gohla, & Keck, 2011). This nanoformulation approach is unique to drug NC platforms and overcomes the characteristic low drug-loading capacities of classical nanoparticles (Gao et al., 2013). In addition, the physicochemical attributes of drug NC, viz. extent of crystalline phases, mean particle size, geometric structure, zeta potential, stiffness, surface texture, and nature of the stabilizer or polymeric coating used in the formulation, direct the overall pharmacokinetic benefits of the drug delivery system (Jarvis et al., 2019; Jaspreet & Sandeep, 2018)

Drug candidates that benefit from the enhanced solubility afforded by drug NC platforms include drugs in Class II and IV within the Biopharmaceutics Classification System (BCS). These are generally hydrophobic drugs or drugs with poor water solubility that cannot be fabricated in an aqueous dispersion medium (Kesisoglou & Mitra, 2012; Müller et al., 2011). Other characteristics of such drugs, viz. high molecular weight, high log P, and high melting points favoring lattice formation, further justify the development of drug NC platforms that benefit from nanocrystallization (Rabinow, 2004; Thipparaboina, Chavan, & Shastri, 2017). Moreover, most BCS II drugs have reached market approval, enabling the commercialization of the corresponding derivative drug NC (Junyaprasert & Morakul, 2015; Sverdlov Arzi & Sosnik, 2018).

There have been several reports on the formulation of drug NCs over the past two decades, with the physiochemical properties of the NC usually tailored to

a specific application context by judicious selection of the fabrication technology (Junghanns & Müller, 2008). NC fabrication is usually carried out via a top-down, bottom-up, or combination approach. The evolution of formulation approaches and corresponding advantages and disadvantages have been extensively reviewed (Couillaud, Espeau, Mignet, & Corvis, 2019; Junghanns & Müller, 2008; Müller et al., 2011; Pardhi, Verma, Flora, Chandasana, & H, 2018; Sinha, Müller, & Möschwitzer, 2013). The bottomup approach usually requires little high energy input, for example, ultrasound, however, this approach is yet to produce a commercialized product (Liu, Yu, & Yin, 2020). NCs have primarily been manufactured by topdown methods (Liu et al., 2020), however, it is known that NC sizes can be more efficiently controlled via a combined technology approach, which has led to increased research and industrial interest in this methodology (Fontana et al., 2018; Pawar, Singh, Meher, Gupta, & Chourasia, 2014). Nonetheless, common to all methodologies is that the surface area to unit mass of the fabricated drug NC is high. This increases the overall energy of the system, rendering it thermodynamically unstable and favoring agglomeration (Rabinow, 2004). The resulting nanosuspension or colloidal dispersion requires stabilization, usually with natural polymers, for example, polysaccharides such as cellulose and derivatives, synthetic polymers, for example, polyvinylpyrrolidone and pluronics, and anionic surfactants, for example, polysorbate (Wang, Mallet, Ricard, & Heng, 2012a). Solid drug NC particles are subsequently obtained by solvent removal, for example, via antisolvent addition or freeze and spray drying methods (Liu, Yu, Yin, & Möschwitzer, 2019).

First generation NCs in the range of 100−1000 nm were predominantly produced via NanoCrystal technology, that is a topdown approach wherein large crystals are broken down into smaller crystals via pearl or bead-driven milling to reduce particle size (Liversidge & Cundy, 1995). Limitations of the topdown milling method include the introduction of impurities in NC particles due to surface erosion and high residence times that result in a slow process that is not amenable to large-scale manufacturing. Other industrial technologies were developed that leveraged homogenization methods such as piston gap homogenization in an aqueous or nonaqueous phase and Microfluidizer technology (Junghanns & Müller, 2008). These techniques promote large-scale production and have been well received in an industrial context, however, material crystallinity is generally affected by the application of high force and heating in such methods, which has led to the development of optimized and improved technologies. Bottomup methods involve nanoprecipitation via controlled and quick nucleation for slow and uniform crystal growth. The pharmaceutical active is solubilized incompatible solvents, and precipitation of amorphous and crystalline nanoparticles occurs after antisolvent addition. Nano spray drying of the solubilized drug solution is also a well-reported technique for the recovery of the solid drug NC (Sosnik & Mühlebach, 2018), wherein polymeric stabilizers or emulsifiers are used to control particle size and crystal growth by coating the growing crystal interfaces to inhibit particle agglomeration (Choi, Yoo, Kwak, Uk Nam, & Lee, 2005).

Developments in bottomup technologies have been geared towards quicker and more controlled nucleation processes. For example, the drug material can be added to the antisolvent under ultrasound in sonoprecipitation techniques, while millisecond mixing has been reported via the use of impinging jet mixers (Sinha et al., 2013). Controlled Precipitation can also be carried out in high gravity reactors, which have been well applied for the preparation of inorganic NCs (Sinha et al., 2013). These techniques have yet to find commercial application primarily due to higher cost however these modified precipitation methods have resulted in the fabrication of some high-quality NCs with uniform size (Mohammad et al., 2019). The advent of SmartCrystals technology (Shegokar & Müller, 2010), a combination of top and bottomdown approaches, gave rise to the second generation of NCs (Keck, Mauludin, & Müller, 2008; Müller et al., 2011). This approach involves initial bead milling and subsequent high-pressure homogenization to decrease manufacturing times and produce NCs with mean particle diameters of <100 nm and with enhanced physio-chemical stability (Shegokar & Müller, 2010).

Irrespective of the technology, the NC formulation approach has been proven to produce effective formulations for improving the bioavailability of sparing water-soluble drugs (Jacob, Nair, & Shah, 2020). For example, nanosizing increases the surface area per unit mass of a material, which results in higher dissolution velocities and improved dissolution rates. Solid NCs, for example, tablets or capsules, are thus associated with enhanced bioavailability, improved dose proportionality, reduced variation in fed or fasting states, minimized intersubject variability, and improved absorption rates—both in vivo and clinical. NC suspensions also have improved biopharmaceutics due to faster dissolution rates, and enhanced cell membrane and surface adhesion (Müller et al., 2011). These characteristics lead to improved pharmacokinetics after oral administration, enhanced dermal permeation after topical application, and smaller injection volumes for parentals—while minimizing side effects commonly observed with traditional formulations after parental administration. The impact of drug NC particle size on dissolution rate and solubility is described by the theoretical equations of Noyes-Whitney, Prandtl, Ostwald Ripening, and Ostwald-Freundlich (Couillaud et al., 2019; Kesisoglou, Panmai, & Wu, 2007; Müller, Jacobs, & Kayser, 2001), with the seminal work of Parks et al. (2017) using computational modeling to predict how solubility and dissolution rate change with size, with Parks et al. (2017) further using polymorph tools for the precise molecular engineering of drug NC systems.

The development of drug NC platforms has also been supported by extensive pharmacokinetic studies that have investigated biodistribution in organs congruent to the route of delivery, either oral (Gao et al., 2013; He et al., 2015), parental (Lu et al., 2016a), oral and parental (Merisko-Liversidge & Liversidge, 2011), intraarticular (Maudens et al., 2018), or via all-routes (Lu, Qi, Dong, Zhao, & Wu, 2017; Pawar et al., 2014). Smaller particle sizes of $<$ 100 nm were observed to give enhanced in vivo performance, with the application to different delivery

routes demonstrating the versatility of the drug NC platform. Moreover, many studies have examined nanomaterials safety in nanomedicine, for example, via bio-predictive Physiologically Based Pharmacokinetic models (Su et al., 2018; Utembe, Clewell, Sanabria, Doganis, & Gulumian, 2020; Yuan, He, Wu, Fan, & Cao, 2019), and in a recent study, Jarvis et al. (2019) reviewed NC platform development from a translational perspective. It was found that translation of NC platforms into clinical reality depends on the physicochemical properties, formulation considerations, and development of targeting or precision delivery systems, which supports the use of smaller NCs systems with mean particle sizes < 100 nm, and with the surface, functionalization to ensure circulation and prevent phagocytosis (Jarvis et al., 2019).

8.2.2 Targeted drug delivery platforms

NCs are potential platforms for targeted drug delivery as their physicochemical attributes can be tuned for the optimum in vivo performance, primarily via functionalization (Boles, Ling, Hyeon, & Talapin, 2016; Zhao et al., 2018). Studies have also shown that surface unmodified NCs similar to other nanoparticle platforms have low circulation in the blood and are rapidly cleared (Gigliobianco et al., 2018; Liu et al., 2019; Lu et al., 2016a). The presence of tight intercellular junctions of the vascular endothelium in nondiseased tissues has been observed to prevent nanoparticle entry (Couillaud et al., 2019). However, there is an increase in vascular permeability in inflammation-prone tumor tissues, where the dilated endothelium enables the passage of nanoparticles via diffusion pathways coupled with the Enhanced Permeability Retention (EPR) effect (Maeda, Wu, Sawa, Matsumura, & Hori, 2000), which supports NC formulation as a promising platform for cancer drug delivery (Hollis et al., 2013a). NCs have thus been applied for the targeted delivery of chemotherapy drugs, with systems that use ultra-small NCs with mean particle sizes < 100 nm gaining prominence (Liu et al., 2019). The use of smaller particle sizes has implications for passive and active targeting platforms for precise cancer nanomedicines, enabling the development of injectable as well as oral drug delivery platforms (Lu et al., 2016a).

It has been reported that nanoparticles accumulate predominantly in the liver and to a lesser extent in the spleen postintravenous (i.v.) administration of injectable NCs formulations (Müller et al., 2011). Although the large surface area of NCs promotes drug solubility, it can also facilitate the absorption of proteins from plasma, particularly opsonins (Lu et al., 2016a). The physicochemical properties of the nanoparticle, viz. dimensions, shape, crystallinity, surface instability, and hydrophobic nature of the surface, underly the adsorption of proteins on nanoparticles (Nel et al., 2009), which triggers phagocytotic uptake of NCs by cells of the mononuclear phagocyte system (Iversen, Skotland, & Sandvig, 2011). However, opsonization can potentially be minimized via surface coating with water-soluble polymers, for example, poly-ethylene-glycol (PEG) or poloxamers (Fuhrmann, Gauthier, & Leroux, 2014; Müller et al., 2011; Wang, Wang, Zhao,

Zhuang, & Wu, 2019a). As such, the design of targeted drug delivery systems using NCs needs to find a compromise between these criteria to ensure adequate bloodstream circulation while promoting the EPR effect for passive targeted pathways.

Active targeting strategies involve NC surface functionalization via coating with stabilizers or moieties for targeting and or cell internalization (Couillaud et al., 2019; Pawar et al., 2014). The targeted release enables low doses that decrease side effects (Junyaprasert & Morakul, 2015), with the targeting groups potentially leading to higher NC tumor localization and uptake, coupled with minimum uptake to organs of the reticulum endothelial system (Agrawal et al., 2018). Early targeted cancer therapy studies with target folate (FA) receptors involved paclitaxel (PTX) NCs coated with folic conjugated Pluronic F127a (Liu et al., 2010). Wang, Li, and Zhang (2012b) prepared FA functionalized docetaxel NC-lipid-based-nanosuspensions, followed by Lu et al. (2014). Who reported on the modification of PTX NCs for Transferrin Receptor targeting. In all reported studies using NC platforms for targeted cancer therapy, cytotoxicity was observed to be lower when using the targeted delivery system. In a recent study, lapatinib NCs were coated with hyaluronic acid for cell surface glycoprotein Cluster of Differentiation 44 (CD44) targeting, where it was found that mouse mammary carcinoma 4T1 led to notable tumor growth retardation, enhanced tumor localization, and increased residence time; which was attributed to the combined active targeting of the CD44 receptors and passive targeting via the EPR effect (Agrawal et al., 2018). Targeting $\alpha v\beta 3$ with peptide motif RGD peptide-binding integrins have been reported to increase the antitumor efficacy of cancer nanomedicines (Nieberler et al., 2017; Wu, Opadele, Onodera, & Nam, 2019). For example, PTX drug NCs functionalized with polydopamine (PDA)-PEG-RGD was accumulated intratumorally at a higher concentration in an A549 model tumor bearing-mice, which resulted in decreased tumor growth relative to free PTX and the unfunctionalized NC (Huang Z-g et al., 2019). Dong, Cho, Lee, and Roman (2014) conjugated FA to Cellulose NCs (CNCs) via heterogeneous chemical synthesis, and results of in vitro assays in cell lines, viz. DBTRG-05MG, H4 human cell lines, and rat brain tumor cells (C6) depicted different cellular uptake pathways for NCs. In DBTRG-05MG and C6 cells, NCs underwent internalization via caveolae-mediated endocytosis, while a clathrin-mediated endocytosis internalization pathway was observed in H4 cells. In addition, FA conjugated CNCs were observed to be cytocompatible with normal cells, indicative of a promising tumor targeting the NC platform (Seabra, Bernardes, Fávaro, Paula, & Durán, 2018; Zhao et al., 2018).

A further targeting approach involves the noncovalent binding of serum proteins on an NC surface, for example, human serum albumin, transferrin, or immunoglobulin G, to serve as stabilizers and targeting moieties (Kratz & Elsadek, 2012; Lu et al., 2014; Sohn, Yoon, Sohn, Park, & Choi, 2017). Park, Sun, and Yeo (2017) have also demonstrated an improvement in antitumor efficacy in B16F10 melanoma in a mice model with albumin-coated PTX NCs, relative to

a market available albumin-based PTX formulation, viz. Abraxane™, which further highlights the impact of functionalized NC platforms in targeted drug delivery. In a separate independent study, PTX drug NCs were decorated via surface adsorption of a monoclonal antibody, viz. Herceptin (HCT) for HER2 receptors targeting, which resulted in stable formulations, with corresponding in vitro studies demonstrating a higher degree of binding and cellular uptake in HER2 positive breast cancer cell lines for PTX-NCs-HCT relative to cells treated with PTX-NCs only (Noh et al., 2016).

Stimuli-responsive NC platforms have also been reported, using either stimuli-responsive polymers as stabilizers in NC formulations or NCs with inherent stimuli-responsive properties that can be harnessed to target NCs to diseased tissues (Lu et al., 2016a). This includes the application of biocompatible Calcium Carbonate ($CaCO_3$) and Hydroxyapatite (HA) NCs, wherein inorganic biomaterials such as $CaCO_3$ degrade in acid media and can be used to target the tumor microenvironment (Lu, Aimetti, Langer, & Gu, 2016b). Shafiu et al. have also reported on the application of $CaCO_3$ NCs loaded with doxorubicin that have demonstrated pH-responsive release at 4.8 pH and enhanced cellular uptake by MDA MB231 breast cancer cells (Shafiu Kamba, Ismail, Tengku Ibrahim, & Zakaria, 2013). Wu, Wang, Hsieh, Sun, and Kang (2016) prepared HA conjugated PEG spacer magnetic field responsive bioceramic HA NCs that demonstrated improved intracellular uptake by epithelial and human breast cancer cell lines for intracellular hyperthermia cancer therapy. Lelli et al. (2016) further reported on direct loaded Pt-bisphosphonate complexes in HA NC suspensions, wherein preliminary in vitro studies demonstrated pH-triggered drug release that could potentially be used for the development of bone-targeted delivery systems. Other surface modifiers for NCs involve efflux inhibitors and functional stabilizers for improved neuroavailability (Chen et al., 2016), and antitumor activity via dual inhibitors (Patel, Patil, Mehta, Gota, & Vavia, 2014).

8.2.3 Theranostic platforms

The physicochemical properties of multifunctional nanomaterials have stimulated significant research into the theranostic applications of these materials, spurring the development of nanotheranostics wherein established nanomedicine strategies have been adapted for these applications. This includes the development of NC theranostic platforms for simultaneous targeted delivery and imaging, wherein hybrid NCs (Hollis, Zhao, & Li, 2013b), multifunctional inorganic NCs (Liong et al., 2008), biomimetic or targeted NCs, and biocompatible CNCs are seen as the most prominent emerging platforms for cancer theranostics and are the primary focus of this review. An ideal nanomaterial for cancer theranostics should elude from the immune system and freely circulate in the bloodstream without inducing toxicity, should allow for efficient targeting of cancer cells via the EPR effect or via the use of targeting moieties conjugated to the nanoparticles, and should be capable of imaging or therapy due to inherent nanoparticle properties,

should be a multifunctional carrier to allow for different theranostic agents, and should be safely eliminated from the patient when the clinical procedure is complete (Noh et al., 2016). Preceding discussions have reviewed active and passive targeting of tumor cells using functionalized NCs. The following discussions review the ability of NCs to offer the ideal nanomaterial properties for cancer theranostics, including a discussion on improved pharmacokinetics due to sustained drug release or drug release from NCs engulfed by phagocytes, which may lead to better cancer patient outcomes.

8.2.3.1 Hybrid nanocrystals for chemotheranostic applications in cancer therapy

In hybrid NCs the imaging agent is physically embedded inside the drug crystal lattice, resulting in potential platforms for dual chemotherapy and imaging in therapeutic drug monitoring applications. This is a valuable tool for early diagnosis and monitoring to improve survival rates in cancer patients (Bittleman, Dong, Roman, & Lee, 2018), and the formulation approach enables the study of circulation and biodistribution of NCs for the establishment of pharmacokinetic models (Li, 2006).

First-generation hybrid drug NCs involved therapeutic drug molecules coupled with fluorescent dyes or water-soluble fluorophores (Hollis et al., 2013a; Hollis, Weiss, Evers, Gemeinhart, & Li, 2014; Zhao et al., 2011), and NCs of camptothecin doped with gold (Au) (Hollis, Dozier, Knutson, & Li, 2019). Other research investigated the use of poor water soluble dyes such as DiR (Lin et al., 2015), DiD (Hu et al., 2015), and Cy5 (Ni et al., 2015) to track NCs after administration. Fig. 8.1A presents an example of first-generation hybrid NCs that have been surface modified for targeting, and embedded with imaging agents that emit fluorescence. Fig. 8.1B presents an example of proof-of-concept NCs of model drugs that have been successfully modified with fluorescein and rhodamine. While this method of incorporating imaging agents in NCs is advantageous relative to conventional methods, it is not without shortcomings as conventional fluorophores in a hybrid NC approach are unable to selectively image NCs over free fluorophores (Lu, Lv, & Li, 2019). For example, both the fluorescent agent inside the NC and the free fluorescent agent contributes to the intensity of the fluorescence signal such that complex processes such as NC dissolution and cell trafficking cannot be accurately tracked (Gao, Lee, Meng, & Li, 2017; Hollis et al., 2014). Moreover, the pharmacokinetic profile of the free probes is different from that of the NCs and drug molecules such that the fluorescent images may lead to errors in biodistribution studies. A possible solution to overcome this is to embed imaging agents that only fluoresce when embedded in the NC.

This spurred the development of the second generation of hybrid NCs, which involved the use of environmentally responsive fluorophores embedded into the NC (Lu et al., 2019). These fluorophores are "switched off" when they are not embedded, enabling self-discrimination of hybrid NCs, Fig. 8.1C. This includes the Aggregation-Induced Emission (AIE) fluorophore tetraphenylethene (TPE)

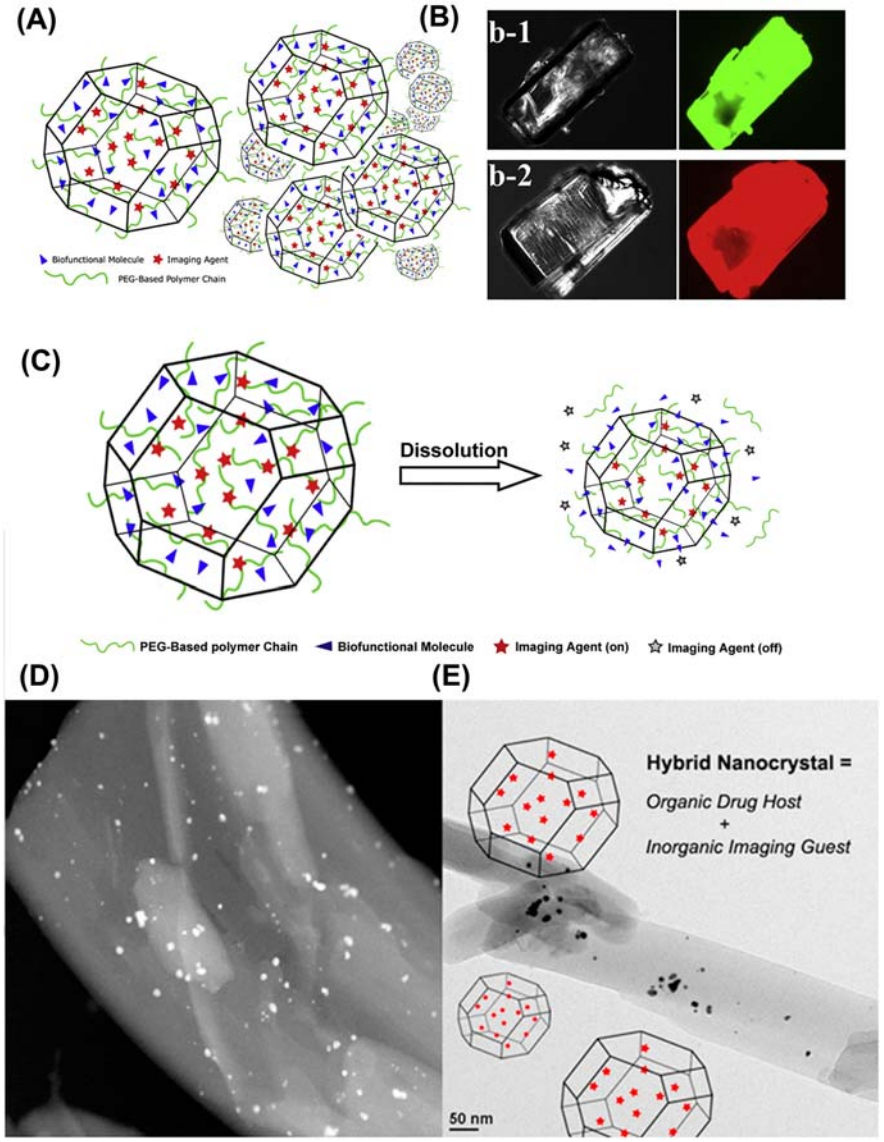

FIGURE 8.1

(A) Graphical impression of a hybrid drug NC that can be surface functionalized with ligands or biopolymers and have embedded imaging agents within the crystal defects. (B) Images of acetylsalicylic acid (B-1) with fluorescein (*green*) and paracetamol NCs (B-2) with rhodamine (*red*). (C) Second generation self-discriminating hybrid drug NCs, (D) STEM and (E) TEM micrographs of Au embedded into hybrid camptothecin.

(A–C) Adapted with permission from Lu, Y., Lv, Y., & Li, T. (2019). Hybrid drug nanocrystals. Advanced Drug Delivery Reviews, 143, *115–133, copyright Elsevier 2019. (D–E) NC reprinted under creative commons license from Hollis, C. P., Dozier, A. K., Knutson, B. L., Li, T. (2019). Preparation and characterization of multimodal hybrid organic and inorganic nanocrystals of camptothecin and gold.* Acta Pharmaceutica Sinica B, 9*(1), 128–134, copyright Elsevier 2019.*

(Gao et al., 2017), and the Aggregation Caused Quench (ACQ) fluorescent probe P2 (Wang et al., 2018). ACQ is a phenomenon that results in the "switching-off" or quenching due to π-π stacking of released ACQ probes from the NC into the medium after dissolution (Hu et al., 2017), whereas AIE fluorophores have minimal toxicity and low or no quantum yield when dispersed in media, but strong lattice fluorescence intensity when aggregated in the NC (Wang, Zhang, Wang, & Liang, 2019b).

The development of NC-based cancer theranostic platforms involves the selection of an appropriate therapeutic drug molecule, imaging agent, ligand or targeting moiety, and biocompatible polymer (Li, 2006). The drug serves as the therapeutic agent, and the crystal nature should allow for the hosting and embedding of guest molecules. The rational molecular design that enables the hosting of an imaging probe inside the crystal lattice in a hybrid NC is underpinned by the principles of solid state chemistry, viz. the concept of guest inclusion (Zhao et al., 2011). Defects in crystal packing serve as spaces to allow for the inclusion of dyes and conjugated chromophores and fluorophores (Kahr & Shtukenberg, 2016). Based on this principle, hybrid NCs have been designed by the physical inclusion of fluorophores within the crystal lattice. During crystallization, random inclusions in the crystal lattice may occur due to the guest molecule having similar size or shape, or being much larger or smaller than the host molecule (Rayahin, Buhrman, & Gemeinhart, 2012). As such, hybrid drug NCs have been predominantly synthesized via bottomup methods, viz. precipitation in antisolvents (Liu et al., 2019). Depending on solubility, the imaging agent can either be dissolved in the organic growth medium or added to the aqueous antisolvent medium. Water soluble fluorophores, for example, FPR-749, fluorescein, and rhodamine B, have also been successfully incorporated into the aqueous phase for the bottomup synthesis of hybrid NCs, wherein the organic phase can be used to solubilize lipophilic fluorophores such as DiD, DiR, Cy5, and P2 (Lu et al., 2019). Imaging agents explored in this context include fluorophore, radionucleotide, or contrast metals—emphasizing the versatility of the design (Lu et al., 2019). The use of PTX, cyclosporine A, and curcumin (CUR) hybrid NCs has been predominantly reported for the preparation of cancer theranostic platforms and has enabled the study of i.v. injection distribution, intratumoral residue and distribution, and intracellular fate of NCs, which further enables the development of precision cancer theranostic platforms (Terreno, Uggeri, & Aime, 2012). Hybrid NCs are not limited to the embedding of organic fluorophores. A recent proof of concept study embedded Au into NCs of camptothecin to function as a Computed Tomography (CT) contrast agent, Fig. 8.1D,E (Hollis et al., 2019). Although in vivo studies have not been reported, good images with low noise-to-signal ratios were obtained at low concentrations of 100 ug/mL of gold, which is far below the toxicity threshold of Au nanoparticles (Hollis et al., 2019). Hybrid drug NCs are thus versatile platforms, and with further comprehensive studies could lead to the market of available theranostic products.

8.2.3.2 Hybrid nanocrystals for phototheranostic applications in cancer therapy

PDT and Photothermal Therapy (PTT) are noninvasive light treatment strategies based on tumor-ablative and necrosis that have a high potential for clinical translation (Gao et al., 2020). PTT interventions are associated with heat generation, whereas in PDT interventions apoptosis is induced by Reactive Oxygen Species (ROS), potentially triggering antitumor immunity to prevent tumor metastasis (Hou et al., 2020). There have been several improvements in multimodal imaging in PDT, PTT, and PDT/PTT combination platforms in cancer therapy, with NCs, considered key contributors (Gao et al., 2020). Inorganic nanoparticles have been well studied in phototheranostic nanoplatforms (Abueva, 2021), and are the primary focus of this discussion. Copper-based NCs serve as a photothermal agent and as a contrast agent for imaging, with the NC surface usually conjugated with a photosensitive (PS) agent to design dual imaging and PDT anticancer theranostic platforms (Dong et al., 2020). Most inorganic NCs have unique photonic, acoustic, and thermal properties, with favorable characteristic dimensions, geometry, and potential for surface modification that renders them extremely versatile. For example, Au nanoparticles have good X-ray attenuating properties that make them excellent contrast CT imaging agents in the biological milieu and have remarkable Surface Plasmon Resonance absorption intensities in the Near-Infrared (NIR) region (Rajkumar & Prabaharan, 2017). Inorganic Colloidal Semiconductor NCs (CSNCs) or quantum dot NCs can also be surface functionalized with a PS agent to enable imaging-guided PDT of cancer (Wang, Zhu, Wan, Zhang, & Zhang, 2020a). This results in the development of "all-in-one" NC platforms that make use of the intrinsic imaging and therapeutic capabilities of inorganic NCs and are considered nascent precision theranostic nanosystems with good potential for clinical translation. The principle underpinning PTT involves the NC absorbing energy at a specific wavelength to produce thermal heat, coupled with the imaging capability of the inorganic NC (Hou et al., 2020). In PDT therapy, PS nanoparticles enter tumor cells via the EPR effect at a low energy state. Under the influence of energy at specific wavelengths, the PS nanoparticles absorb energy and move to a higher excited state and produce ROS in the presence of oxygen, which results in cell death (Wang et al., 2020a).

8.3 Theranostic applications of nanocrystals

The in vivo behavior and interactions of NCs in theranostic applications are generally understood via Transmission Electron Microscopy (TEM), which assesses the presence of NCs in excised tissues, and via the tracking of inherent fluorescing drug NCs (Lu et al., 2017). However, there are limited self-fluorescing drugs

such that the real-time in vivo imaging and tracking processes enabled by hybrid NCs are beneficial for clinical diagnostics and the development of chemotherapeutics in cancer theranostics.

8.3.1 Chemotheranostics

8.3.1.1 Hybrid drug nanocrystals

NCs of PTX have proven to be versatile platforms for the study of the real-time in vivo fate of NCs via i.v. injection or injection at the tumor site (Lu et al., 2019). Although PTX-NCs-fluorophore (FPI-749) has been reported to show tumor suppression effects relative to Taxol, a variation in biodistribution data and whole-body imaging was observed with low tumor accumulation, as compared to results obtained via ^{3}H spiked PTX hybrid NCs (Hollis et al., 2013a). Results obtained for PTX-NCs with a combination of NIR and matrix metalloproteinase (MMP) bioactivatable fluorescent imaging probe were similarly affected by embedded and free fluorophores (Hollis et al., 2014), with these discrepancies attributed to the nondiscriminative nature of the fluorophore (Ni et al., 2015). Gao et al. (2017) thus utilized the AIE fluorophore TPE to investigate the mechanism of cellular uptake of PTX-TPE-NCs in human cancer cell models. TPE emits photons of energy when embedded within the PTX-NCs, and upon release has molecular motion which impedes fluorescence, thereby rendering the system self-discriminating. PTX-TPE-NCs were subsequently coated with polyoxyethylene-polyoxypropylene block copolymer, viz. Pluronic F-68, for enhanced circulation in KB (nasopharyngeal epidermal carcinoma) and HT-29 (human colon adenocarcinoma) cell lines. It was observed that PTX-TPE-NCs trafficked directly into cells in the solid state, followed by dissolution (Gao et al., 2017). It was further reported that both the surface coated and uncoated PTX embedded TPE-NCs underwent cellular internalization in both tested cell lines, however, cellular uptake was enhanced by the polymeric coating in HT-29 due to stronger transmembrane interactions (Gao et al., 2017). After an incubation period of 3 h, cells were withdrawn, resuspended in fresh media, and incubated for a further period of ca. 24 h (Gao et al., 2017). Corresponding Confocal Laser Scanning Microscopy images for the study showed diminishing emissions that were more pronounced in the KB cells, indicative of dissolution and potential exocytosis of NCs. In addition, the coated NCs afforded slower dissolution in the HT-29 cells (Gao et al., 2017). The disparity in intracellular environments can explain these differences, nonetheless, NC cellular uptake was found to be influenced by drug concentration, incubation period, and the presence of surface-coated NCs. It was further reported that ca. 20%−50% of NCs retained crystallinity after internalization, such that dissolution occurred inside the cells, directed by the type of cell and drug concentration (Gao et al., 2017). This study was limited by the water solubility of TPE, which could potentially result in recrystallization once released from the hybrid NCs, and may have contributed to the measured fluorescence intensity. In a recent study, a TPE derivative

with increased water solubility was prepared and fabricated into NCs for evaluation as an AIE probe (Zhang, Corpstein, & Li, 2020), where it was observed that the fluorescence diminished with increasing concentration of ethanol: water, Fig. 8.2A–C (Zhang et al., 2020). In vitro cellular uptake studies were also

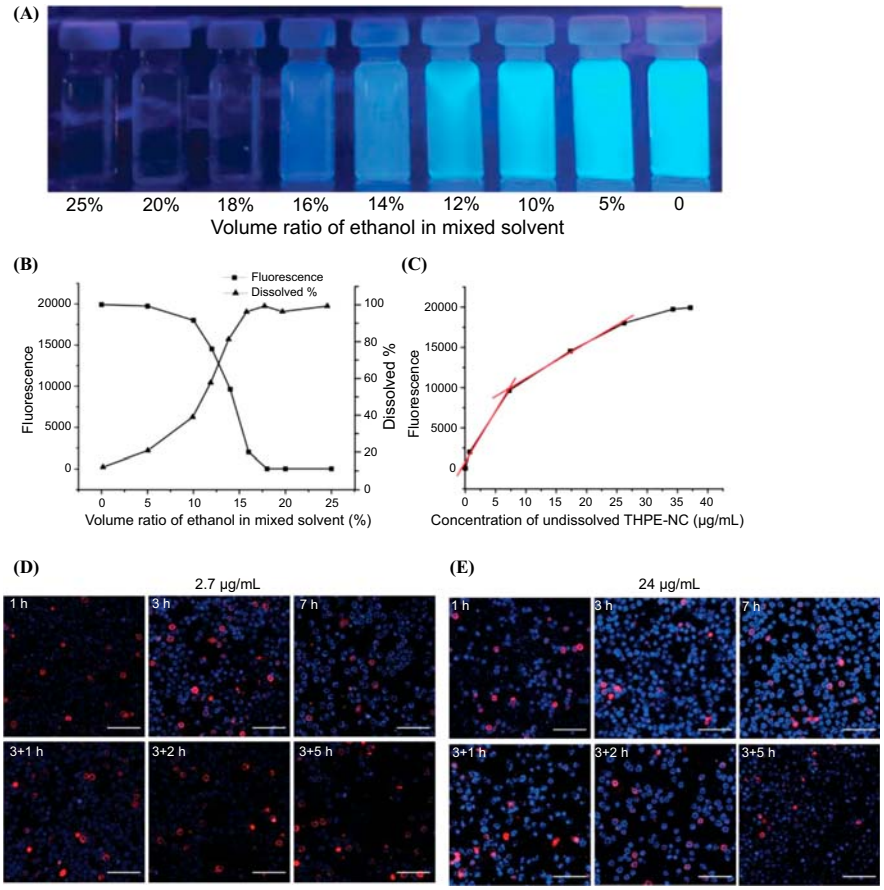

FIGURE 8.2

(A) Digital images of the fluorescence emission of a series of THPE-NCs dissolved in increasing % v/v ethanol aqueous solution, (B) Plot of fluorescent intensity, and (C) Graphical visualization of fluorescence intensity as a function of the concentration of AIE probe NCs. Confocal microscopy images at incubation periods 1, 2, 3, and 7 h, and then up to 24 h incubation in fresh media of a derivative of TPE-NCs in KB cells. (D) 2.7 μg/mL, and (E) 24 μg/mL. Blue is indicative of the derivative of TPE-NCs red depicted the cell membrane. Scale bar Z 100 mm.

Reprinted under Creative common license, copyright Elsevier Zhang, J., Corpstein, C. D., Li, T. (2020). Intracellular uptake of nanocrystals: Probing with aggregation-induced emission of fluorescence and kinetic modeling. Acta Pharmaceutica Sinica B.

conducted, Fig. 8.2D–E (Zhang et al., 2020), and concurred with the results of Gao et al. (2017), where exocytosis of intracellular water-soluble derivatives of TPE-NCs exhibited different kinetics and mechanisms compared to endocytosis. Both empirical and simulated data provided complementary evidence that cellular uptake of NCs was easier, relative to release from cells (Gao et al., 2017). Although the cited study focused on the in vitro performance of the AIE probe with no therapeutic drug integrated into the design of the delivery system, the future of hybrid NCs looks promising as the molecular design of probes is being leveraged to enhance the reliability of in vivo optical imaging.

A further example of self-discriminating hybrid NCs for two different NC particle sizes, viz. 300 and 600 nm, were reported for CUR with P2 ACQ probes (Wang et al., 2018). NCs were coated with Poloxamer (P188) and administered i.v. to promote longer blood circulation. It was reported that NCs were distributed throughout the body for up to 48 h postinjection, Fig. 8.3, with the NCs accumulated in the reticuloendothelial organs and tissues. Notably, NCs with 600 nm particle size were more suspectable to reticuloendothelial accumulation. To accurately assess real-time translocation, a reference experiment was carried out in the cited study with quenched P2, with some emissions observed at 8 h (Wang et al., 2018). Nonetheless, P2 has been shown to have high sensitivity to quenching in an aqueous environment, with a lower affinity for emission rekindling (Huang et al., 2020), and may thus serve as a future distinguishable probe for live imaging of hybrid NCs.

Mei et al. (2020) have reported a precise NC platform, wherein hybrid PTX-NCs formed the core within a coating of platelet membranes to augment efficient delivery in fortuitous postsurgery tumor metastasis. Relative to a nonbiomimetic NC platform, the precision-engineered site-specific platform demonstrated effective in vitro cellular uptake and antitumor activity, coupled with high drug loading and lower systemic toxicity due to efficient targeting (Mei et al., 2020).

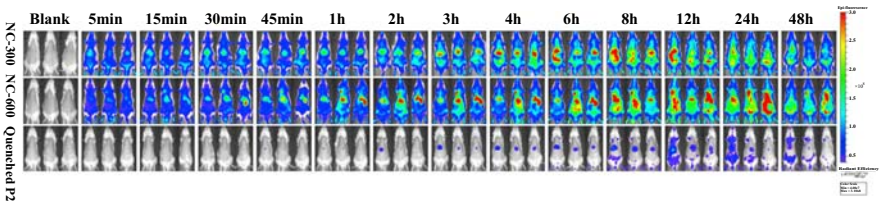

FIGURE 8.3

Exploration of in vivo fate of NCs using live imaging postintravenous dosing of CUR NCs of 300 and 600 nm respectively. For validation, a quenched P2 system was used in a parallel experiment in a rat model.

Reprinted with permission copyright Elsevier 2018. Wang, T., Qi, J., Ding, N., Dong, X., Zhao, W., Lu, Y., et al. (2018). Tracking translocation of self-discriminating curcumin hybrid nanocrystals following intravenous delivery. International Journal of Pharmaceutics, 546*(1), 10–19.*

Shen, Shen, Zhu, and Yuan (2021) have recently investigated the impact of integral NCs on overall bioavailability in a rat model. Quercetin hybrid NCs with mean particle sizes of 280 and 550 nm were embedded with an environment discerning ACQ-probe P2. It was reported that the nondissolved NCs remained in vivo for up to ca. 48 h post oral and i.v. dosing in the rat model, Fig. 8.4A,B (Shen et al., 2021), and it was found that the nondissolved NCs added to the oral bioavailability of the drug by ca. 40.27% and 50.65% for the 280 nm and 550 nm NCs respectively.

Based on cited studies, the hybrid NC premise provides a versatile platform or tool for therapy and in vivo imaging of NCs. Although the conjugation of PTX with imaging probes and formulation of nanocomposites with NCs are well reported (Guo et al., 2017; Wang et al., 2017), the hybrid drug NC platform offers a simple fabrication route to obtain both imaging and therapy. Nonetheless, targeted systems should also be considered to prevent or minimize side effects.

8.3.1.2 Multifunctional cellulose and iron based nanocrystals

Polysaccharide-based CNCs offer the potential for surface multifunctionality bestowed by reactive hydroxyl groups, enabling covalent or noncovalent bonding of functionalized groups for targeting ligands, labeling via image probes, and for drug loading; resulting in a versatile NC platform for theranostics (Raja et al., 2021; Seabra et al., 2018). CNCs also have characteristic rod-like shapes, which allow for high length-to-volume aspect ratios and unique cell uptake. Ribbon NCs have also been reported in the literature (Elazzouzi-Hafraoui et al., 2008), but to the best of our knowledge, no theranostic applications exist for these NCs. CNCs were recently investigated at a preclinical level for dual chemotherapy and radiotherapy in mice allograft in a lung metastasis model (Imlimthan et al., 2021). The surface of the CNCs was covalently linked to a complex of the theranostic radioisotope of ^{177}Lu and capped with polypeptide (poly-L-lysine) for adsorption of a therapeutic drug, Fig. 8.5A (Imlimthan et al., 2021). Biodistribution studies

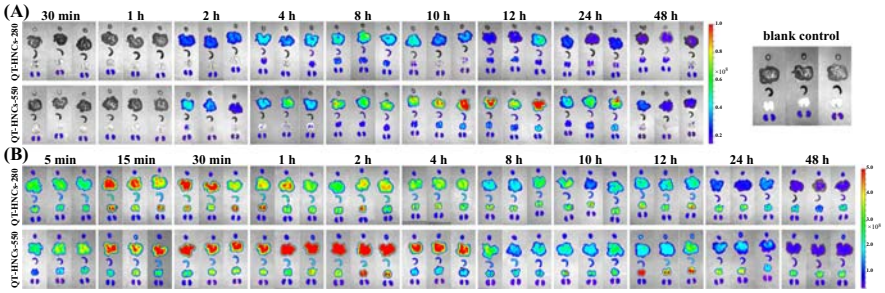

FIGURE 8.4

Imaging of excised RES organs after (A) Oral, and (B) Intravenous dosing.

Reprinted under Creative Common License, copyright Elsevier Shen, B., Shen, C., Zhu, W., & Yuan, H. (2021). The contribution of absorption of integral nanocrystals to enhancement of oral bioavailability of quercetin. Acta Pharm Sinca B.

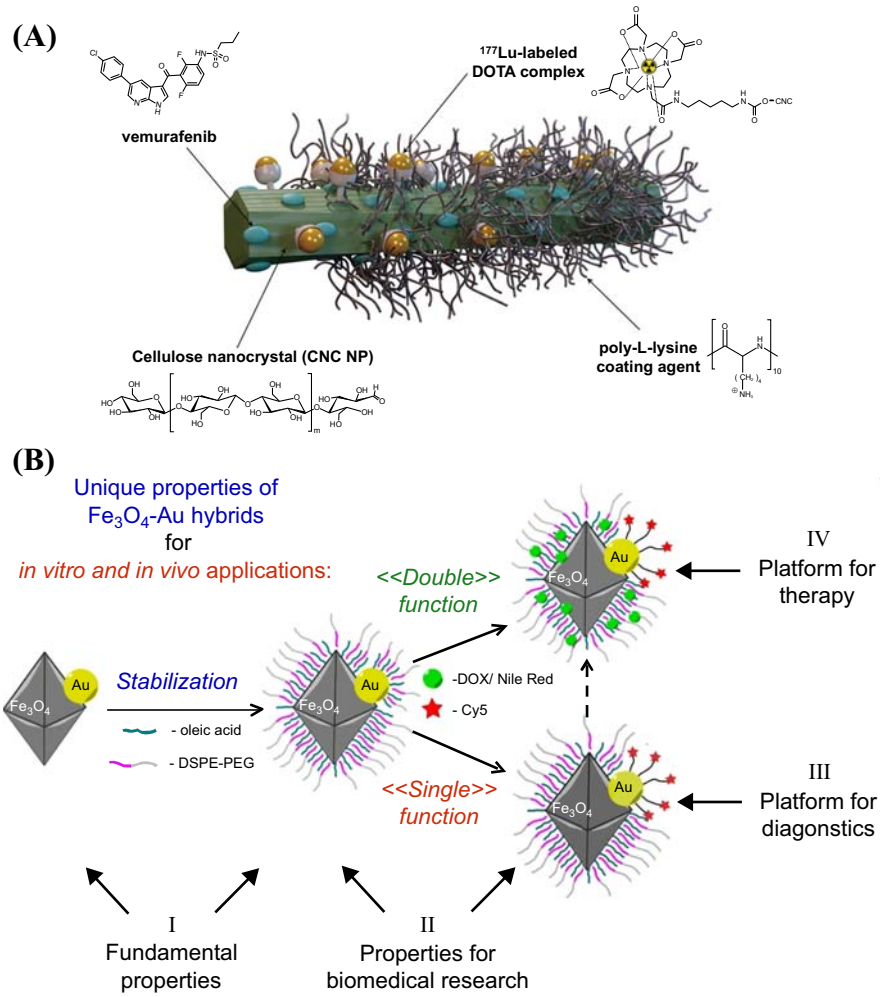

FIGURE 8.5

(A) Multifunctionalization of a CNC (*green rod*) derived from cotton with surface adsorption of Vemurafenib and surface conjugation of DOTA-NH2 for completion to ^{177}Lu surfaced and capped with poly-L-lysine. (B) Design of a Fe_3O_4 hybrid NC platform for therapy and diagnosis.

(A) Reprinted under Creative common license, Wiley 2021 Imlimthan, S., Khng, Y. C., Keinänen, O., Zhang, W., Airaksinen, A. J., Kostiainen, M. A., et al. (2021). A theranostic cellulose nanocrystal-based drug delivery system with enhanced retention in pulmonary metastasis of melanoma. 2007705, *1–23. https://doi.org/10.1002/smll.202007705 and (B) Reprinted under Creative common license, Nature 2018 Efremova, M. V., Naumenko, V. A., Spasova, M., Garanina, A. S., Abakumov, M. A., Blokhina, A. D., et al. (2018). Magnetite-gold nanohybrids as ideal all-in-one platforms for theranostics.* Scientific Reports, 8*(1), 11295.*

were further conducted via measurement of ionizing radiation in ex vivo samples. Although in vivo imaging was not reported, the use of long-lived ^{177}Lu radioisotopes or radiotherapy endowed by beta emission is a promising platform that highlights the versatility of CNC platforms towards surface functionalization.

Ferrite NCs are sensitive Magnetic Resonance Imaging (MRI) agents that are also multifunctional nanoplatforms (Li & Zhang, 2019). Mn and Zn doped Ferrite NCs (MZF) have been reported to show improved biocompatibility, with the ability to adjust the magnetic moment towards more accurate MRI by varying the composition of the doped metals (Xie et al., 2014). The coated MZF NCs allowed for encapsulation of PTX and integrin $\alpha v\beta 3$ targeting (RGD) peptides, with real-time MRI of breast cancer tumor-bearing mice indicating that serval injections were required for effective magnetic hyperthermia (Xie et al., 2014). This suggested that the NCs should be further modified with appropriate moieties to target tumor vasculature and tumor cells for improved overall accumulation. Although this platform is still in the early developmental phase, Mn and Zn doped iron oxide NCs can potentially serve as high contrast probes for the design of biosensors for the detection of viruses and variants thereof. Fe_3O_4 NCs (Fig. 8.5B) have also been surface modified with either fluorophores or fluorophore and a drug to enable concurrent imaging for the study of the in vivo fate of drug and carrier (Efremova et al., 2018). Notably, the hexagonal shape was reported to sustain the inherent magnetism of the NCs (Efremova et al., 2018).

8.3.2 Photodynamic therapy and photothermal therapy theranostics

Inorganic-based NCs are versatile due to the ability to design corresponding platforms incorporating 2 or more imaging modalities, viz. CT imaging, fluorescence, and high-contrast X-ray imaging, with a combination of PTT/PDT and chemotherapy. Different imaging modalities can also be combined into one platform. Copper-based nanosystems are considered an emerging platform for theranostics (Dong et al., 2020). For example, Copper Sulfide (CuS) nanoparticles inclusive of NCs are versatile and adaptable platforms as CuS has optical and electronic attributes that enable it to act as a contrasting agent for MRI, as well as intrinsic fluorescence for bioimaging (Poudel et al., 2019). CuS can also produce heat and ROS for multimodal cancer therapy, with a recent study on CuS-NCs demonstrating that the thermo- and photo-responsive behavior of this material endowed trimodal imaging, photothermal therapy, and Chemo-PTT (Poulose et al., 2015). Cu_2S-NCs can also be coated with PEG-lipid and FA ligands to minimize aggregation and improve circulation and biocompatibility (Poulose et al., 2015). Wang et al. (2015) conducted a recent study conceptually similar to Poulose et al. (2015) that leverages the inherent NIR responsive properties of CuS-NCs for PDT and PTT platforms. Stable plasmonic NCs were assessed for melanoma therapy

using the relatively safer NIR, coupled with the enhanced ability to reach deeper lying diseased tissue with a better 3D resolution of the tumor (Wang et al., 2015). Fabrication methods that give uniform shape dispersity and size of CuS-NCs have also been reviewed in the literature, and have concluded that future clinical translation can only be released through standardized fabrication methods (Giancaspro et al., 2021). Lv et al. (2016) reported on CuInS/ZnS quantum dot core-shell NCs for intrinsic fluorescence and Multispectral Optoacoustic Tomography (MSOT) dual-modal imaging ability for high-resolution imaging, which included an assessment of the penetration and retention of the platform at the tumor site. Yang et al. (2019). recently reported on biocompatible $CuFeSe_2$ (CSNCs) nanoplatform coated with hydrophilicity bestowing PDA, and loaded with Bovine Serum Albumin (BSA) and ascorbic acid, 2,2-azobis[2-(2-imidazolin-2-yl)propane] dihydrochloride (AIPH) to produce free radicals for image-guided PDT, coupled with NIR-II-induced therapy for overall enhanced cancer therapy.

Inorganic-based NCs can also serve as imaging probes and Au NCs have been decorated on CuS in this regard. A recent study by Wang et al. (2020b) is conceptually built on this approach, using galvanic exchange mediated template growth to minimize the use of chemical reducing agents for the decoration of Au NCs on CuS nanoplates. The nanocomposite-based dispersions were found to be relatively stable post i.v. injection into the tumor site in a mice model, imaged via CT and thermal imaging, followed by thermal ablation with NIRII laser irradiation (Wang et al., 2020b). However, NIR-responsive anisotropic Au NCs have shown a lack of photothermal stability (Poudel et al., 2019). As such, alternative platforms for bioimaging have been investigated, including the doping of established plasmonic Au NCs into copper chalcogenide NCs, and is considered a promising adaptive theranostic platform (Ding, Li, & Jiang, 2020).

Although multifunctional copper NCs have been extensively investigated for cancer diagnosis and therapy, clinical translation has been greatly impeded by the reticuloendothelial system accumulation and toxicity concerns (Dong et al., 2020). Biocompatible PEGylated bismuth NCs derived from biocompatible Bi is a good attenuator of X-rays, and can potentially function as a CT contrast agent (Li et al., 2013). Moreover, Bi-based NCs are NIR responsive and display good photothermal conversion for PTT. Li et al. (2017). applied PEGylated bismuth NCs for three imaging modalities, viz. high-contrast CT, photoacoustic (PA), and infrared thermal (IRT). In vivo imaging in tumor-bearing mice revelated photothermal ablation was efficiently applied to tumors, Fig. 8.6 (Li et al., 2017). Chalcogenide-based NCs are also considered to be quite versatile, and have been used for the preparation of NIR responsive lipid-PEG coated Cu/Bi chalcogenide (Cu_3BiS_3) for combinational PTT/PDT and X-ray imaging of a subcutaneous tumor-bearing mice model (Veeranarayanan et al., 2018). Notably, ultra-low laser intensity resulted in ROS formation, resulting in the hindrance of angiogenesis with relatively no skin tissue scarring, which can be interpreted as a promising step toward the clinical application of image-guided PDT (Veeranarayanan et al., 2018).

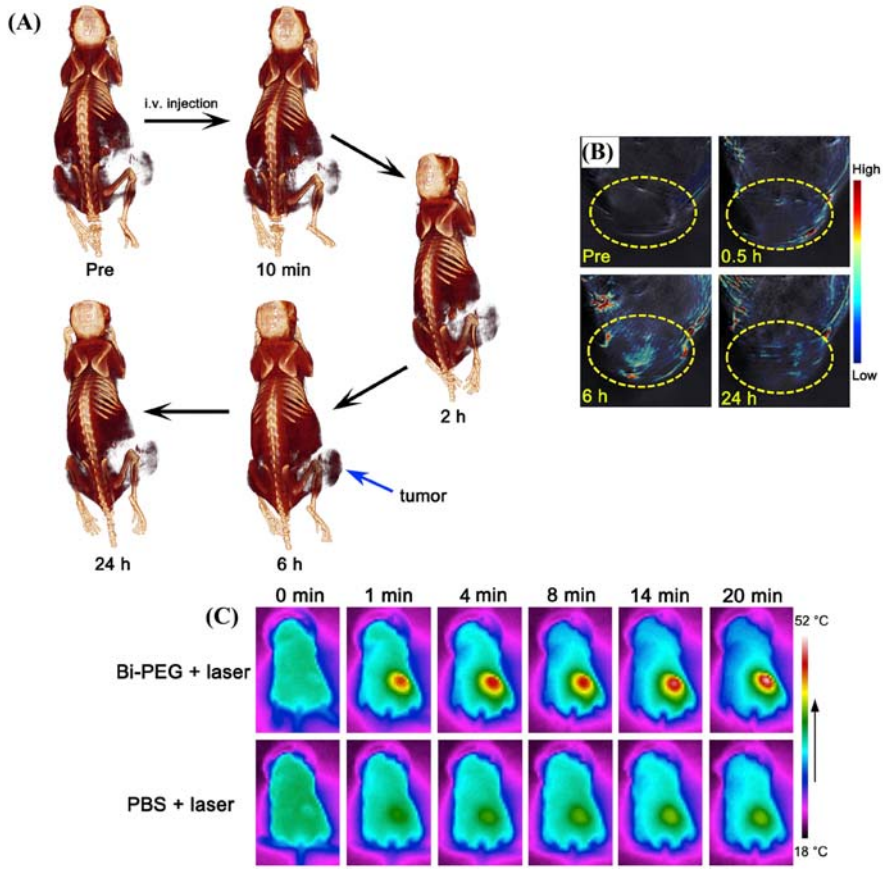

FIGURE 8.6

Illustration of tri-modal imaging: (A) 3D CT images, (B) PA images with the tumor demarcated by dotted ovals for mice bearing tumor models after intravenous dosing with PEGylated-Bi platform, and (C) IRT images for photothermal ablation induced by NIR laser.

Adapted with permission from ref Li, Z., Liu, J., Hu, Y., Li, Z., Fan, X., Sun, Y., et al. (2017). Biocompatible PEGylated bismuth nanocrystals: "All-in-one" theranostic agent with triple-modal imaging and efficient in vivo photothermal ablation of tumors. Biomaterials, 141, *284–295, copyright Elsevier.*

A study reported on the use of dopants by exchanging iron with metal ions, for example, Co and Gd, for improving transversal and longitudinal relaxivities for MRI and using Bi and Au as contrast agents (Luengo et al., 2019). Photothermal therapy of Au NCs at relatively low doses has also been reported for multimodal high sensitive Surface Enhanced Raman Scattering imaging, high-resolution PA imaging, and live Mr imaging (Huang et al., 2015). Several studies have also examined the use of a Lanthanide-doped inorganic upconversion (UCN) NC platform

due to higher penetration dept for irradiation, fluorescence imaging ability, high spatial resolution, and low signal-to-noise ratios, and biocompatibility (Peng et al., 2020). However, these systems are limited by not having a precise targeting mechanism for the effective localization of UCN NCs within the diseased tissue. A further study has investigated the use of the enzymatic degradation of Cathepsin B responsive peptide-functionalized on the surface of UCN NC (Ai et al., 2016).

8.3.3 Other applications of nanocrystal platforms in theranostics

In addition to nanomedicine and tumor nanotherapeutics, NCs have also been applied to other theranostic applications. Hybrid NCs of Chloro-3-phenylsulfonylindole-2-carboxamide containing fluorophore agents were investigated for vaginal delivery of anti-HIV drugs (Gong, Patel, Parniak, Ballou, & Rohan, 2019). Confocal microscopy images revealed that the bioactive gradually moved from the cell surface to the cytoplasm after administration. Rod-like ^{177}Lutetium labeled bismuth sulfide NCs have also been applied for rilpivirine biodistribution studies and multimodal imaging in HIV theranostics (Kevadiya et al., 2020); and ultra-small quantum dots have been investigated as potential biosensors and a theranostics platform against infectious viral diseases, for example, COVID-19 (Vahedifard & Chakravarthy, 2021). CNCs have also been labeled with ultrasmall superparamagnetic iron oxide to enable MRI-guided tissue engineering (Chen et al., 2018a). Cui et al. (2016). has further reported on an NC platform comprising UCN nanoparticles of $NaYF_4$:Yb/Er/Tm NCs functionalized with an 8-hydroxyquinoline-2-carboxylic PEGlyated lipid (DSPE-PEG) to endow imaging, therapy, and biocompatibility for Alzheimer's Disease (AD) applications. Notably, NaYF4:Yb/Er/Tm NCs demonstrated targeting and sequestration of Copper (II) cations via Cu^{2+}—Aβ42 complexes (Cui et al., 2016). A further adapted NC platform for AD theranostics involved mesoporous silica nanoparticles that were designed as a multifunctional vehicle for surface-bound Ceria NCs and iron oxide NCs, and for the loading of methylene blue and a PET tau tracer (amino-T807) (Chen et al., 2018b). The antioxidant properties of Ceria NCs enabled therapy, with MRI enabled by the iron oxide NCs. Although the corresponding in vivo studies showed promising results, further studies are required to assess the neuroavailability of the nanoplatform to enable translation into clinical reality. Nonetheless, the cited studies highlight the versatility of NC platforms towards targeted and theranostic potential in general, supporting the preceding discussions that were focused primarily on cancer theranostic platforms.

8.4 Conclusions and outlook

NCs are versatile nanomaterials for the development of theranostic platforms. This is primarily due to the unique physicochemical properties of these materials,

viz. particle size, surface area, morphology, and magnetic, thermal, and optical properties; and additional functionality that can be induced by judicious functionalization of the NC surface, viz. bioimaging, targeting, and controlled drug release. The NC approach has been used for the precision molecular engineering of diverse platforms comprising hybrid drug NCs, cellulose, and several inorganic NC platforms, as summarized in Table 8.1. Hybrid drug NC platforms are enabling platforms for cancer theranostics, in particular, however, most research is at a qualitative or preliminary level, requiring further studies to understand detailed cellular uptake, in vivo biosafety, imaging accuracy, and practical fabrication. For example, in-depth quantification and assessment of the in vivo fate of NCs is required in general to further platform development, and future work should involve investigating how cellular uptake varies with NC particle size and crystallinity to enable the development of standardized manufacturing techniques to aid clinical translation. Future research should also focus on NC platforms that provide accurate and reliable in vivo imaging and data. Alternate probes such as metal contrast agents, radioisotopes, and superparamagnetic particles could potentially be embedded within a drug NC network to access other noninvasive imaging modalities and enable a more reliable and deeper understanding of in vivo behavior, drug kinetics, and cellular interactions at a molecule level. Moreover, all components of a hybrid drug NC, and released drug and imaging agents, need to be independently mapped and differentiated via in vivo studies, potentially using multiple or distinguishable probes. The fabrication of drug NCs may be limited due to the chemical nature of the drug molecule, and may potentially increase the cost of a corresponding chemotherapeutic molecule. However, drug NCs can be used to tune the overall properties, bioavailability, and stability of the drug, and augment therapy for resistant tumors. Moreover, the development of hybrid NC platforms that offer controlled drug delivery and live monitoring via visualization of drug release using MRI is a path towards the development of precise nanotheranostics. Similarly, hybrid inorganic NC platforms are enabling platforms for phototheranostics in cancer therapy, but are limited by poor biodegradability, and complex synthesis, and require size and shape tailoring coupled with additional surface functionalization steps. In addition, the duration of in vivo behavior and biological interactions in inorganic NC platforms are limiting towards comprehensive validation of pharm pharmacokinetics, immunogenicity, cytotoxicity, and biosafety and require further research in this regard. Advancements in the design of NC platforms for theranostic applications should be directed towards size and shape-controlled fabrication of NCs, surface functionalization with ligands to improve residence time, leveraging of computational modeling and Artificial Intelligence machine learning for ligand surface modification with biomolecules, leveraging on multimodal imaging for holistic patient therapy and diagnosis, and the harnessing of high content quantitative imaging for in vitro studies to support further quantitative measurements to fully unravel the in vivo fate of the hybrid NC platforms.

Table 8.1 Summary of engineered hybrid NC platforms.

NC core	Imaging modalities	Size and shape	Imaging agents	Theranostic Application	References
Hybrid nanocrystals for Chemotheranostic applications					
PTX	OI NIRFI	200 nm (SEM) rod-like	FPR-749	Distribution via i.v. injection.	Hollis et al. (2013a)
PTX	OI NIRFI	380 ± 140 nm (SEM) 282 ± 21 (DLS) rod-like	FPR-648 MMPSense 750 FAST	Distribution via i.v. injection.	Hollis et al. (2014)
PTX	OI NIRFI	120 nm (DLS) rod-like	DiR	Intratumoral residue and distribution.	Lin et al. (2015)
PTX	OI NIRFI	150–200 nm (DLS) rod-like	DiD	Intratumoral residue.	Hu et al. (2015)
PTX	OI NIRFI	10 nm dots	Cy5	Distribution via i.v. injection. Cellular uptake.	Ni et al. (2015)
PTX	OI NIRFI	270.26 ± 21.09 nm to 432.97 ± 51.94 nm spheres	DiR	Distribution via i.v. injection.	Mei et al. (2020)
CPT	CT	10 nm (Au)	Au	–	Hollis et al. (2019)
PTX	OI NIRFI	280 ± 6.2 nm 550 ± 13.5 nm rod-like	TPE	Intracellular fate.	Gao et al. (2017)
CUR	OI NIRFI	280 and 580 nm rod-like	P2	Tracking translocation.	Wang et al. (2018)
Cellulose	SPECT	9–14 nm 136–158 nm length rods	^{177}Lu-DOTA	Cellular uptake and internalization and ex vivo biodistribution.	Imlimthan et al. (2021)
Mn–Zn Fe_3O_4	MRI	14 nm (core), 166 nm spherical	Fe_3O_4	Tumor vascular localization and tissue accumulation.	Xie et al. (2014)

Hybrid nanocrystals in phototheranostic applications in cancer therapy

Cu_2S	PAI, X-ray CT	15−20 nm spherical	Cu_2S	PTT and Chemo-PTT Intracellular drug trafficking.	Poulose et al. (2015)
Cu_{2-xS}	Infrared thermal imaging	6.5 ± 0.4 nm spherical	$Cu_{2-x}S$	Photothermal efficacy.	Wang et al. (2015)
CuInS/ ZnS	MSOT OI NIRFI	25 and 80 nm, spherical	CuInS/ZnS	PTT/PDT 660 nm In vivo distribution after injection at the tumor site.	Lv et al. (2016)
$CuFeSe_2$	Infrared thermal imaging	15.2 nm (core) 21.5 nm (coating BSA) spherical	$CuFeSe_2$	Image-guided PDT.	Yang et al. (2019)
Au-CuS	CT	10−45 nm Irregular NCs on hexagonal CuS	Au	PTT 1064 nm NIR laser Distribution of Cu post intratumoral injection.	Wang et al. (2020b)
PEG- Bi	CT	~41 nm hexagonal	Bi	PTT 808-nm laser Distribution via i.v. injection.	Li et al. (2017)
Cu_3BiS_3	MicroCT	12 ± 4 nm polygonal/ spherical	Bi	PTT 800 nm NIR laser. PDT 808 nm @(10 mW).	Veeranarayanan et al. (2018)

Bioactive: CMT, *chemotherapy;* CPT, *camptothecin;* CUR, *curcumin;* PTX, *paclitaxel. Therapy:* PDT, *photodynamic therapy;* PTT, *photothermal therapy. Imaging modality:* MRI, *magnetic resonance imaging;* MSOT, *multispectral optoacoustic tomography;* NIRFI, *near-infrared fluorescence imaging;* OI, *optical imaging,* PAI, *photoacoustic imaging. Imaging agents:* DiD, *1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine perchlorate;* DiR, *1,1-dioctadecyltetramethylindotricarbocyanine iodide.*

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CHAPTER

Niosome as a promising vesicular tool for therapy and diagnosis

9

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9.1 Introduction

Vesicles that are prepared with the help of nonionic surfactants are called niosomes (NSVs). The NSVs depict quite a resemblance to liposomes as far as the structure and their physical properties are concerned (Kaur, Garg, Singla, & Aggarwal, 2004; Paecharoenchai et al., 2014; Panda et al., 2019) even though the methods of preparation are similar to liposomal preparations (Kumar panda, Gour, Saraf, & Jain, 2019) Vesicles comprising one or more surfactant bilayers confining aqueous spaces are called nonionic surfactant vesicles or NSVs. The NSVs are popular carriers because of the various advantages over liposomes such as greater chemical stability, better skin permeation potential, sustained-release characteristic (Kaur et al., 2004; Uchegbu & Florence, 1995), quite economic due to the ease of access and handling of the formulation ingredients, biocompatible and non-immunogenic (Kaur et al., 2004). In niosomal formulations, the derivatization of surfactants is quite easy. Various physiochemical characteristics and thermodynamic parameters like the hydrophilic-lipophilic balance (HLB), the structure of the molecule, etc. are the primary factors exerting a significant role in the development of the vesicular system (Marianecci et al., 2014). A few other factors, which are, ought to be considered while formulating NSVs are lipid chain length, chain packing, and asymmetry of the membrane. The surface energy and chemical energy contribute to the energy needed for the formation of vesicles with amphiphilic molecules (Verma et al., 2019). On hydration, the monomers of nonionic surfactant associate with vesicular structure due to the high interfacial tension developed between water and the hydrocarbon part of the amphiphile. Simultaneously, the steric, hydrophilic, and/or ionic repulsion amidst the head groups ascertain the contact of these groups with water. The concentration of the monomer and temperature depict a very essential role in the formation of the vesicle (Verma et al., 2020a).

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The niosomal drug delivery systems have a size range from 10 to 1000 nm. These nonionic surfactant vesicular systems have amphiphilic characteristics, which leads to the encapsulation of hydrophilic drugs in the core and hydrophobic drugs within the bilayer structure (Tiwari, Saraf, Verma, Panda, & Jain, 2018).

9.2 Benefits of niosomes over other carriers

The NSV is the surfactant-based vesicular drug delivery system. It can overcome the several drawbacks that are related to other vesicular formulations resulting in NSV as a successful drug delivery system. It is an alternative controlled drug delivery approach to liposomes in the context of sterilization, large-scale production, and stability. The niosomal drug delivery system has encapsulation efficacy of any type of drug that is, hydrophilic, lipophilic, and amphoteric (Verma et al., 2020b). The NSVs show maximum stability with greater patient compliances. In the present scenario, NSVs can be a suitable and safe drug delivery system for any type of medicament with effective bioavailability. The NSVs can serve as a reservoir for drug(s) and deliver the medicament in a controlled manner. Alteration in their components or surface modifications can permit them to effective for targeting. The advantages of the NSVs carrier system are diagrammatically shown in Fig. 9.1.

9.3 Formulation considerations for niosomes

The components of a drug delivery system are to be used in the development of a carrier system, which ought to be characterized in terms of physicochemical properties including biocompatibility.

Non-ionic surfactant-based vesicular systems are microscopic lamellar organizations that developed on the admixture of a nonionic surfactant such as alkyl or dialkyl polyglycerol ether, cholesterol (CHOL), and sometimes charge inducers with subsequent hydration with suitable aqueous media.

9.3.1 Role of nonionic surfactants

Surfactants of the nonionic category, such as sorbitan esters (Span series), polysorbates (Tween series), etc. are employed for the preparation of NSVs. These surfactants are assigned as GRAS (generally regarded as safe) excipients for the formulation of NSVs. As compared to anionic and cationic surfactants, nonionic surfactants have very less toxic potential. Nonionic surfactants are amphiphilic molecules, similar to ionic surfactants possessing two definite parts in their chemical structure, among which one is hydrophilic, and the other is hydropho-

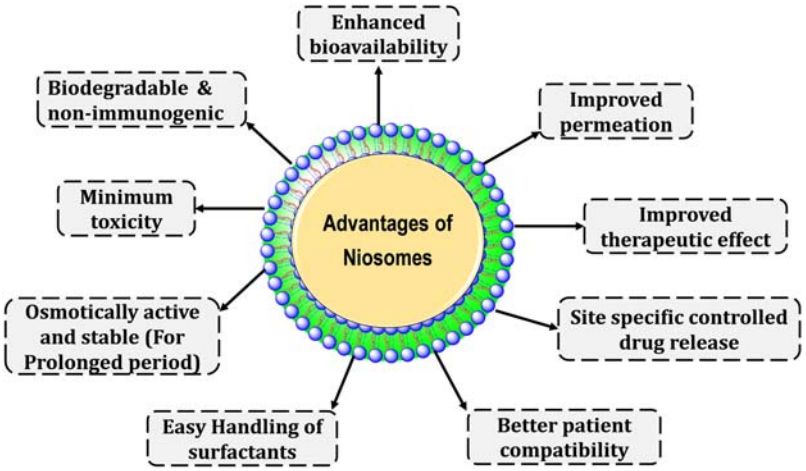

FIGURE 9.1

Advantages of niosomes.

bic. Ether, amide, or ester bonds might be employed to link these two parts of the surfactant molecule. Various types of molecules like amino acids, alkyl esters, amides, alkyl ether surfactants, etc. may be employed in the development of a nonionic surfactant-based vesicular system. In addition, the most essential and commonly used molecules amongst them are alkyl ether surfactants. Based on the properties of the hydrophilic head part, alkyl ether surfactants could be categorized into two broad categories: One is alkyl ethers in which the hydrophilic head part is made up of reiterating glycerol subunits, related isomers, or larger sugar molecules and the second category comprises those in which the repeated ethylene oxide subunits make up the hydrophilic head. The initial NSVs were developed employing CHOL and single-chain surfactants like alkyl (usually from C12 to C18) oxyethylene (Uchegbu & Florence, 1995). Polyglycerol monoalkyl ethers and polyoxylate analogs are the most useful single-chain surfactants for the formation of NSVs. Ether-based surfactants are usually employed in the formulation of noisome and their lipophilic part is comprised of monoalkyl or dialkyl chains. The recent surfactants depict higher entrapment efficiency. Ester-type amphiphilic molecules are also employed in the preparation of NSVs. They undergo degradation by enzyme esterases to form triglycerides and fatty acids. Despite the low solubility of these surfactants over the ether types, they have less toxicity (Kumari, Jain, Hurkat, Verma, & Jain, 2016). Besides, glucosides of myristyl, cetyl, and stearyl alcohols may be employed in the niosomal formulation. Although the numerous hydrophobic permutations are quite restricted presently, a broad range of hydrophilic head

groups are found in vesicle-forming surfactants, and the development of novel compounds is in evolution, based on particular vesicular structures that are required. It has been accounted that the preparation and evaluation of novel surfactants having specific physiochemical characteristics have depicted application potential in the arena of colloid pharmaceutical sciences (Saraf et al., 2020; Verma et al., 2020a).

9.3.2 Role of cholesterol

The physical properties of NSVs are affected by CHOL due to its bonding with the surfactants. It has been observed that the addition of CHOL (approx. 30−50 mol%) leads to the generation of vesicles by a few surface-active agents. The HLB value of the amphiphile molecules determines the amount of CHOL to be added. With a rise in the HLB value above 10, it becomes mandatory to raise the concentration of CHOL for compensating the effect of bulky head groups on the critical packing parameter (Bandyopadhyay, 2007; Talsma, Van Steenbergen, Borchert, & Crommelin, 1994).

CHOL enhances the cohesive forces among the nonpolar parts of the bilayer structure, thereby improving the integrity of the membrane of vesicles. The CHOL usually modulates the mechanical strength and water permeability characteristics that become principally crucial under severe stress conditions (e.g., in vivo). However, it should be noted that published outcomes are not always in agreement evidenced that the entrapment efficiency of NSVs and hydrodynamic diameter which are formulated using nonionic surfactants were enhanced by the addition of CHOL (Manosroi et al., 2003). CHOL is one of the commonly employed formulation additives to formulate NSVs of stable nature. CHOL stabilizes bilayer structure, avoids leakiness, as well as slows down the rate of diffusion of moiety present in the core region (aqueous) of these carrier systems from inside to the outside. CHOL is known to reduce the chances of phase transition from gel to lipid in niosomal systems resulting in the potential inhibition of leakage of drugs from NSVs (Carafa et al., 1998). In most of the niosomal formulations, the CHOL is commonly incorporated in a 1:1 molar ratio (nonionic surfactant: CHOL). However, even after the inclusion of CHOL in the formulation, the intrinsic phase transition characteristic of surfactants still affects certain properties of the vesicular system; especially the membrane permeability, encapsulation potential, and rigidity of bilayer structure (Saraf et al., 2020).

9.3.3 Role of charged molecules

A unique technique has been used for stabilizing the NSVs that is based on adding a charged moiety to the bilayer. The particles which are frequently employed for prohibiting the accumulation of NSVs, some are anionic such as Dicetyl phosphate (DCP) and phosphatidic acid, whereas stearylamine and

cetylpyridinium chloride are cationic (Uchegbu & Vyas, 1998). Usually, 2.5−5 mol.% of radicals are added in the prepared niosomal formulation since it can be suppressed by a higher amount of molecules with charge or radicals (Junyaprasert, Teeranachaideekul, & Supaperm, 2008). It has been documented that zeta (ζ)-potential over |30| mV is necessitated for complete electrostatic stabilization; potentials between |5| and |15| mV present the site of limited flocculation; whereas between |5| and |3| mV represents the flocculation with a higher degree. So, the probability of particle aggregation rarely happens because of charged moiety (high zeta potential) and their electric repulsions. Though this phenomenon cannot be so firmly implemented for systems having steric stabilizers due to the adsorption of such steric stabilizers, shift in the shear plane of the particle, and decreased zeta potential (Panda, Verma, & Jain, 2020; Verma et al., 2019) Moreover, the strong dependence of the zeta potential on the ions found in the medium should be considered (Heurtault, Saulnier, Pech, Proust, & Benoit, 2003). For the enhancement of drug encapsulation efficiency and improvement of penetration via a different barrier, the charged species could be incorporated into the structure of NSVs (Sennato et al., 2008).

9.4 Development of niosomes

9.4.1 Vesicle preparation

There are different techniques reported in the literature for the preparation of a niosomal drug delivery system. The formulation of NSVs is generally easier due to the resistance of surfactants against oxidative degradation. The temperatures for hydration are working for the niosomal formulation is ought to be above the phase transition temperature of gel to the liquid system. The procedure of development of vesicular structure necessitates the energy input and all the methods implemented mainly hydration of a blend of the surfactant: CHOL at raised temperature followed by optional size reduction to achieve a colloidal system (Lasic & Papahadjopoulos, 1998). Further, the raised temperature, which is employed, does not portray an issue, since the nonionic surfactant is more stable than the phospholipid (Verma et al., 2019). Moreover, the hydration temperature can be selected based on the melting point of the drug.

9.4.2 Methods of preparation

Numerous techniques like the reverse phase evaporation, organic solvent injection, thin film hydration, the bubble method, etc. have been earlier reported for the development of niosomal formulations (Jain & Jain 2016a, 2018; Uchegbu & Vyas, 1998).

9.4.2.1 Thin film hydration method

The NSVs using the thin film hydration method can be prepared using the thin film hydration method with the help of either a rotary evaporator or simple handshaking. In a flask (usually round bottom) surfactant and CHOL are taken in a fixed proportion and both dissolved in chloroform and methanol mixture to get a clear solution. Then, the prepared solution is subjected to rotation either by hand or in a rotary evaporator to obtain a film onto the surface of the flask. The temperature and rotation speed should be optimum for the formation of the thin film. When the film becomes completely dry, a suitable hydration medium is added and is kept overnight at 4°C for proper hydration. The next day flask is rotated for an adequate time at proper hydration temperature without applying pressure for complete hydration. In the case of handshaking, it should be rotated for a sufficient period till the complete removal of flakes from the surface of the flask and converted into niosomal suspension (El-Sayed, Hussein, Sarhan, & Mansour, 2017). Verma et al. (2019) developed the trimethyl chitosan (TMC) coated NSVs by thin film hydration method for ocular delivery of natamycin for the treatment of fungal disease. The developed NSVs were having high encapsulation efficiency as well as optimum vesicle size (Verma et al., 2019; Verma et al., 2020b).

9.4.2.2 Reverse phase evaporation method

The components for niosomal formulation are dissolved in a combination of nonpolar solvents of volatile nature that is, chloroform and ether, and the drug of interest is added in the aqueous phase. Both organic and nonaqueous solvents are mixed properly with the help of bath sonication which results in the formation of water in oil emulsion. In this technology, the fundamental principle is the evaporation of organic solvent to form NSVs. At a definite temperature and for a suitable period this emulsion should be dried in an apparatus (rotary evaporator) to get a semisolid gel with large vesicles of NSVs. Then, hydration media or buffer in small quantities is added and this semisolid formulation is subjected to sonication to get small unilamellar vesicles of NSVs (Fig. 9.2) (Zidan, Mokhtar Ibrahim, & Megrab, 2017).

9.4.2.3 Sonication method

This is another efficient method for the formulation of a NSV drug delivery system. In this technique, a buffer solution of the drug is introduced into the glass vial of 10 mL. Then, this solution is incorporated into the mixture of surfactant and CHOL in a glass vial of the same volume. The whole mixture was subjected to probe sonication at a definite time and temperature using a titanium probe to yield niosomal suspension. The prepared niosomal vesicles were usually small and unilamellar (Fig. 9.3) (Hao, Zhao, Li, & Yang, 2002; Greiner, Roohinejad, Oey, & Wen, 2018).

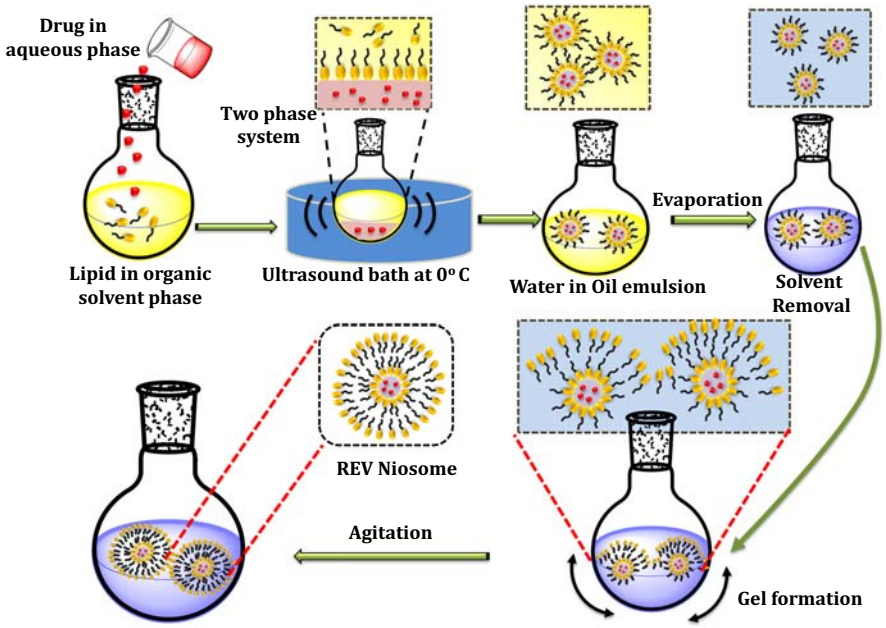

FIGURE 9.2

Reverse phase evaporation method.

9.4.2.4 Proniosome method

Nonionic surfactants coated with a water-soluble carrier molecule such as sucrose or mannitol beads to the production of NSVs from proniosomes. A thin film of components formulation component of NSVs (i.e., surfactant and lipid) are coated on water-soluble beads which are called proniosomes. These proniosomes upon hydration are converted into NSVs (Sankar, Ruckmani, Durga, & Jailani, 2010; Yasam, Jakki, Natarajan, & Kuppusamy, 2014). Various benefits of proniosome-derived NSV include the deduction in the physical instability of NSVs such as flocculation, fusion, and leakage of the active principle, and the enhancement of drug entrapment efficiency (Blazek-Welsh & Rhodes, 2001). Besides, proniosomes as being dry powder can be formulated in the form of beads or capsules as unit dosage forms, and at the point of use, they are hydrated with aqueous media and NSVs would be formed. (Fig. 9.4) (Khalil, Abdelbary, Basha, Awad, & El-Hashemy, 2017).

9.4.2.5 Heating technique

This heating technique was introduced by Mozafari to prepare the nonionic vesicles. In this method, hydration of amphipathic molecules is preceded at high temperature in an aqueous solution that has 3 v/v% of polyol. This technique is one-

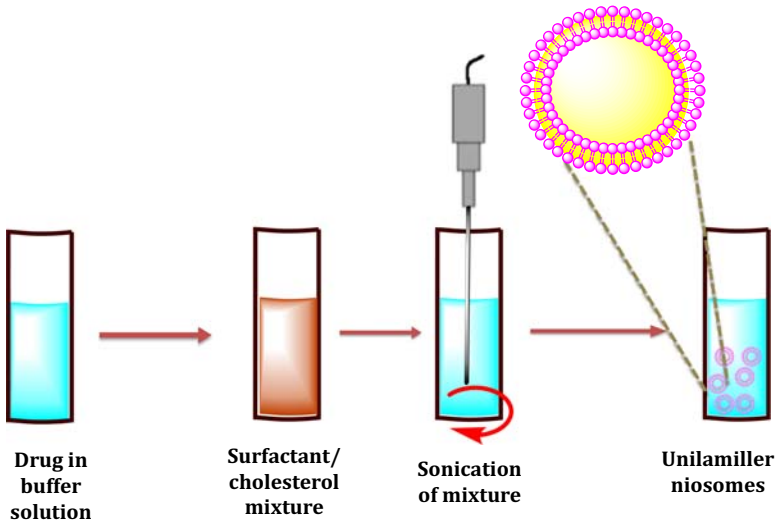

FIGURE 9.3

Sonication method.

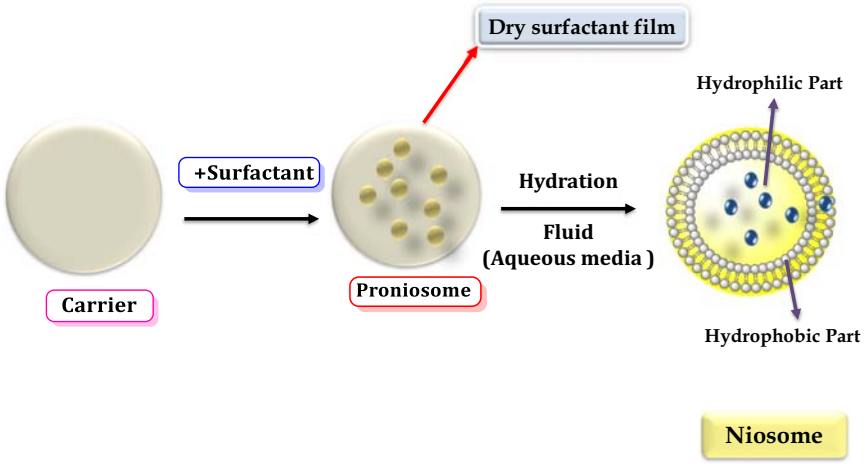

FIGURE 9.4

Formation of niosomes from proniosomes.

step, scalable, and nontoxic (no use of any organic solvent) (Mozafari, 2005). A modified heating method has also been used to develop α-tocopherol-loaded NSVs. Several parameters like type and concentration of surfactants, presence or absence of CHOL, and use of DCP, as well as different amounts of α-tocopherol

(α-TOC) have been investigated and reported the impact of them on mean vesicle size, polydispersity index (PDI), zeta potential, and entrapment efficacy. Percent entrapment efficiency and stability of the NSVs increased with decreasing the HLB value of the surfactant molecule. The physicochemical properties of NSVs are improved with an optimum amount of DCP and CHOL (Basiri, Rajabzadeh, & Bostan, 2017).

9.4.2.6 Supercritical carbon dioxide fluid (scCO2) method

Manosroi developed a new technique known as the supercritical reverse phase evaporation method with the help of supercritical fluid, for the preparation of NSVs (Manosroi, Chutoprapat, Abe, Manosroi, & Manosroi, 2012). The main advantages of this method include applications of noninflammable, volatile, and nontoxic organic solvents, one-step, and easy scaleup production of NSVs. Although, the NSVs were prepared with this method. possess vesicles with a large unilamellar structure of 100−440 nm size range. By combining this method with a few other methods like sonication or polycarbonate extrusion, the narrow size distribution of the vesicles can be obtained.

9.5 Loading of the drug in niosomes

Usually, two loading methods of drugs have been employed for the NSVs that is, direct loading and remote loading. The efficacy of these loading techniques depends on to physicochemical properties of the drug as well as other excipients. Physical trapping, ionic interaction between drugs and vesicles, nature of chemical bonds, and, adsorption mechanisms have also been influenced by the loading of drugs in the NSVs.

9.5.1 Direct entrapment (passive loading)

The direct entrapment method is the most common and simplest loading technique where drugs of lipophilic nature are solubilized in an organic solvent and water-soluble active principles are solubilized in the aqueous phase during the preparation of NSVs (Buchner et al., 1991). These methods are:

9.5.2 Remote loading (active loading)

This method increases the loading potential of the drug with the help of pH and ions. The main reason for the material transition by the membrane is the difference between pH and ion (Manconi, Sinico, Valenti, Lai, & Fadda, 2006). In the remote loading with the help of transmembrane pH gradient (in the acidic range), a basic drug in a unionized state travels through the niosomal membrane. If the pH is higher on the outside of NSV vesicles; the basic drug undergoes ionization

and precipitates, because of the lower pH inside the NSV (Jain, Tiwari, Verma, Saraf, & Jain, 2020). This method can experimentally be attained by hydrating the thin film of nonionic surfactant and CHOL with citric acid at pH 4.0 by vortexing. MLVs are frozen and thawed and suspension is vortexed after the addition of the drug. The pH is then elevated to 7.0−7.2 and heated at 60°C for 10 min (Buchner et al., 1991; Saraf, Jain, Hurkat, & Jain, 2016).

9.6 Vesicle purification

Irrespective of the drug-loading optimization process, there might be incomplete loading of the drug in NSVs by hydration (Verma et al., 2019). Therefore, it becomes mandatory to eliminate the unentrapped drug. The various techniques employed for the removal of unentrapped drugs are discussed below.

9.6.1 Dialysis

Diffusion and osmosis of solutes and fluid, respectively, are occurring across a semipermeable membrane that is the basis for dialysis. Few scientists have utilized this technique for purifying the formulation from unreacted and/or unentrapped materials. The prepared NSVs are placed into dialysis bags and the free drug molecules are allowed to dialyze against buffer saline to remove the unentrapped drug or solutes (Manconi et al., 2006; Muzzalupo et al., 2007).

9.6.2 Gel filtration

Gel-filtration on Sephadex- G75 or G50 or G25 was utilized for isolating the encapsulated NSVs from unencapsulated substances (Abdelbary & AbouGhaly, 2015; Agarwal et al., 2018; Alemi et al., 2018; De et al., 2018; Junyaprasert, Singhsa, Suksiriworapong, & Chantasart, 2012; Sandeep, Reddy, & Devireddy, 2014; Sita, Jadhav, & Vavia, 2020). Gel-filtration chromatography is a very common and versatile method that permits the separation of free drug molecules effectively with high yield (Carafa et al., 2006; Tabbakhian, Tavakoli, Jaafari, & Daneshamouz, 2006; Terzano, Allegra, Alhaique, Marianecci, & Carafa, 2005).

9.6.3 Centrifugation/ultracentrifugation

Purification of NSVs is proceeded using centrifugation and ultracentrifugation separation techniques. The centrifugation process is employed for removing the unentrapped material from NSVs, proceeding with the gradient density centrifugation technique (Manvi, Gupta, Srikanth, & Devanna, 2012; Vyas et al., 2005). Few researchers combined the earlier mentioned centrifugation processes with the Sephadex chromatography mini-columns (Minicolumn centrifugation) method

(Gupta et al., 2005; Singh et al., 2004). This purification process is suitable for numerous solutes and 92%−100% recovery can be obtained for NSVs without any dilution of the niosomal dispersion. As all these mentioned techniques depict advantages as well as disadvantages, therefore, various factors ought to be taken into account while selecting the method. For example, for industrial purposes it might be more suitable to focus efforts and resources on the attainment of high levels of drug loading, therefore avoiding separation steps Jain (Jain & Jain, 2018; Jain et al., 2018).

9.7 Characterization of niosomes

Any suitable method is adopted for the formulation of a surfactant-based vesicular carrier system. Different characterization parameters have been evaluated for this type of formulation to analyze their ability to deliver the therapeutic moiety. Nonionic surfactant-based vesicular systems is evaluated with different parameters like entrapment efficiency, drug release, vesicle size, rheological behavior, stability, and toxicological studies to consider them successful and safer drugs delivery systems (Drakulevska, Angelovska, Cvetkovski, & Stefanovska, 2018).

9.7.1 Entrapment efficiency

After the development of NSVs, the unentrapped drug is isolated by various methods like dialysis, centrifugation, etc. (Devi & Udupa, 2000; Szoka & Papahadjopoulos, 1980; Yoshioka, Sternberg, & Florence, 1994). The NSVs were lysed with help of 50% propanol or 0.1% triton X-100 and then centrifuged and supernatant fluid is analyzed for drug content using a suitable analytical technique. Then, the percent drug entrapment efficacy is calculated using the following formula:

$$\%EE = \left(\frac{\text{The total amount of drug added-drug present in supernatant}}{\text{The total amount of drug added}}\right) \times 100$$

The %EE is higher for the lipophilic drug whereas the hydrophilic drug has lower entrapment potential. The %EE is also influenced by the method employed for the development of NSVs. It was concluded (Baillie, Florence, Hume, Muirhead, & Rogerson, 1985) that NSVs prepared by ether injection method led to higher entrapment efficacies of carboxyfluorescein than the vesicles prepared by handshaking (Verma, Jain, Hurkat, & Jain, 2016). Entrapment efficacy is also affected by the concentration of surfactants and CHOL (Jain & Jain, 2016b) Higher percentage of CHOL prevents the leakage of NSVs thus it retains the high amount of encapsulated moiety.

9.7.2 Vesicle size and shape

NSVs are spherical shape vesicular drug delivery systems. Transmission electron microscopy is commonly used to determine the shape, size, and lamellarity of the NSVs. Different techniques have also been employed for the measurement of the size and shape of niosomal vesicles such as light microscopy, photon correlation microscopy, and freeze-fracture electron microscopy (Verma et al., 2020b).

9.7.3 Osmotic shock

The influence of osmotic shock on NSVs with and without DCP is assessed by observing the alteration in vesicular diameter after incubation of vesicles in a medium of different tonicity (Carafa et al., 1998) that is, 1 M sodium iodide (hypertonic), 0.9% sodium chloride (normal) and 0.5% sodium chloride (hypotonic). Niosomal suspensions are incubated in these media for a definite time and the alteration in size of vesicles is assessed by microscopy (Patel, Kumar, & Thakkar, 2012).

9.7.4 Determination of viscosity

The measurement of viscosity of niosomal dispersion is required for topical, nasal, and ophthalmic purposes where retention of NSV at the application site is beneficial. Verma et al. (2021) prepared a modified chitosan coated niosomal system for the ocular administration where the determination of viscosity of this system is played an important role in retention of preparation in the cul-de-sac for a longer period, the same time it should not affect the behavior of tear fluid and reduce the irritation of the eye. The viscosity of NSVs is measured by the Brookfield viscometer at 37°C ± 2°C (Verma et al., 2020b).

9.7.5 In vitro release

An in vitro release rate study was carried out using the dialysis tubing. A dialysis sac is washed and permitted to soak in distilled water. The noisome formulation is then pipetted into a dialysis bag composed of the tubing and sealed. The bag is then placed in 200 mL of buffer solution in a 250 mL beaker with continuous shaking at 25°C or 37°C. At different time intervals, the buffer is analyzed for the drug content using a suitable assay method (Jain & Jain, 2016b; Yoshioka et al., 1994).

9.7.6 Stability and toxicity studies

Concerning to liposomes, NSVs are comparatively stable structures; few concerns have been shown regarding the in vitro stability and in vivo toxicity profile of NSV (Moghassemi & Hadjizadeh, 2014) employment of surfactants in the preparation of NSVs may lead to toxicity. Nevertheless, no reports on the in vivo

toxicity of NSVs associated with the concentration of ether or esters surfactants employed in the preparation of vesicles have been found. The toxicity of CnEOm surfactants was studied by Hofland et al. with the help of two models (Hofland et al., 1992) which include:

1. The ciliary beat frequency (CBF) of the trachea, is significant for intranasal administration and
2. The cell proliferation of keratinocytes is essential for the transdermal application of vesicles. The toxicity of the preparation was determined by the reduction in CBF.

Azmin et al. conducted the first in vivo experiment on the NSV drug delivery system and accounted that no adverse effects were noted in the experiment carried out (Azmin et al., 1986). Rogerson et al. performed in vivo experiment in 70 male BALB/C mice and concluded that no fatalities were observed that could be attributed to the preparation. The toxic effects directly related to the drug are decreased (Rogerson, Cummings, & Florence, 1987).

9.8 Application of niosomes in different drug delivery systems

9.8.1 Niosomes in oral drug delivery

The oral route is one of the most preferred routes for the administration of all kinds of drugs. The prime problem associated with the oral delivery of NSVs is the acidic environment and the digestive enzymes (Sharma, Kushwaha, Repka, & Murthy, 2021). However, many niosomal formulations have been investigated to deliver the drugs successfully in the gastrointestinal tract. Onochie et al. developed NSVs in which benzylpenicillin was used for oral drug delivery where the microbial activity and duration of action of benzylpenicillin were improved (Onochie et al., 2013). Samyukta et al. synthesized NSVs of orlistat from proniosomes for oral drug delivery. It increased the solubility and duration of action of the drug, which was intended for controlled drug delivery action (Samyuktha & Vedha, 2011). Moghassemi et al. prepared TMC-coated NSVs of insulin to increase the permeation of insulin across the intestinal membrane (Moghassemi et al., 2015).

9.8.2 Niosomes in ocular drug delivery

Ocular delivery is mainly favored when a drug has intended to deliver in the anterior section of the eye. It has been noticed that only 1%−3% of drugs reached to desired ocular site and it has to face precorneal loss due to the formation of tear and insufficient residence time in the conjunctiva region (Biswas & Majee, 2017; Verma et al., 2020a,b). Abdelkader et al. developed a controlled release niosomal and disomal

systems to deliver the drug naltrexone to ocular sites. They found anionic NSVs are superior as compared to neutral NSVs because the anionic NSVs improved the permeation of naltrexone across the cornea (Abdelkader, Ismail, Kamal, & Alany, 2011). Zeng et al. synthesized NSVs in which they incorporated tacrolimus that was further coated with mucoadhesive hyaluronic acid. Tacrolimus has high lipophilicity and high mol. wt. (822.5 D) and hence, the corneal permeation is very poor. To solve this problem, they were coated with hyaluronic acid to improve corneal permeation. In addition to this, it also prolonged the ocular contact time (Zeng et al., 2016). Abdelkader and his team developed unique NSVs of elastic nature and nano-sized to deliver in the ocular region. They used prednisolone acetate and prednisolone sodium phosphate as model drugs. They accomplished different tests such as bioavailability and anti-inflammatory effects and ocular irritation tests. The outcomes of the experiment were compared with the results of the conventional eye drops (both suspension and solution). Besides, a modified Draize test was also performed and it was observed better ocular tolerability as well as bioavailability for prednisolones. Besides, the NSVs effectively reduced intraocular pressure due to suppressing rapid ocular absorption peaking without conceding the ocular bioavailability and the therapeutic efficacy of prednisolone (Gaafar, Abdallah, Farid, & Abdelkader, 2014).

9.8.3 Niosomes in dermal and transdermal drug delivery

The dermal route is the most preferable route used especially in skin diseases for the local delivery of a wide range of drugs. The main advantage of this route is that the drugs do not arrive in the blood circulation and hence have fewer side effects. In transdermal delivery, the drug may reach the blood circulation but the drugs do not cross the skin barrier. Both dermal, as well as transdermal delivery, have revealed that the vesicular systems including the niosomal systems are more efficient in the delivery of drugs (Manosroi et al., 2003, 2010; Marianecci et al., 2014). NSVs improve the penetration of drugs in the dermis and epidermis via the transdermal delivery system. Manosroi et al. developed niosomal systems in which gallidermin was used as a model drug for transdermal drug delivery. They demonstrated that more accumulation of the drug took place in the skin and no systemic side effects occurred (Manosroi et al., 2010). Patel et al. prepared niosomal gel for the efficient delivery of lopinavir via the transdermal route. They proved that the niosomal gel is better than ethosomal gel with the help of ex vivo permeation studies. Moreover, histopathological studies were also carried out that indicate that the NSVs are quite safer than the ethosomes. Besides, it was observed that the in vivo bioavailability was considerably better than the lopinavir oral suspension.

Sandeep et al. developed fluconazole-containing proniosomal gel for topical drug delivery. Both the ex vivo skin penetration and permeation studies were performed and the outcomes displayed a greater amount of drug had accumulated in the skin. It improved the local delivery of fluconazole which has a prolonged duration of action (Sandeep et al., 2014). Junyaprasert et al. prepared NSVs of

ellagic acid using different surfactants (span 60 and tween 60) and solubilizers (propylene glycol 400, propylene glycol, and methanol). These affect the size of NSVs, entrapment efficiency, and permeation of the drug. The NSVs prepared using propylene glycol 400 and propylene glycol gave a significantly higher concentration of ellagic acid in the epidermis as compared to methanol NSVs. It may be because of the higher entrapment efficiency and the penetration enhancing capability of propylene glycol and propylene glycol 400 (increases partition and permeation by the salvation of keratin of stratum corneum and occupying the hydrogen bonding sites). It also studied the effect of chemical penetration enhancers on the skin permeability of ellagic acid. The in vitro skin permeation studies in the human epidermis have been exhibited that found that the NSVs enhance the permeation of ellagic acid (Junyaprasert et al., 2012). Abdelbary et al. prepared NSVs of methotrexate for targeted delivery in psoriasis via topical application. This preparation has reported the highest percentage of drug deposition (22.45%) in the skin (Abdelbary & AbouGhaly, 2015).

9.9 Application of niosomes in targeting

9.9.1 Niosomes in cancer targeting

Delivery of anticancer drugs can be achieved using NSVs. This targeting could be passive (deposition of NSVs within the tumor using the special properties of the tumor cells not existing in the normal cells), physical (delivery based on specific environmental conditions like pH or magnetic fields), or active type (active uptake of NSVs by the tumor cell). Active targeting can be achieved either by modifying the structure of the surface or by attaching the ligand to the nisomes. For ligand attachment either CHOL-PEG-ligand conjugate be incorporated into the NSVs or it can be attached to CHOL or the end of the polyethylene glycol chain. NSVs bearing paclitaxel is successfully prepared for oral delivery to enhance bioavailability and stability. Lin et al. prepared PEGylated NSVs of gambogenic acid by the modified ethanol injection method. These PEGylated NSVs are used as the carrier for anticancer therapy and to enhance the stability of gambogenic acid. Sharma et al. prepared self-degrading NSV for multidrug delivery. In this work curcumin (hydrophobic) and doxorubicin hydrochloride (hydrophilic), drugs were encapsulated in NSVs for anticancer treatment. They observed release phases; in the initial phase, the release of doxorubicin was observed for the first two days and then curcumin was released for 7 days. An enhanced (synergistic) cytotoxic effect was observed against HeLa cell lines. Alemi et al. prepared cationic PEGylated NSVs for the coadministration of curcumin and paclitaxel. These NSVs reported enhanced synergistic antitumor efficacy (Alemi et al., 2018). Agarwal et al. prepared the NSV of morusin for the potentiation of anticancer therapy. They observed a pH-dependent release of the drug. At pH 7.4 release of morusin from NSVs was less as compared to the release at pH 4.5. After 120 h, the drug release was 58.1% in acidic

conditions, pH 4.5 whereas at physiological pH 7.4 only 43.3% release was obtained. It indicates high drug release can be obtained in the acidic conditions of the cancer cell (Agarwal et al., 2018).

9.9.2 Niosomes for brain targeting

Drug delivery to the brain is a very tough task due to the low permeability of blood−brain barrier. Therefore, it is quite challenging to transport the optimum concentration of therapeutic agents into the brain. De et al. have shown enhanced delivery of the drug to the brain in glioblastoma by forming smart NSVs of temozolomide. To achieve the specific targeting of gliomas, a peptide chlorotoxin (36 amino-acid containing small peptides) was identified from the venom of the scorpion, (*Leiurus quinquestriatus*) and used for surface modification. Pentamidine is an antiprotozoal drug that is also having an anti-inflammatory and neuroprotective effect on Alzheimer's disease. Its clinical efficacy is limited due to poor permeability across the blood-brain-barrier and high hepatotoxicity. To overcome these issues chitosan-glutamate-coated pentamidine NSVs were prepared for intranasal drug delivery to reach the brain, which bypasses the first-pass hepatic metabolism and blood-brain barrier (De et al., 2018).

A stable nonionic surfactant-based niosomal system was prepared by taking bromocriptine mesylate as a model drug. The prepared formulation was intended to deliver into the brain through the nasal pathway. The formulation was prepared by the ethanol injection method. The NSVs synthesized have shown their size in the desired range which is about 180 $\pm$ 4.5 nm. Besides, they have exhibited a good encapsulation efficiency that is 75.8 $\pm$ 3.6 and a zeta potential of -14.2 ± 1.8 mV. The ex vivo study was also performed using goat nasal mucosa for 24 h and compared to the drug suspension and found to be six times higher. In addition to this, the relative brain bioavailability, drug targeting efficiency, and direct transport percentage of the prepared NSVs were observed. The outcomes suggested that the niosomal formulations have a wide potential for brain targeting ability via the nasal route and help in the management of Parkinson's disease (Sita et al., 2020).

9.9.3 Niosomes for lungs targeting

Pulmonary administration of niosomal drugs through the pulmonary route offers several advantages improved permeation through mucus, sustained delivery of the drug, targeting, and better therapeutic benefits. NSVs containing glucocorticoid were prepared and evaluated for their interaction with the human lung fibroblast. These NSVs did not show any significant toxicity in the concentration range of 0.01 to 1 μM. With the help of confocal laser scanning, these niosomal systems are localized in the cytoplasm (site for glucocorticoid receptors). These NSVs were found to be efficient for drug uptake and could able to target human lung fibroblasts (Bhardwaj, Tripathi, Gupta, & Pandey, 2020). Rifampicin-containing NSVs were prepared using Span-85 and CHOL in different molar ratios. The various process variables that affect the

physical parameters and in vitro release of the drug from the NSVs were studied and optimized. Moreover, the in vivo distribution studies were also studied which found that 65% of the drug could be localized in the lungs through the niosomal preparation by controlling their size up to 8−15 μm in their diameters Jain (Jain & Vyas, 1995).

Gemcitabine and cisplatin-containing NSVs were developed the targeting lung cancer. NSVs were prepared using the common heating method and optimized by a D-optimal mixture design. The prepared formulations were characterized for particle size, PDI, and zeta potential which are about 166.45 nm, 0.16, and −15.28 mV, respectively. Further, the stability study was also performed for 90 days. It was found to be stable at 27°C with no phase separation. An in vitro drug release study was conducted by dialysis bag diffusion technique and the result indicated the controlled release of both drugs for up to 24 h. A cytotoxicity study against normal lung (MRC5) and lung cancer (A549) cell lines was studied. The results displayed that the niosomal systems had efficiently reduced cytotoxicity against both MRC5 and A549. These findings suggested that the prepared NSVs have a remarkable ability to treat lung cancer (Mohamad Saimi, Salim, Ahmad, Abdulmalek, & Abdul Rahman, 2021).

9.10 Conclusion and prospects

The NSV is an important drug delivery carrier, which can be used for the controlled, sustained, and targeted delivery of the drug. It is an emerging drug carrier because of its ability to encapsulate both hydrophilic as well as hydrophobic drugs and they have good storage stability. They have the potential to encapsulate all varieties of drugs including anticancer drugs. Not just the drug, they provide wider flexibility in the route of administration also. Their advantages over liposomes and other vesicular systems' for being nontoxic and stable create them more appropriate for drug delivery. Thus, it appears that the research in the arena of NSVs will upsurge further and may lead to a worthy market formulation in the pharmaceutical industry.

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CHAPTER

Cubosomes for enhanced drug delivery and targeting therapeutics

10

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10.1 Introduction

Nonlamellar liquid crystalline (NLC) systems have shown great promise in the field of drug delivery and theranostic systems which involve therapeutic and diagnostic approaches. NLC phases could be classified into cubic, hexagonal, and sponge internal structures (Urandur et al., 2018). Liquid crystals (LCs) the so-called mesophases are an intermediate stage between the true ordinary liquid and solid crystal phase. LCs are classified into thermotropic, lyotropic, and metallotropic mesophases. Thermotropic phases are solvent-free induced by changes in temperature, whereas lyotropic phases are solvent based. Notably, lyotropic liquid crystals are classified into lamellar, cubic, and hexagonal phases based on their internal structures. The cubic structures can exist in the P-surface (primitive), G-surface (groid), and D-surface (diamond) (Fig. 10.1) (Gaballa, El Garhy, & Abdelkader, 2020; Urandur et al., 2018).

Cubosomes are lyotropic cubic liquid crystalline nanoparticles (LCNPs) that combine the merits of both particulate and fluidic delivery systems in which they can diffuse like liquid molecules but still ordered to a certain extent just like solid phase (Nazaruk, Majkowska-Pilip, & Bilewicz, 2017). These nanosystems are based on amphiphilic lipids such as glycerol monooleate (GMO), glyceryl monolinoleate (GML), oleyl glyceryl (OG), and phytantriol (PHYT), which can easily self-assemble in water in the presence of suitable stabilizer (e.g., poloxamer 470). Cubosomes are formulated upon dispersing liquid crystalline cubic aggregates in

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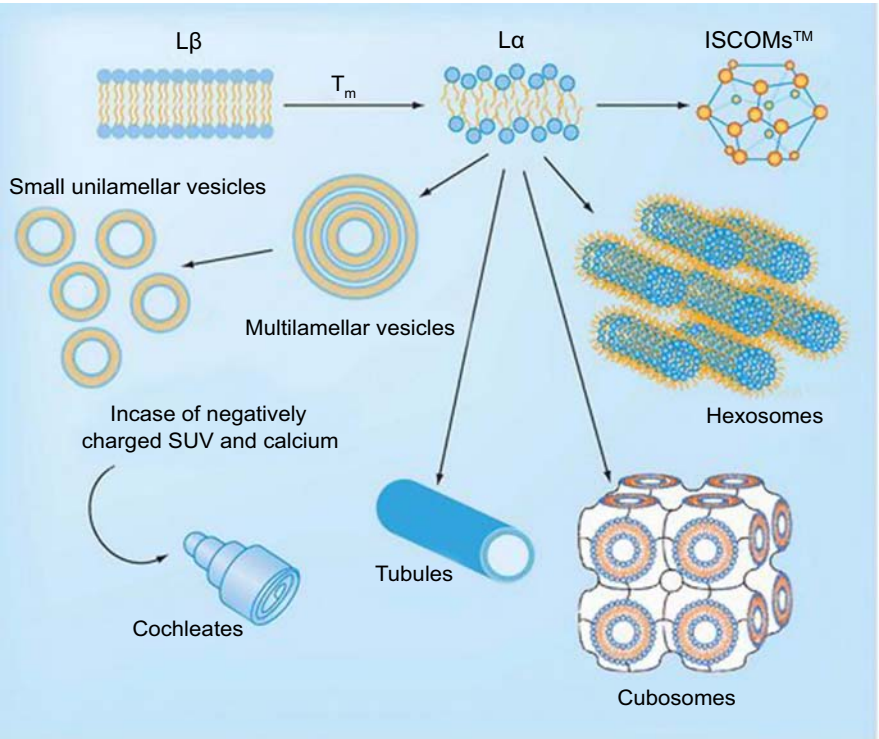

FIGURE 10.1

Schematic illustration of different lyotropic phases and liquid crystalline phases.

aqueous media. They are highly advantageous and characterized by their cost-effectiveness, ease of preparation, biocompatibility, biodegradability, bioadhesion, high internal surface area, high drug loading capacity, microstructure similarity with the parent cubic aggregates, low viscosity relative to parent cubic phases, high colloidal and thermodynamical stability with targeted and prolonged release profile (Abdelaziz et al., 2019; Freag, Elnaggar, Abdelmonsif, & Abdallah, 2016). Thanks to their honeycomb (cavernous) structures with curved bicontinuous lipid bilayer enclosing two identical, nonintersecting water channels that efficiently encapsulate hydrophilic, hydrophobic as well as amphiphilic drugs (Gaballa et al., 2020; Naveentaj & Muzib, 2020; Urandur et al., 2018). Notably, the viscosity imparted by cubosomes and their highly organized internal structures resulted in the powerful immobilization of incorporated drugs with prolonged release profiles and enhanced physical stability. Therefore cubosomes prove their superiority over other lipid-based drug delivery systems including liposomes and micelles especially in biological fluids and in vivo applications. Cubosomes could withstand excessive dilution levels in water without possible drug leakage (Gaballa et al., 2020; Nazaruk et al., 2017).

Cubosomes have witnessed a special interest as a superlative drug delivery system. They can significantly contribute to reducing the dose, minimizing toxicity, and improving therapeutic efficiency either in the form of nanoparticles, colloidal dispersion, or gels. They are widely employed in oral, ocular, transdermal, and chemotherapy drug delivery (Gaballa et al., 2020; Naveentaj & Muzib, 2020). In addition to their applications in cosmetics drive famous companies like L'Oreal and Nivea to serve trials to scale up innovative efficient and cost-effective nano cosmeceutical products (Sherif, Bendas, & Badawy, 2014).

Despite the above-mentioned advantages of cubosomes and their promising applications, their large-scale production is still a major hurdle facing scientists due to the high viscosity of cubic phases. Moreover, it is difficult to entrap large amounts of hydrophilic drugs due to excessive water content already in cubosomal structures during their formation (Naveentaj & Muzib, 2020).

10.2 Narration/the past

Early in the 1980s, the cubosome was first introduced by Larsson in his review on cubic lipid/water phases. The term "Cubosome" was based on the structural resemblance to cubic molecular crystals and liposomes. He found that cubosomes can be obtained upon the dispersion of bulk cubic structure of amphiphilic lipids in water in the presence of appropriate stabilizers. The resulting submicron particles possessed internal structures similar to the parent cubic structure. Cubosomes are self-assembled and organized in 3D models as a "honeycomb" structure. Several attempts were performed to scale up the production of cubosomes. However, it was difficult due to the high viscosity (Gaballa et al., 2020).

10.3 Theories of the self-assembling of amphiphilic lipids

The amphiphilic lipid forming cubosomes mainly comprising of polar head and non-polar tail tend to self-assemble in water into highly organized structures with one, two, or three-dimensional structures. The structures could be micelles, open lipid bilayer, closed lipid bilayer, and inverted micelles. There are two major approaches explaining the self-assembly of surfactants either the principle of opposing forces or the principle of packing parameter. In the case of cubosomes, they follow the principle of packing parameters in which amphiphilic lipids (e.g., GMO and PHYT) are capable of self-assembly into bulk cubic crystalline structures that can be furtherly dispersed into the water in the presence of appropriate stabilizer in the sake of forming our promising cubosomes (Gaballa et al., 2020).

10.4 Components of cubosomes

10.4.1 Amphiphilic lipids

Various types of amphiphilic lipid vehicles are utilized during the preparation of cubosomes including glyceryl monooleate (GMO), phytantriol, glycolipids, glycerates, and others (Freag et al., 2016).

10.4.1.1 Glycerol monooleate

Glyceryl monooleate (GMO), generally referred to as monoolein (MO), is FDA approved amphiphilic lipid that is widely used during the synthesis of cubosomes. GMO is a polar unsaturated monoglyceride that can self-assemble in water into bicontinuous cubic structures (Gaballa et al., 2020; Naveentaj & Muzib, 2020). The amphiphilic chemical structure of GMO with hydrophilic polar head (hydroxyl groups) and hydrophobic chains in the tail facilitates H-bond formation with water. GMOs are classified as GRAS (generally recognized as safe) due to their biodegradability, biocompatibility, and minimal toxicity. The biodegradability was attributed to the lipolysis of GMOs in different tissues via esterase activity. The GMO-based systems, specially bicontinuous cubic and hexagonal LC phases, were highly reported as promising drug delivery. Thanks to their unique features including their minimal toxicity, prolonged release rate, and thermodynamic stability upon exposure to excess water (Urandur et al., 2018).

10.4.1.2 Phytantriol

Phytantriol (PHYT), is an aliphatic alcohol mainly used in cosmetic products. PHYT is capable of forming a bicontinuous cubic structure in aqueous media under physiological conditions and temperature. The superiority of PHYT over GMO during the preparation of cubosomes is attributed to its improved chemical stability due to the absence of an ester bond from the backbone of PHYT, enhanced skin penetration, high moisture retention, and commercial availability with high purity (95%). Moreover, PHYT-based systems provide sustained release profiles for hydrophilic drugs thus considered a promising drug delivery system (Ali, Noguchi, Iwao, Oka, & Itai, 2016).

10.4.2 Stabilizers

It is an essential prerequisite to use a suitable stabilizer during the synthesis of cubic liquid crystalline nanoparticles (cubosomes) to guarantee their stability and prevent the re-coalescence of dispersed particles when subjected to excessive dilution (Abdelaziz et al., 2019). Herein, the stabilizer acts as an electrostatic barrier between particles in the lipid-water system without affecting the cubic liquid crystallinity. Among the widely used stabilizers is the water-soluble triblock copolymer pluronic F127 (Poloxamer 407) which is mainly composed of polyethylene oxide (PEO) and polypropylene (PPO) arranged in PEO-PPO-PEO configuration so that PPO represents

hydrophobic, whereas PEO represents a hydrophilic segment. Notably, the stabilizing effect of F127 in cubosomes is exerted via adsorption of hydrophobic (PPO) onto the surface of the particles, whereas hydrophilic (PEO) protrudes to outside and exposure to aqueous media thus maintaining steric shielding (Naveentaj & Muzib, 2020).

10.5 Manufacture of cubosomes

There are two major techniques for the preparation of cubosomes, the top-down and bottom-up, both techniques depend mainly on the presence of appropriate stabilizer such as Poloxamer 407 (Pluronic F127) to avoid cubosomal dispersion aggregation (Fig. 10.2) (Abdelaziz et al., 2019). The choice of optimum preparation approach is based on stability, biocompatibility, and optimal drug release. Worth mentioning that

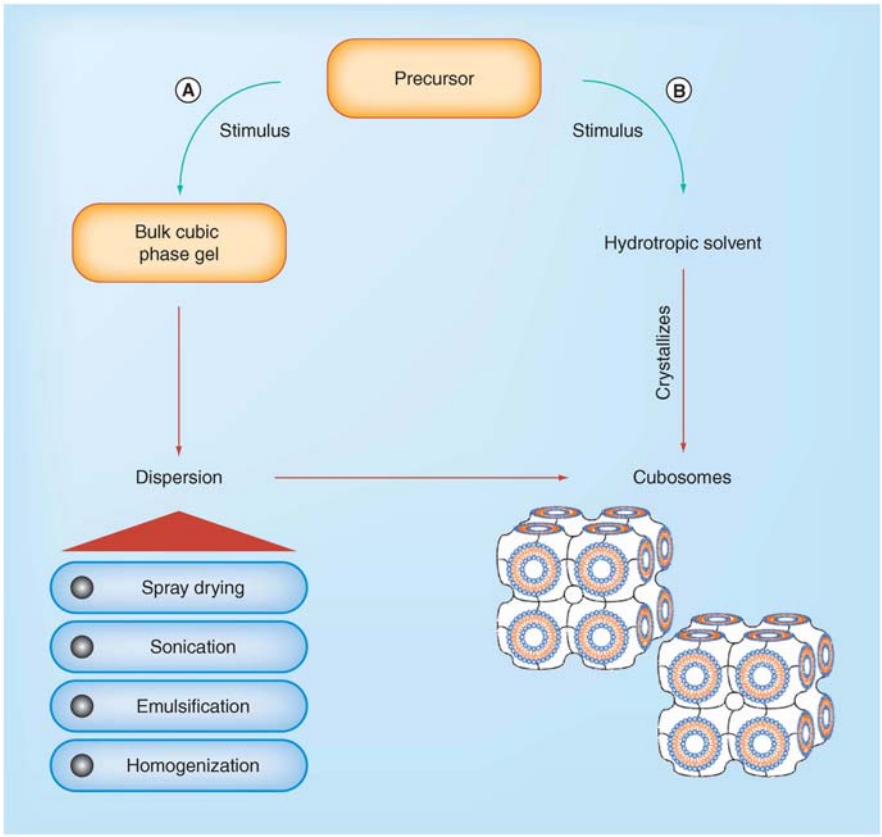

FIGURE 10.2

Schematic illustration of different approaches to preparing cubosomes (A) Top-down, (B) Bottom-up (Shanmugam & Banerjee, 2011).

the morphology and the internal structure of cubosomes could be determined via different techniques including polarized microscopy, cryo-transmission electron microscopy (TEM), and small angle X-ray scattering (SAXS) to confirm their cubic liquid crystalline structure (Freag et al., 2016).

10.5.1 Top-down technique

The top-down approach is widely involved during the preparation of cubosomes. This is accomplished via two main steps. Firstly, the formation of self-assembled bulk viscous cubic aggregates upon hydration of cubosomes forming lipid (amphiphile) with appropriate stabilizer. Secondly, the cubosomes are obtained upon the dispersion of the bulk cubic aggregates into aqueous media via mechanical shearing or high energy input (e.g., high-pressure homogenizer or ultrasonicator). This approach guarantees the cubosomes stability against aggregation for up to a year. However, this approach can not be used on large scale, especially for thermolabile bioactive agents such as peptides and proteins due to the high energy input required to maintain the stability of cubic viscous aggregates during their dispersion into cubosomes. The heat generated during preparation may damage the amphiphilic molecules as well as encapsulated payloads (Naveentaj & Muzib, 2020; Urandur et al., 2018).

10.5.2 Bottom-up approach

The bottom-up approach is known as the solvent dilution method. This method depends mainly on the appropriate selection of hydrotrope which is capable of solubilizing water-insoluble lipids (amphiphiles) and reducing their viscosity to form lipid precursors thus inhibiting liquid crystals production at high concentrations. GMO swells in water forming various lyotropic liquid crystalline structures including cubic, hexagonal and lamellar phases based on the concentration of GMO and temperature used. Therefore, hydrotropes play a major role in improving the solubility and stability of cubosomes. Notably, the dispersion of lipid precursors composed of cubosomes forming lipid, stabilizer, and a hydrotrope into excess water requires minimal energy input. Therefore, this method can be used safely for the production of thermolabile-loaded cubosomes with promising long-term stability in which stabilizers are dispersed on the surface of nanovesicles. However, due care is essential during using hydrotropes as they might induce toxicity (Naveentaj & Muzib, 2020; Urandur et al., 2018).

10.6 Applications of cubosomes in drug delivery

Cubic liquid crystalline phases (Cubosomes) open the frontiers to overcome pharmaceutical challenges facing lipophilic drugs to ensure organ or tissue-specific drug

delivery. They contribute to improving bioavailability, biocompatibility, bioadhesivity, and biodegradability. The global application of cubosomes is as drug delivery vehicles with controlled release profiles. New insights are interested in the application of cubosomes in cosmetics and personal care products such as skin care, hair care, and antiperspirants (Naveentaj & Muzib, 2020; Urandur et al., 2018).

10.6.1 Ocular applications

When it comes to eye diseases and especially anterior segment eye diseases, topical ophthalmic administration of drugs is the preferred route (Lallemand, Felt-Baeyens, Besseghir, Behar-Cohen, & Gurny, 2003). It delivers the drugs directly at the site of action with a high ocular-to-systemic drug concentration ratio. It has the advantages of convenient administration and patient compliance (Lallemand et al., 2003; Suresh & Sah, 2014). Unfortunately, this route of administration encounters several anatomical obstacles, which can lower the drug bioavailability. Tears dilution, tears turnover, and nasolacrimal route drainage cause precorneal drug elimination (Gaballa, El Garhy, Moharram, & Abdelkader, 2020). The different layers of the eye possess different polarities, which can resist lipophilic drugs such as the hydrophilic stroma and hydrophilic drugs such as the lipophilic corneal epithelium. Thus, amphiphilic permeants offer a solution as they can permeate through the different layers. Also, the tight junctional complexes surrounding the corneal epithelial cells slow down the permeation of drugs paracellularly into the corneal inner layers (Gaudana, Ananthula, Parenky, & Mitra, 2010). Cubosomes can act as an excellent ocular drug delivery system, since they are bioadhesive, can incorporate both hydrophilic and lipophilic drugs, and can enhance drug permeation (Gan et al., 2010; Suresh & Sah, 2014). Cubosomes have been studied to deliver various antifungal drugs for fungal keratitis in the form of eye drops or eye gels (Table 10.1) (Elfaky et al., 2021; Nasr, Teiama, Ismail, Ebada, & Saber, 2020; Younes, Abdel-Halim, & Elassasy, 2018). Nasr et al. (2020) found that hydrophilic fluconazole (FCZ) loaded cubosomes eye drops had 2-fold higher corneal permeation in rabbits cornea, with a greater reduction in inflammation score in rats in different eye tissues versus FCZ solution. Elfaky et al. (2021) developed a cubosomal gel loaded with ketoconazole (KCZ) that showed better antifungal activity with a reduction in minimum inhibitory concentration (MIC) values than the commercially available topical KCZ. Younes et al. (2018) overcame the poor aqueous solubility of sertaconazole nitrate (STZ) by loading it into cubosomes and using the penetration enhancer limonene. The cubosomes didn't show signs of irritation in rabbits' eyes with 2.3-fold enhanced permeation in vivo study more than the reference solution (Rhodamine B dye). Some antiglaucoma drugs loaded cubosomes have been reported in the literature that resulted in better intraocular pressure (IOP)-lowering effect than commercial eye drops with longer retention time (Bessone et al., 2021; Huang et al., 2017; Sayed, Abdel-Moteleb, Amin, & Khowessah, 2021). Timolol maleate (TM) cubosomes reduced IOP in rabbits' eyes from 27.8 ~ 39.7 to 21.4 ~ 32.6 mmHg after 1-week administration without signs of cytotoxicity and

Table 10.1 Examples of applications of cubosomes as an ocular drug delivery system.

Drug loaded system	Pharmacological uses	Key outcomes	References
FCZ-cub	Fungal keratitis	Greater reduction in inflammation score than FCZ solution	Nasr et al. (2020)
KTZ-cubogel	Fungal keratitis	Better bioavailability and antifungal action than topical KCZ	Elfaky et al. (2021)
STZ-cub	Fungal keratitis	No signs of irritation and enhanced permeation	Younes et al. (2018)
TM-cub	Glaucoma	No signs of cytotoxicity and reduction of IOP	Huang et al. (2017)
Cub-LNP	Glaucoma	Reduction of IOP by 30% after 24 h	Bessone et al. (2021)
DZ-cubogel	Glaucoma	Higher bioavailability and greater reduction in IOP than Trusopt eye drops	Sayed et al. (2021)
BDP-cub & cubogel	Uveitis	More corneal permeability and bioavailability than BDP suspension (BDP-cubogel > BDP-cubosomes > BDP suspension)	Gaballa et al. (2020)
DEX-cub	Eye inflammation	Longer preocular retention and greater bioavailability than DEX-Na phosphate eye drops and DEX suspension	Gan et al. (2010)

FCZ, *Fluconazole;* Cub, *cubosomes;* KTZ, *ketoconazole;* Cubogel, *cubosomal gel;* STZ, *sertaconazole nitrate;* TM, *timolol maleate;* IOP, *intraocular pressure;* Cub-LNP, *latanoprost-loaded cubosomes;* DZ, *dorzolamide hydrochloride;* BDP, *beclomethasone dipropionate;* DEX, *dexamethasone.*

histological alterations (Huang et al., 2017). In the same vein, Latanoprost-loaded cubosomes (Lnp-loaded cubosomes) were able to cause a 30% more reduction in IOP after 24 h and a 20% reduction in IOP for 9 days at low concentrations (Bessone et al., 2021). Moreover, Dorzolamide hydrochloride (DZ) was loaded in cubosomal gel (cubogel) for glaucoma treatment. DZ-loaded cubogel showed a 2.5-fold higher bioavailability and 43.18% greater reduction in IOP at t_{max} 6 h compared to Trusopt eye drops (Sayed et al., 2021). Corticosteroids are used in the treatment of various ophthalmic conditions. Unfortunately, their lipophilic nature can cause their absorption via conjunctival blood vessels and nasal mucosa causing unwanted systemic side effects (Gaballa et al., 2020). Corticosteroids can cause systemic side effects such as Cushing syndrome and may cause negative feedback on the production of endogenous corticosteroids from the adrenal gland (Gaballa et al., 2020). Thus, cubosomes can be considered a suitable drug delivery system because of their bioadhesive nature and sustained release effect (Suresh & Sah, 2014). In an in vitro and an in vivo study, Gaballa et al. compared beclomethasone dipropionate (BDP) suspension to BDP-loaded cubosomes and BDP-loaded cubogel for uveitis. The cubosomes and cubogel showed more controlled in vitro drug release, corneal

permeability, and relative bioavailability than BDP suspension (BDP-cubogel > BDP-cubosomes > BDP suspension) (Gaballa et al., 2020). In another study, Dexamethasone (DEX) loaded cubosome was fabricated and tested against eye inflammation. It gave longer preocular retention and greater bioavailability than DEX-Na phosphate eye drops and DEX suspension (1.8- and 8-fold increase in AUC, respectively) (Gan et al., 2010).

10.6.2 Oral applications

One of the most remarkable applications of cubosomes is the oral delivery of active pharmaceutical agents. Cubosomes as a very effective drug delivery carrier were utilized for the delivery of many active therapeutic agents with enhanced pharmacokinetics, bioavailability as well as safety profiles of the loaded drugs (Table 10.2). Thanks to the unique properties of cubosomes such as high drug payloads, large surface area, and the ability for controlling the release of the drug, they have been selected for improving the oral bioavailability of many therapeutic drugs that have limited oral absorption. This can be explained by the bioadhesion characteristic of ribosomes that enhance the secretion of surface active agents which enhance the digestion of lipids in the gastrointestinal tract (Kossena, Charman, Boyd, & Porter, 2004). In an attempt the delivery insulin, insulin-loaded cubosomes were prepared with special care to maintain the activity of insulin they were prepared at a low-temperature range also were in the form of large aggregates, they concluded that cubosomes can provide a relatively stable oral delivery of insulin with efficient hypoglycemic activity can be explained by the mucoadhesiveness of glyceryl monooleate (GMO). Furthermore, cubosomes were used for improving the bioavailability of ibuprofen which has been extensively used as an analgesic and anti-inflammatory, the entrapment efficiency of ibuprofen in the cubic nanoparticles was high reach to 86% with a very optimum particle size of about 238 nm. A sustained release profile resulted in the ibuprofen cubosomes formula, authors have concluded that the entrapment of

Table 10.2 Examples of applications of cubosomes in oral drug delivery.

Drug loaded system	Pharmacological uses	Key outcomes	References
Insulin-cub	Hyperglycemia	Stable oral delivery of insulin with efficient hypoglycemic activity	Kossena et al. (2004)
IBP-cub	Pain and inflammation	Efficient pain relief activity with a sustained release profile	Dian et al. (2013)
PIP-cub	Alzheimer	Improvement in cognition and reduction of the inflammatory markers	Elnaggar et al. (2015)
Dox-cubic NPs	Breast cancer	Enhancement of anticancer activity of Dox with reduced cardiotoxicity	Swarnakar et al. (2014)

IBP, *Ibuprofen;* PIP, *piperine;* Dox, *doxorubicin;* NPs, *nanoparticles;* Cub, *cubosomes.*

ibuprofen into the cubic structure of cubosomes resulted in an enhancement of bioavailability and as result, it provides a good drug delivery carrier with efficient therapeutic activity (Dian et al., 2013).

Recently cubosomes showed a promising result in the treatment of Alzheimer's disease (AD) that impact on CNS of human especially elder persons, cubosomes were found to be effective in the delivery of drugs that can improve memory potential by overcoming brain barriers, for example for these drugs is Piperine (PIP) but unfortunately, it suffers from poor aqueous solubility and as a result bad bioavailability as wee as first-pass metabolism so modified cubosomes were fabricated for actively targeting PIP to the brain cells. Fabricated cubosomes were formulated by using monoolein modified with tween and other surface active agents, T-cubs showed excellent drug loading with 86.67% ± 0.62%, sustained release profile for PIP. In conclusion, the fabricated drug delivery system was revealed to improve cognition and decrease inflammatory markers. The most important issue is the safety of the formula on the liver, brain, and kidney (Elnaggar, Etman, Abdelmonsif, & Abdallah, 2015).

In an attempt for enhancing the therapeutic efficacy of doxorubicin cubic liquid crystalline NPs were developed by using the hydrotropic method, the fabricated formula showed cubic structure and high stability for approximately 6 months at gastrointestinal fluids. The cytotoxicity results of Dox-loaded cubic NPs were high in comparison with a free solution this may refer to the localization of nanoformula in the nucleus. The bioavailability study revealed the superiority of the Dox-loaded nanoformula by about 17.7-fold in comparison with a free solution. Furthermore, the cardiotoxicity caused by doxorubicin was found to be reduced by incorporation into the cubic liquid crystal NPs when compared with Adriamycin. Cubic NPs encapsulating Dox were found to enhance the anticancer activity of Dox, its efficacy as well as its safety profile and provide an effective option for chemotherapy to be taken orally (Swarnakar, Thanki, & Jain, 2014).

10.6.3 Transdermal applications

Transdermal drug delivery is usually hampered by the highly organized formidable outer layer of skin known as the stratum corneum (SC). Cubosomes are widely used as a potential non-invasive transdermal drug delivery system (Table 10.3). They are well-known skin penetration enhancers. This could be attributed to the structural similarity between the cubic phases of the SC and cubosomes which resulted in the fluidization of SC upon contact with the lipid part of the nanoparticles. Moreover, the intimate bioadhesive interaction between cubosomes and SC could be efficiently used in topical and mucosal drug delivery (Peng et al., 2015).

Vaccination through transcutaneous (TCI) immunization is one of the dermatological approaches based on taking the benefits of the synergistic combinational approaches of microneedles (MNs) and cubosomes to ensure local delivery of antigens to the targeted cells in the skin. This could be attributed to MNs crucial

Table 10.3 Examples of applications of cubosomes in transdermal drug delivery.

Drug loaded system	Pharmacological uses	Key outcomes	References
Capsaicin-cub	Pain management	Bypass metabolic degradation in GIT and provide sustained release	Peng et al. (2015)
Etodolac-cub	Relief pain of arthritis	Improved in vivo bioavailability of etodolac relative to the oral capsules (266.11%) with a longer half-life	Salah et al. (2017)
Cl-cubogel	Management of epileptic seizures	Compared to Cl-suspension, Cubogel displayed high transdermal permeation, retention time, and skin deposition.	El-Enin and Al-Shanbari (2018)

Cub, *Cubosomes;* Cl, *clonazepam;* Cubogel, *cubosomal gel.*

role in improving the permeation of the aqueous peptide via the skin layers whereas peptide-loaded cubosomes helped in providing longer skin retention (Rattanapak et al., 2013).

Peng et al. (2015) developed a study for the delivery of capsaicin within PHYT- and GMO-based cubosomes. This study aimed to overcome the pharmaceutical hurdles facing capsaicin upon oral or intravenous administration. So that the transdermal capsaicin-loaded cubosomes could successfully bypass metabolic degradation in GIT and provide a sustained release profile thus attenuating post-incision pain without the need for overdosing and undesirable systemic side effects. Notably, GMO-based cubosomes displayed the least permeation rate of capsaicin (0.18 ± 0.02 μg/cm^2/h) among PHYT-based cubosomes (0.32 ± 0.05 μg/cm^2/h) and conventional cream (0.28 ± 0.05 μg/cm^2/h). GMOs hampered capsaicin diffusion rate into the skin resulting in high sustained skin retention that is frequently applied on mouse skin (Peng et al., 2015).

Similarly, the transdermal delivery of GMO-based cubosomes managed to provide a sustained release profile of etodolac to control the terrible pain and joint stiffness associated with arthritis (Salah et al., 2017). Herein, the high negativity of cubosomes with zeta potential value up to −36.10 mV was attributed to multiple factors including the presence of the fatty acid (GMO), Poly(vinyl alcohol) hydroxyl group on the surface of the cubosomes and poloxamer-407 hydroxyl ions that interact with an aqueous medium. The particle size of cubosomes is inversely proportional to the increase in poloxamer concentration and zeta potential values. Notably, cubosomes showed biphasic penetration behavior across excited mice skin in which initial fast etodolac penetration was followed by slower penetration for up to 24 h. The in vivo evaluations of the proposed system in human volunteers manifested improved bioavailability of etodolac relative to the oral capsules (266.11%) with a longer half-life (Salah et al., 2017).

10.6.3.1 In the treatment of epileptic children patients

Unfortunately, the spread of epilepsy in children is very high in which epileptic children represent 22% of whole epileptic patients. Tailoring novel acceptable dosage forms for children is of great challenge. Transdermal drug delivery is a highly attractive approach for epileptic children based mainly on the ease of application of transdermal patches. Normally the skin of children as well as neonates possess occlusive features to control water loss. The transdermal multilayered patch system is composed of polymer and drug incorporated into the drug reservoir layer (El-Enin & Al-Shanbari, 2018).

GMO-based cubosomes can be successfully used as a drug reservoir system via the transdermal route. In this study, the cubosomal-gel patch reservoir was used to deliver the antiepileptic clonazepam (Cl) via the transdermal route (El-Enin & Al-Shanbari, 2018). This system was prepared in the presence of Pluronic-F127(PF127) along with different stabilizers either Ethanol (Et) or polyvinyl alcohol (PVA). This regimen highly influenced the particle size and entrapment efficiency. So that PVA or Et with PF127 resulted in a remarkable reduction in particle size (352 ± 2.8 and 264 ± 2.16 nm) and elevation in entrapment efficiency ($58.97\% \pm 4.57\%$ and $54.21\% \pm 3.89\%$). Moreover, cubosomes displayed biphasic release characterized by an initial burst release of Cl (37.39% and 46.04% in the first hour) followed by sustained release up to 4 h. The results emphasized the superiority of Cubosomal-gel containing PVA over Cl-suspension in terms of transdermal permeation, retention time, and skin deposition thus controlling the epileptic seizures.

10.6.4 Transnasal applications

The optimum delivery of drugs to treat brain-related disorders is handicapped by the formidable blood-brain barrier (BBB) that impedes some small molecules from entering the brain. Cubosomes are promising non-invasive lipid-based nanoparticulate drug carrier that plays a crucial role in increasing drug loading into the brain, improving encapsulation efficacy, and enhancing drug stability (Table 10.4) (Ahirrao & Shrotriya, 2017).

Table 10.4 Examples of applications of cubosomes in transnasal drug delivery.

Drug loaded system	Pharmacological uses	Key outcomes	References
RSV-cubogel	Brain disorder	In vivo biodistribution confirmed transnasal permeation and distribution of cubogel to the brain	Ahirrao and Shrotriya (2017)
Risperidone-cubogel	Schizophrenia and bipolar disorder	Using 0.4% w/v polyox during the preparation of cubogel increases its mucoadhesive properties and enhances transnasal permeation	Abdelrahman et al. (2015)

RSV, *Resveratrol.*

GMO-based cubosomes were fabricated to deliver the potent polyphenolic resveratrol (RSV) to the brain via the transnasal route (Ahirrao & Shrotriya, 2017). The optimized cubosomes were prepared using GMO (4% w/v) and Poloxamer 407 (Lutrol F127) (1.5% w/v) by probe sonication process. The resulting in situ cubosomal gel with particle size 161.5 ± 0.12 nm was suitable for nasal use. Ex vivo permeation and in vivo biodistribution tests confirmed the superiority of the optimal cubosomal gel in transnasal permeation and distribution to the brain over drug solution (i.n.) and drug solution (oral).

Similarly, the transnasal route could pave the way to treat other CNS disorders such as schizophrenia and bipolar disorder. GMO-based cubosomal gel was developed to improve risperidone delivery to the brain (Abdelrahman et al., 2015). The proposed formula was optimized in the presence of 15% w/v PF127, 20% w/v T80, and gelling mucoadhesive polymer (0.4% w/v polyox) resulting in particle size 160.4 ± 3.79 nm, zeta potential -23.6 ± 1.57 mV and entrapment efficiency $43.25\% \pm 5.29\%$. The ex vivo and in vivo biodistribution studies supported the superb transnasal permeation and better distribution of risperidone-loaded cubosomal gel to the brain.

10.6.5 Anticancer applications

Recently, cubosomes withdraw the attention of researchers as a promising approach for the delivery of targeted chemotherapeutic agents to treat various types of tumors that threaten mankind. Based on the unique above-mentioned characteristics, cubosomes could successfully enhance the bioavailability, pharmacokinetics, and safety profiles of the therapeutic payloads (Table 10.5). Thus reducing the possibility of undesirable toxic effects and nonspecific drug delivery to off-target healthy cells (Gaballa et al., 2020).

Various targeting approaches have been elaborated for delivering anticancer-loaded cubosomes to cancer cells. Nanocarriers' uptake and cellular internalization could be achieved via two major pathways paracellular and transcellular (Abdel-Bar & El Basset Sanad, 2017). Paracellular transport is a passive diffusion based on intercellular spaces and tight junctions, whereas transcellular endocytosis includes macropinocytosis, clathrin-mediated endocytosis, caveolae-mediated endocytosis or caveolae- and clathrin-independent endocytosis. These difference among the various endocytic pathways was based on the size it could internalize and the fate of the penetrated nanocarrier. This could be demonstrated in this study in which GMO-based cubosomes were developed in an attempt to overcome pharmaceutical hurdles facing RSV. The prepared RSV cubosomes possessed particle size ranging from 22 ± 1.26 to 195 ± 2.99 nm, PDI < 0.2, and zeta potential ranging from -17.32 ± 2.71 to -32.12 ± 1.41 mV. Notably, increasing GMO ratio during cubosomes synthesis was associated with a significant increase in their negativity. This could be attributed to free fatty acids in GMOs. The particle size variation highly influenced the cellular penetration and the endocytic pathway in which cubosomes with high particle size (97.65 ± 4.50 nm) obeyed

Table 10.5 Examples of applications of cubosomes for anticancer drug delivery.

Drug loaded system	Pharmacological uses	Key outcomes	References
TQ-cubs	Breast cancer	The promising antitumor activity showed in the ability to trace and detect the cancer cell	Mehanna et al. (2020)
ETP-cubs-FA	Breast cancer	Enhanced uptake with a remarkable accumulation in the cancer cells.	Tian et al. (2017)
5-FU-cubs	Liver cancer	Selective antitumor efficacy with lower adverse effects	Cheng et al. (2012b)
RSV-cubs	Liver cancer	Enhanced cytotoxicity and targeted cellular uptake	Abdel-Bar and Abd el Basset Sanad (2017)
LbL-LCNPs/SF	Liver cancer	pH-sensitive release maximize drug concentration in the acidic environment of tumor cells	Thapa et al. (2015)
LF-CaP-Cs	Lung cancer	LF-CaP-Cs displayed high anti-angiogenic activity, high apoptotic effect, and improved cellular uptake	Sethuraman et al. (2019)
LF/CS-coated PEM-RSV LCNPs	Lung cancer	Inhalable DPI nanocomposites 2.72 μm MMAD and 61.6% FPF guarantee deep lung deposition	Abdelaziz et al. (2020)
ICA-Cubs	Ovarian cancer	ICA-Cubs proved cytotoxic activity with increased apoptotic activity compared to free ICA.	Fahmy et al. (2021)
DOX-cub	Brain cancer	They displayed high antitumor activity with minimal cardiac toxicity	Nazaruk et al. (2017)
miR-7-5p and TMZ or DOX-cub	Brain cancer	The miR-7-5p sensitizes the glioma cells to the chemotherapeutic agent to overcome MDR	Gajda et al. (2020)

TQ, *Thymoquinone;* ETP, *etoposide;* FA, *folic acid;* 5-FU, *5-fluorouracil;* RSV, *resveratrol;* SF, *sorafenib;* LCNPs, *liquid crystalline nanoparticles;* LbL, *layer-by-layer;* LF, *lumefantrine;* CaP, *calcium phosphate nanoparticles;* Cs, *cubosomes;* LF, *lactoferrin;* CS, *chondroitin sulfate;* PEM, *pemetrexed;* ICA, *icariin;* DOX, *doxorubicin;* TMZ, *temozolomide;* miR-7-5p, *microRNA.*

micropinocytosis only, whereas cubosomes with low particle size (28 nm) obeyed clathrin-mediated endocytosis mechanism only. The optimum cytotoxicity and cellular uptake of RSV-loaded cubosomes against HepG2 human hepatoma cells were achieved with a particle size range of 45−76 nm that facilitates additional internalization via caveolae-mediated endocytosis (Abdel-Bar & El Basset Sanad, 2017).

Generally speaking, nanoparticles are passively targeted to tumor cells based on their particle size in which they follow a diffusion-mediated transport process. The recommended size for cubosomes ranges from 10 to 150 nm. The systemically delivered colloidal particles accumulated in tumor cells via enhanced permeation and retention (EPR) effect based on leaky vasculature and poor lymphatic drainage. However, we can not depend on passive targeting as the main targeting approach because the EPR effect is highly influenced by the degree of angiogenesis that differs corresponding to tumor type and status (Urandur et al., 2018).

The passive targeting approach was adopted by Ali et al. (2016) who prepared PHYT-based cubosomes to deliver SN-38 (chemotherapy). This system in the presence of six monoglyceride additives managed to handle the poor aqueous solubility and chemical instability of SN-38. In vitro evaluations demonstrated the slow release of 55% of the drug under physiological conditions. This was attributed to drug entanglement within the cubosomal lipid bilayer. Notably, the drug released was in its active lactone and reached the tumor cells via the EPR effect.

On the other hand, active targeting is the major clue that can overcome the limitations associated with passive targeting. The surface of nanoparticles is decorated with specific targeting ligands (e.g, biotin, lactoferrin, hyaluronic acid, chondroitin sulfate, or folate groups) to the overexpressed receptors on tumor cells thus triggering receptor-mediated endocytosis and guaranteeing drug localization and penetration within tumor sites (Urandur et al., 2018).

Another targeting approach for the tumor cell is based on the combination between metabolic labeling and copper-free click chemistry for effective drug delivery. This approach depends mainly on the covalent interaction between azide groups and the complimentary octyne probe such as dibenzocyclooctyne (DBCO). The surface of PHYT-based cubosomes was decorated with phospholipids containing azide or DBCO group (Alcaraz et al., 2018). The "clickability" was evaluated using size exclusion chromatography in which the clickable cubosomes reacted specifically and efficiently with complementary dye (azide-Cy5) with minor changes in the internal structure of cubosomes. The results open the frontiers for utilizing copper-free click chemistry and metabolic labeling with cubosomes for efficient targeted drug delivery and imaging purposes (Alcaraz et al., 2018).

10.6.5.1 Cubosomes for breast cancer

Cubosomes are a powerful platform that can incorporate various types of chemotherapeutic agents that can help in the treatment of breast cancer. Cubosomes loaded with Thymoquinone (TQ) formulation showed promising antitumor activity with the ability to trace and detect the cancer cell within the human cells. In this study cubosomes were prepared by emulsification method, the used lipid phase was glyceryl monooleate (GMO) and pluronic F127 (PLX) was the stabilizer. The result showed that the anticancer activity of thymoquinone was improved when encapsulated in the formulated Cubosomes NPs as well as its binding to plasma proteins and low bioavailability was enhanced. TQ-loaded liquid NPs showed a reduction in the viability of breast cancer cell lines MDA-MB-231 and MCF-7. The fabricated

NPs showed excellent uptake by the cancer cells by endocytosis mechanism. The improved antitumor activity was observed with elevation in caspase-3 and apoptotic markers when MDA-MB-231 cell lines were treated with TQ-cubosomes NPs (Mehanna et al., 2020).

In another approach etoposide (ETP) due to its anticancer activity and the antiproliferating effect was encapsulated into cubosomes that were stabilized by copolymer P407 as well as its folic acid modified form, and sustained release profile resulted for both ETP-Cubs-FA as well as ETP-Cubs. The entrapment efficiency of ETP in Cubs was relatively higher than that for Cubs-FA it was 87.3% for the first and 84.7% for the last mentioned. The most promising result was that the uptake of Rhodamine B-encapsulated Cubs-FA showed a remarkable accumulation in the cancer cells in comparison with Rhodamine B encapsulated cubosomes, this was attributed to the targeting ability of the folic acid to the tumor cells, interestingly that the result of cytotoxicity of the optimized formulation ETP-encapsulated Cubs decorated by folic acid was superior in comparison with unmodified cubosomes. The authors concluded that the folic acid-modified cubs can be utilized as an efficient drug delivery system for breast cancer (Tian et al., 2017).

10.6.5.2 Cubosomes for liver cancer

Cubosomes nowadays have gained great attention in the treatment of cancer due to their attractive properties, they have been used for the delivery of different compounds with poor bioavailability and solubility. Cubosomes showed a good result in the targeted delivery of 5-Fluorouracil (5-FU) to liver cancer (Cheng et al., 2012a). It was reported that cubosomes loaded with 5-FU showed selective antitumor efficacy with lower adverse effects. The release profile showed a biphasic pattern starting with a fast release followed by a relatively slow release that maintains for about 4−5 h. Moreover, in the comparison with the 5-FU solution, 5-FU cubosomes showed increased liver concentration. This may be due to the enhanced systematic absorption of 5-FU-cubsomal formulation resulting from cubosomes' higher permeability via an epithelial membrane.

RSV was incorporated recently into cubosomes NPs to decrease some of its limitations such as its poor solubility. cubosomes were prepared using GMO and P407, they were optimized in their size and entrapping efficiency. The formulated cubosomes were reported to maintain RSV release for a prolonged period. An in vitro cytotoxicity study on HepG2 cell lines showed an enhanced cytotoxicity result in comparison with an RSV-free solution. Moreover, cubosomes encapsulated RSV showed promising results in the targeted cellular uptake to the cancer cells via different endocytosis mechanisms (Abdel-Bar & El Basset Sanad, 2017).

Thapa et al. (2015) adopted the layer-by-layer (LbL) self-assembly technique for surface functionalization of sorafenib (SF)-loaded LCNPs (LbL-LCNPs/SF). Herein, multiple layers of poly-l-lysine (PLL) and polyethylene glycol-b-polyaspartic acid (PEG-b-PAsp) were used to enable active targeting and thus constraining hepatocellular carcinoma (HepG2 cells). PEGylation helped in escaping from immune recognition. This system managed to enhance SF stability,

provide prolonged release, upgrade its therapeutic activity and minimize its toxicity. Furthermore, LbL-LCNPs/SF displayed pH-sensitive release that guarantees maximum drug concentration in the acidic environment of tumor cells.

10.6.5.3 Cubosomes for lung cancer

A novel study reported site-specific delivery of antimalarial lumefantrine based on pH-responsive properties of calcium phosphate nanoparticles loaded cubosomes (LF-CaP-Cs) to treat lung cancer (Sethuraman et al., 2019). The optimized formulation with cubic phase structure possessed an average particle size of 259.4 ± 19 nm, zeta potential −2.28 ± 0.7 mV, and encapsulation efficiency of 78.76% ± 0.5%. The LF release was potentiated in response to the slightly acidic environment of lung cancer cells A549 (pH 4.0). The in vitro release of LF-CaP-Cs displayed a significant increase from (48.32% ± 1.6%) at pH 7.4 to (84.04% ± 0.4%) at pH 4.0. Compared to LF-Cs/blank Cs, LF-CaP-Cs manifested enhanced anti-angiogenic activity, higher apoptotic effect, higher cytotoxic influence, and improved cellular uptake (Sethuraman et al., 2019).

Abdelaziz et al. (2020) managed to synthesize novel inhalable dry powder (DPI) nanocomposites to combat lung cancer. Firstly layer-by-layer (LbL) of lactoferrin-Chondroitin sulfate (LF/CS) self-assembled GMO-based LCNPs were prepared for codelivery of the synergistic chemo-herbal combination of pemetrexed-resveratrol (PEM-RSV). Then the resulting LF/CS-coated PEM-RSV LCNPs were incorporated into the matrix of multicarrier mannitol:leucine: insulin (1:1:1 w/w) via spray drying technique to obtain nano-in-microparticles (nanocomposites) dry powder. Notably, the optimized DPI possessed a promising in vitro aerodynamic profile (2.72 μm of MMAD and 61.6% FPF) which guarantees deep lung deposition. Further in vitro (A549 lung cancer cell line) and in vivo (urethane-induced lung cancer-bearing mice) evaluations confirmed potent antitumor activity relative to the spray-dried free PEM/RES powder mixture and positive control. The results were supported by in vivo fluorescence bioimaging that displayed the proper lung deposition.

10.6.5.4 Cubosomes for ovarian cancer

The anticancer activity of icariin (ICA) against ovarian cancer was potentiated when delivered via cubosomes which could overcome its low aqueous solubility (Fahmy et al., 2021). The ICA-loaded cubosomes (ICA-Cubs) were optimized via the Box-Behnken statistical design in which the particle sizes ranged from 73 to 183 nm and the entrapment efficiency ranged from 78.3% to 97.3%. Screening the optimized ICA-Cubs against ovarian cancer cell lines (SKOV-3 and Caov 3) displayed elevated cytotoxic activity, increased production of reactive oxygen species (ROS), and the overexpression of p53 and caspase-3 associated with superior apoptotic activity over free ICA. This could be explained through the enhanced solubility and cellular permeability of ICA within cubosomes. Moreover, ICA-Cubs manifested a promising role in pre-G1 and G2/M phases relative to free ICA. Notably, the optimized ICA-Cubs are cytosafe to normal EA. hy926 endothelial cells (Fahmy et al., 2021).

10.6.5.5 Cubosomes for brain cancer

Interestingly, the role of cubosomes was extended to in vitro trials to manage drastic brain cancer. Despite the potency of antineoplastic doxorubicin, its wide applications are hampered by its induced cardiac toxicity. Therefore, stimuli-responsive cubosome was considered a magical clue to deliver DOX with minimal side effects (Nazaruk et al., 2017). These phenomena guarantee specific drug release at the acidic pH of the tumor microenvironment without affecting normal cells. DOX-loaded cubosomes at the tumor site (pH 5) had mean particle size (150 ± 10 nm), PDI close to 0.2 ± 0.02, and zeta potential -15 mV. The pH-dependent encapsulation efficiency (EE %) of DOX represented ($92\% \pm 4\%$ at acidic pH versus $95\% \pm 2\%$ at neutral pH). Further, investigations of DOX-loaded cubosomes on the Glioblastoma T98G cell line displayed superior antitumor activity over free DOX.

The resistant glioblastoma cells can be tamed via co-administration of microRNA (miRNA) miR-7-5p and TMZ or doxorubicin (DOX) within cubosomes (Gajda et al., 2020). The effects of drug/miRNA-loaded vehicle on glioma- (A172, T98G), papillary thyroid- (TPC-1), and cervical carcinoma-derived (HeLa) cells were investigated. The miR-7-5p managed to increase the sensitivity of the glioma cells to the chemotherapeutic agent. The associated alteration in the genetic expression of multidrug resistance (MDR) proteins resulted in improved antitumor efficacy, elevated apoptotic level, and reduced MDR relative to using the free drug alone. Collectively, the influence of miR-7-5p in increasing chemosensitivity is universal and independent of the cancer cell origin or the used drug.

10.6.6 Gene therapy

It is the use of nucleic acids (RNA or DNA) to treat or prevent human diseases. A functional therapeutic gene is delivered to replace a defective or deficient gene or levels of the harmful defective gene product are reduced by using either naked oligonucleotides or non-viral or viral vectors (Kaufmann et al., 2013). Ideally, Gene delivery systems should penetrate specifically the targeted cell, evade endosomal/lysosomal degradation, and deliver the nucleic acids cargo to the nucleus (Nishikawa & Hashida, 2002). Although viral vectors have high transduction efficiency with long-term transgene expression, they are immunogenic, oncogenic, and expensive. On the other hand, non-viral vectors are safer, easy to prepare, and can carry large genes (Boulaiz et al., 2005). Unfortunately, non-viral vectors have short-term transgene expression but that can be tackled by the optimization of the structure of the construct (Nishikawa & Hashida, 2002). Cubosomes have a high potential for being non-viral vectors because of their intrinsic properties (Table 10.6). The liquid crystalline 3-dimensional internal structure provides a large internal surface area that can carry amphiphilic, hydrophilic, and hydrophobic materials. It is flexible and its structure is tunable, thus can be adjusted according to cargo sizes (Tajik-Ahmadabad et al., 2019). They are thermodynamically stable and their lipid-based structure has an inherent ability for fusion with

Table 10.6 Examples of applications of cubosomes in gene therapy.

Drug loaded system	Key outcomes	References
miR-7-5p/DOX or TMZ-cub	miR-7-5p caused glioma cells to be more sensitive to the chemotherapeutic drugs	Gajda et al. (2020)
siRNA-cub	Successfully loading siRNA into cubosomes by forming siRNA-Zn_2BDPA complex	Tajik-Ahmadabad et al. (2019)
siRNA-PEGylated cuboplexes	Enhanced siRNA delivery compared to traditional lipoplex system	Kim and Leal (2015)

miR-7-5p, *microRNA-7-5p;* DOX, *doxorubicin;* TMZ, *temozolomide;* siRNA, *small interfering RNA; Zn2BDPA, zinc (II)-bis (dipicolylamine).*

cellular membranes (Gajda et al., 2020; Sarkar et al., 2020). Gajda et al. studied the codelivery of microRNA-7-5p (miR-7-5p) with doxorubicin (DOX) or temozolomide (TMZ) via cubosomes for the treatment of glioblastoma (GB). It was designed to overcome the multidrug resistance (MDR) that is linked to the downregulation of miR-7-5p in GB. They found that the codelivery of miR-7-5p and the chemotherapeutic drug by cubosomes showed great cytotoxicity. They deduced that miR-7-5p made the glioma cells more sensitive to the chemotherapeutic drugs by reduction of the expression of MDR proteins. The drug-sensitive cell line A172 and TPC-1 treated with DOX/miR cubosomes showed significant cytotoxicity, while the drug-resistant cell line HeLa and T98G showed less but still statistically significant cytotoxicity ($P < .05$) (Gajda et al., 2020). Additionally, Zinc metallo-cubosomes were prepared as a novel noncationic lipid-based delivery system to deliver small interfering RNA (siRNA). Where, the affinity of lipidic zinc (II)-bis (dipicolylamine) (Zn_2BDPA) for the phosphodiester groups present on the siRNA backbone was exploited to form a complex with siRNA and then loaded into the cubosomes (Tajik-Ahmadabad et al., 2019). Kim and Leal developed siRNA-loaded PEGylated cuboplexes which are PEGylated lipid particles with internal cubic symmetry. Cuboplexes were made by indirect, low power, and ultra-chilled sonication of a tri-component system of GMO, 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP), and GMO-PEG conjugate. Where polyethylene glycol was conjugated to GMO for steric stability and DOTAP is a positively charged lipid that is used to entrap siRNA by electrostatic interaction. Cuboplex provided a unique topology that can enhance fusion with the membrane of endosomes and lower the free energy required to form pores in them, escape the entrapment, and thus efficiently deliver siRNA to the cell. The PEGylated cuboplex showed superior siRNA delivery compared to the traditional lipoplex system (Kim & Leal, 2015).

10.6.7 Theranostics

Developing of nanomedicine has led to nanoparticles that can deliver therapeutics and act as imaging probes simultaneously, known as nanotheranostics (Murgia et al., 2015). Caltagirone et al. reported on in vitro uptake of cubosomes targeted

Table 10.7 Examples of applications of cubosomes as a theranostic drug delivery system.

Drug loaded system	Key outcomes	References
CPT and NIR-FA-cub	Better cellular uptake than nontargeted cubosomes	Caltagirone et al. (2014)
QUE and dansyl-cub	Better cellular uptake of cubosomes-D than PF108-cubosomes, with biocompatibility for both formulations	Murgia et al. (2015)
QUE, dansyl and fluorescein-cub	Successfully loading two fluorophores and an anticancer drug in the cubosomes for future theranostic applications	Murgia et al. (2013)
DTX and rhodamine B-loaded, FA-targeted cub	Significant cytotoxicity effect against HeLa cancer cells compared to free DTX (at 4 h of incubation)	Meli et al. (2015)

CPT, *Camptothecin;* QUE, *quercetin;* DTX, *docetaxel;* FA, *folic acid.*

with folic acid, loaded with anticancer drug camptothecin (CPT) and NIR-emitting fluorophore (a squaraine) in HeLa cells (Table 10.7) (Caltagirone et al., 2014). In vitro cellular uptake study by lipid droplet evaluation showed that targeted cubosomes demonstrated a 1.8-fold increase ($P < .001$) in integrated optical density (IOD) per cell than nontargeted cubosomes after 4 h of incubation. After 24 h, there was a 1.2-fold increase ($P < .05$) in IOD per cell in targeted cubosomes than the nontargeted cubosomes. While nontargeted and targeted cubosomes showed 2.9- and 3.5-fold increases in IOD/cell more than non-treated control ($P < .0001$) with no signs of morphological alterations. Moreover, confocal microscopy showed consistent findings (Caltagirone et al., 2014). Another fluorescent imaging agent and anticancer drug combination that was used was dansyl and quercetin (QUE) by Murgia et al. (2015). The fluorophore was conjugated to the copolymer pluronic PF108 (hydrophilic moiety) that is used to stabilize the cubosomes while the cytotoxic drug was physically loaded. The cubosomes were tested for their in vitro cytotoxicity and cellular uptake against HeLa cells (Murgia et al., 2015). In the in vitro cellular uptake study, cubosomes made with PF108 (cubosomes) and cubosomes made with PF108/PF108-D mixture (Cubosomes-D) were confirmed to cause lipid droplet growth (IOD/cell as follows; Cubosomes-D > cubosomes > control cells) at 4 h of incubation. Cubosomes-D may have shown the greatest IOD/cell because the dansyl moiety may have changed the cubosomes interaction with the cells by changing their molecular recognition characteristics. In the in vitro cytotoxicity study, the two formulations showed comparable cell viability to the control cells after 4 h of incubation (Murgia et al., 2015). In another in vitro cellular uptake study, Murgia et al. also loaded quercetin and dansyl with another fluorescent probe, fluorescein in cubosomes (Murgia et al., 2013). Both fluorescein and dansyl have slight water solubility, thus, both were altered individually to increase their hydrophobicity for better encapsulation (Murgia et al., 2013). Moreover, targeted theranostic cubosomes were successfully developed using the cytotoxic docetaxel (DTX) as a therapeutic, rhodamine B as a fluorescent imaging agent, and folic acid as a

targeting ligand. These cubosomes had a significant cytotoxicity effect against HeLa cancer cells compared to free DTX (at 4 h of incubation) and were able (without the drug) to image living HeLa cells (Meli et al., 2015).

In another study, the surface of fluorescent-MO based cubosomes (MO-Fluo) was functionalized by biotin to guarantee active targeting of antineoplastic paclitaxel (PX) thus avoiding toxicity associated with commercial PX using Cremophor EL and dehydrated ethanol (Aleandri et al., 2015). The efficacy of the optimized formulation was determined on human adenocarcinoma cell lines (HeLa). The theranostic biotinylated cubosomes helped in the diagnosis, drug delivery, and therapeutic monitoring of drugs.

Other drug-loaded theranostic cubosomes were investigated for codelivery of the synergistic combination of cisplatin and paclitaxel (PX) (Zhang et al., 2020). The surface of cubosomes was coated with a layer of poly-ε-lysine to prevent the initial burst release of the drug thus giving the chance for sustained release and enhanced efficacy. The analyses confirmed the uniform dispersion of drugs along cubosomes. The coated cubosomes were nontoxic against the human hepatoma HepG2 cell line. The therapeutic efficacy of coated combinatorial cubosomes against the HeLa cell line was confirmed by the reduction in the impedance of cells associated with HeLa cell impairment as visualized by fluorescence microscopy.

10.6.8 Nanocosmeceuticals

Cubosomes are one of the promising nanocosmeceuticals that can overcome the drawbacks related to traditional products (Sherif et al., 2014). Thanks to their unique features including ease of preparation, lipid biodegradability, high drug loading capacities, controlled and targeted release profile, and high skin permeability. Therefore, cosmetic giants (L'Oreal and Nivea) are paying great attention to fulfilling patents concerning cosmeceutical applications of cubosomes in the market (Sherif et al., 2014). Cubosomes are utilized in cosmetics as oil-in-water (O/W) emulsion stabilizers and pollutant absorbents. They have been introduced in several personal care products (e.g., skincare, hair care, antiperspirants). Despite the notable progress in the applications of nanocosmeceuticals, their wide utility is still constrained due to the fear of their possibility to penetrate through the skin and cause nanotoxicological health hazards.

It was reported that alpha-lipoic acid (ALA) loaded cubosomes managed to successfully minimize the facial lines and ameliorate both skin texture and color in the majority of volunteers. This could be attributed to the potent antioxidant activity of that naturally occurring fatty acid (ALA) (Sherif et al., 2014).

10.7 Conclusion and future perspective

Cubosomes are considered a promising lipid-based nanosystem. Their unique cubic liquid crystalline multicompartmental nanostructure allows the delivery of

hydrophilic, hydrophobic, and amphiphilic bioactive agents. Their fruitful applications are prominent in the cosmeceutical and pharmaceutical fields. Cubosomes have displayed an effective role in ocular, oral, brain, and transdermal drug delivery. Their potential controlled release role is extended to the targeted delivery of chemotherapeutic agents to combat various dreadful cancers. Researchers pay a great effort to overcome drawbacks associated with cubosome scaling up in industries.

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CHAPTER

Aquasomes: a novel nanocarrier system for drug delivery

11

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11.1 Introduction

Scientists have explored the use of nanoparticles for potential applications in drug delivery with the first proposal reported by Gregory Gregoriadis et al. about four decades ago where they investigated the possibility of liposomes as drug carriers in cancer patients (Gregoriadis, Swain, Wills, & Tavill, 1974). Lipids (Lamprecht, Saumet, Roux, & Benoit, 2004), graphene and graphene oxide (Liu, Cui, & Losic, 2013), exosomes (Batrakova & Kim, 2015), dendrimers (Cheng, Wang, Rao, He, & Xu, 2008), polymers (Nicolas, Mura, Brambilla, & Mackiewicz, 2013), metal-organic frameworks (Sun, Zheng, & Yang, 2020a), nanogels (Rajput, Narkhede, & Naik, 2020), magnetic nanoparticles (Gholami et al., 2020; Mirza, Ahmad, Shah, & Ateeq, 2019), quantum dots (Zavareh, Pourmadadi, Moradi, Yazdian, & Omidi, 2020) and aquasomes (Hasanein, 2021) are some of the examples other than liposomes that have been employed as nanocarriers in drug delivery. This process of drug delivery requires the incorporation of the drug by adsorption, diffusion, or copolymerization onto the surface of the nanoparticles and the subsequent spatial, intermittent, or steady delivery to the targeted site in the body. The strengths of this method of drug delivery over other traditional methods among others include; improved drug delivery (targeted delivery), bio-distribution, solubility, stability, sensitivity, selectivity, efficiency, and reduced side effects and toxicity (Chamundeeswari, Jeslin, & Verma, 2019; Huda, Alam, & Sharma, 2020).

Aquasomes which means "water bodies" are nanoparticles that are self-assembled and suitable as carriers or vehicles for drug delivery. They consist of three distinctive layers namely; the inner core, a polyhydroxyl carbohydrate layer, and an outer layer (Fig. 11.1). The nanocrystalline solid inner core is usually coated with an oligomeric film (polyhydroxyl carbohydrate) which by copolymerization, adsorption or diffusion carries a targeted biochemically active molecule (drug). These self-assembled layers are normally held together by non-covalent bonds, ionic bonds, or van der Waals forces and do not only have the required structural integrity but also have a high surface area which is exploited

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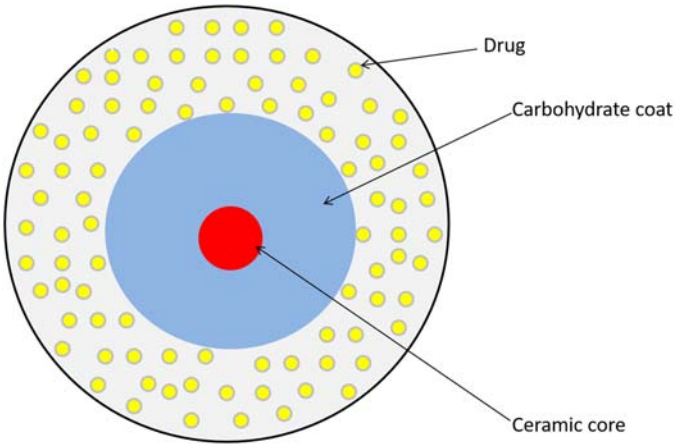

FIGURE 11.1

Structure of aquasome.

Table 11.1 Different drug vehicles.

Vehicle	Description	Relevance
Aquasomes	Self-assembling particles with three layers of inner core, carbohydrate layer, and drug.	Drug carriers with specificity and sensitivity
Novasomes	Made up of cholesterol, polyoxyethylene 10-stearyl ether, and glyceryl dilaurate	Drug delivery
Virosomes	Spiked liposomes	Immunological adjuvants
Archaeosomes	Contain glycerolipids	Adjuvant
Genosomes	Artificial complex for gene transfer	Gene transfer
Proteasomes	High molecular weight enzymatic complexes	Adjuvant, protein carrier
Vesosomes	Nested bilayer compartments	Protection
Cryptosomes	Lipid vesicles	Drug targeting
Enzymosomes	Liposomes	Drug delivery
Transferosomes	Modified lipids	Drug delivery
Discomes	Niosomes	Drug targeting
Photosomes	Liposomes	Photodynamic therapy
Erythrosomes	Cytoskeletons	Drug targeting
Eulsomes	Lipids	Drug delivery

in drug delivery, especially for targeted proteins, genes, antibodies, antigens, enzymes and so on (Banerjee & Sen, 2018). Table. 11.1 gives a summary of some drug carriers or vehicles which have found use in different areas (Umashankar, Sachdeva, & Gulati, 2010).

11.2 Composition of aquasomes

11.2.1 Inner core material

The inner core of aquasomes is solid and usually prepared to employ a ceramic or polymeric material. Ceramic materials like tin oxide, calcium phosphate, carbon ceramic (diamond particles), hydroxyapatite, and polymeric materials like albumin, acrylates, and gelatin have been widely used to prepare the core (Ahluwalia et al., 2020). The crystalline nature of ceramics affords structural order and crystallinity which in turn provides a high surface area for the binding (coating) of the carbohydrate layer. The strength of calcium phosphate and hydroxyapatite as a core layer in aquasomes lies in their natural occurrence in the body. Calcium phosphate and hydroxyapatite have therefore been explored in different forms and shapes including biocomposites (Beherei, El-Bassyouni, & Mohamed, 2008; El-Kady, Mohamed, & El-Bassyouni, 2009; Kato, Lee, & Nagata, 2020; Prokopowicz, Szewczyk, Skwira, Sądej, & Walker, 2020; Schnepp, Gonzalez-McQuire, & Mann, 2006), scaffolds (Hong et al., n.d.; Meißner, Bertol, Rehman, dos Santos, & Boccaccini, 2019; Mi et al., 2020; Radwan, Nasr, Ishak, Abdeltawab, & Awad, 2020; Sun et al., 2020b), nanorods (Li et al., 2018; Wang et al., 2020a; Yi, Li, Ma, & Zhu, 2020), hydroxyapatite whiskers (Matinfar, Mesgar, & Mohammadi, 2019; Nouri-Felekori et al., 2020) and nanoparticles (Niu et al., 2020; Wang et al., 2020b; Zhang et al., 2020) as effective cores in aquasomes. It is important to note that ceramics are ideal candidates for preparing aquasomes because they are stable, safe, biodegradable, biocompatible, cheap, and easy to manufacture (Beherei et al., 2008).

11.3 Polyhydroxyl carbohydrate layer

Carbohydrate materials like lactose, sucrose, trehalose, cellobiose, citrate, chitosan, and pyridoxal-5-phosphate are usually employed to coat the inner core (carbohydrate coat) (Hasanein, 2021; Wang et al., 2020b). The carbohydrate coat serves as a glassy layer which is useful in (1) absorbing small molecules while maintaining the conformational integrity of structurally soft drugs (Banerjee & Sen, 2018) (2) delivery of aqua like environment essential for biochemically active molecules (3) protecting the key three-dimensional conformation of targeted drug molecules (Goyal et al., 2008; Kossovsky et al., 1996; Sachin & Simran, 2020) and (4) acting as a natural stabilization agent. It is important to mention that the polyhydroxy carbohydrate layer is coated (attached) to the inner core by adsorption.

11.4 Outer layer

The outer layer of the aquasome is usually the bioactive material or drug of a target. It can interact with the coated inner core through non-covalent or ionic interactions.

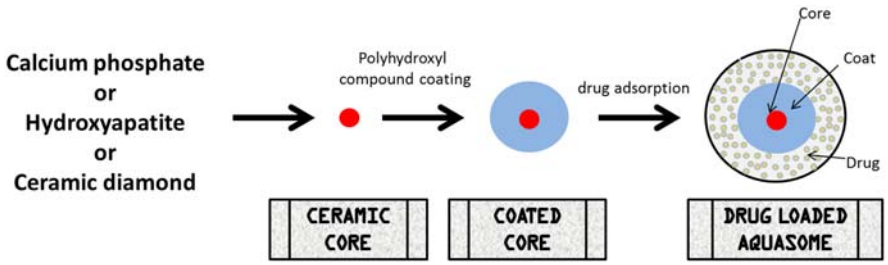

FIGURE 11.2

Schematic illustration of the preparation of aquasomes.

11.5 Preparation of aquasomes

Aquasomes are generally prepared by the principle of self-assembly (Sachin & Simran, 2020) consisting of the preparation of the inner core followed by the coating of the inner core with polyhydroxyl carbohydrates and the subsequent loading of the bioactive agent or drug. Fig. 11.2 gives a schematic illustration of the preparation of aquasomes.

11.6 Preparation of the inner core material

The formulation of aquasomes begins with the preparation of the inner core which could be an inorganic ceramic, calcium phosphate, carbon ceramic, or tin oxide. The most important question in the formation of the ceramic core is the suitability of materials to be employed which are usually hydroxyapatite, calcium phosphate, and diamond. The suitability of these materials lies in their high crystallinity and high surface area which affords a good platform for coating and regular/ordered structures. The ceramic inner core is generally prepared by precipitation, plasma condensation, sputtering or sonication, and subsequent isolation by centrifugation. Isolated ceramic cores are usually washed properly to remove any impurities arising from their preparation.

Nanocrystalline calcium phosphates have also been prepared as inner core materials for the fabrication of aquasomes. These highly ordered materials have been prepared by either sonication (Sahoo, Ramana, Satyanarayana, & Mohanty, n.d.; Vengala, Vanamala, & Subrahmanyam, 2017) or precipitation (Cherian, Rana, & Jain, 2000; Goyal et al., 2008; Khopade, Khopade, & Jain, 2002; Sahoo, Ramana, Satyanarayana, & Mohanty, n.d.; Vengala et al., 2017). Prepared calcium phosphate cores have to be isolated by centrifugation and thorough washing. It is also important to note that the crystallinity of these calcium phosphate inner cores provides the required high surface area needed for the subsequent coating of carbohydrates.

Diamond particles or carbon ceramic can also be used in the fabrication of inner core materials for the preparation of aquasomes. The highly ordered

crystalline particles of diamond are usefully exploited in the fabrication of inner cores in providing the required surface area for carbohydrate coating. Prepared inner cores of diamond particles usually by precipitation are washed thoroughly after isolation. Diamond particles as inner cores of aquasomes can be prepared in specific or required sizes.

11.7 Coating of the inner core with polyhydroxy carbohydrate

Prepared inner cores of aquasomes are usually covered or coated with a polyhydroxy carbohydrate which serves to (1) provide the suitable medium for drug loading (2) improve drug loading, (3) shield loaded drugs against dehydration and denaturation, and (4) facilitate drug release to the targeted site by the provision of a suitable environment. The inner cores are first dispersed in an aqueous medium and then sonicated with polyhydroxy carbohydrates to achieve the coating of the inner cores. The coated inner cores are then freeze-dried to enhance the irreversible adsorption of the carbohydrate onto the inner core (Goyal et al., 2008).

11.8 Loading of the bioactive agent or drug

The final step in the formulation of aquasomes is the adsorption or loading of the drug or the bioactive agent onto the inner core (Goyal et al., 2008; Shailendra, Pancholi, Agrawal, & Agrawal, 2008; Vengala et al., 2017). Inner cores coated with carbohydrates are usually dispersed in a known concentration of the drug at a suitable temperature and pH in this process of drug loading. Drug concentration and temperature are the key factors that affect the loading of drugs on coated inner cores.

11.9 Properties of aquasomes

Aquasomes afford a large surface area that provides a platform for drug loading and subsequent release to the targeted area. The release of the drug at the targeted site could be continuous or intermittent.

They also offer a suitable environment for the preservation of the biochemical stability of the adsorbed drug. The integrity of the drug conformation, which is essential for the efficacy of the drug, is maintained in aquasomes due to its aqueous properties and surface chemistry.

Aquasomes also exhibit self-stabilization properties as the inner core can be protected by the polyhydroxy carbohydrate layer. This self-stabilization process

in aquasomes is a function of the interactions between the inner core and the carbohydrate layer which could be ionic or non-covalent.

The biodegradability of aquasomes is another important property of these nanocarriers of drugs. This property of aquasomes hinges on the nature of materials that are used in the formulation of the core.

11.10 Characterization of aquasomes

Aquasomes are characterized based on factors like morphology, particle size and distribution, and drug loading capacity.

11.11 Particle size and distribution

Particle size and distribution are vital characterization indices for aquasomes since they are nanoparticles tailored toward drug loading and release. These nanoparticles should be in the size range of 60−300 nm (Sachin & Simran, 2020), and their distribution also affects physical properties like surface area, morphology, and packing density.

Scanning electron and transmission electron microscopy characterization techniques are useful characterization techniques in the particle size and distribution analysis of aquasomes. Photo correlation spectroscopy is a characterization technique that is employed in particle size and distribution analysis especially for particle mean size and zeta potential (Hasanein, 2021). Rojas-Oviedo et al. formulated aquasomes where the drug indomethacin was loaded on a lactose film-coated calcium phosphate core. They employed scanning electron and transmission electron spectroscopy to determine the size of the aquasomes to range between 60 and 120 nm and an average size of 90 nm (Rojas-Oviedo, Salazar-López, Reyes-Gasga, & Quirino-Barreda, 2007). Similarly, Basvaray et al. prepared aquasome nanoparticles with a calcium phosphate inner core, lactose film carbohydrate layer, and an etoposide drug layer. The surface morphology and zeta potential characteristics of these prepared nanoparticles were analyzed with particles ascertained to be spherically shaped with a particle size range of 150−250 nm (Nanjwade, Hiremath, Manvi, & Srichana, 2013).

11.12 Particle crystallinity

Crystallinity is a key property sought in the choice of a material in the formulation of the inner core of aquasomes. This is because a crystalline inner core affords a higher surface area for the carbohydrate coating and subsequent drug loading. The powder X-ray diffraction analysis (PXRD) technique is useful in the crystallinity and purity analysis of formulated inner cores. In this technique, the obtained powder X-ray diffraction patterns are matched with standard reference patterns which then inform of

the crystallinity and purity of prepared inner cores. It is important to note that PXRD analysis is also used in ascertaining the crystallinity and purity of prepared aquasomes. The process of the coating prepared inner cores with carbohydrates can however affect the crystallinity of prepared aquasomes. For example, Kamaljeet et al. prepared calcium hydrogen phosphate ($CaHPO_4$) as an inner core and characterized them with PXRD analysis that showed sharp and intense peaks indicative of crystallinity which was matched to standard reference patterns of $CaHPO_4$. The prepared $CaHPO_4$ was then coated with trehalose, cellobiose, and pyridoxal-5-phosphate, and the PXRD analysis of the mixture was determined. The PXRD peaks of the mixture showed the deformation of the peaks compared to that of $CaHPO_4$ indicative of an amorphous mixture (Kaur et al., 2015). It is important to note that the X-ray diffraction technique is used for particle crystallinity in other areas of nanochemistry (Ayom et al., 2020).

11.13 Particle structure

Information about the particle structure of aquasomes is useful in showing the capability of formulated aquasomes to withstand internal and external forces of stress. Fourier-transform infrared (FT-IR) spectroscopy usually expressed in a range of wavenumbers is the common technique used in the determination of particle structure properties of aquasomes. The acceptable FT-IR wavenumber range for aquasomes is $400-4000\ cm^{-1}$ (Sachin & Simran, 2020). In the potassium bromide (KBr) sample, the disk FT-IR method is usually employed and obtained bends are matched with standard patterns to get more information about particle structure. FT-IR spectroscopy results can also show whether the carbohydrate layer has been coated onto the inner core or the drug loaded onto the coated inner core and the drug stability. Duan et al. characterized their formulated nanomicelles targeted at ovarian cancer cells by FT-IR with characteristic wavenumbers of $2891\ cm^{-1}$ for CH_3/CH_2 groups, $1685\ cm^{-1}$ for $O-C=O$ groups, $3281\ cm^{-1}$ for primary amide groups, $1689\ cm^{-1}$ for amide linkage groups and $846/890\ cm^{-1}$ for para-substituted benzene groups (Jie, Ligang, Jiao, Li, & Xiao, 2020). Duan et al. could by this FT-IR spectroscopic results confirm their self-assembled three-layer nanomicelles.

A summary of how useful FT-IR results can be in the characterization of chemical groups of aquasomes' inner core, polyhydroxy carbohydrate, and drug layer is shown in Table. 11.2. Raman spectroscopy is another technique recently employed in the structural analysis of aquasome particles and found to be superior to the FT-IR (Damera, Kaja, Janardhanam, Alim, & Venuganti, 2019).

11.14 Polyhydroxy carbohydrate coating

The polyhydroxyl carbohydrate coating of the inner core of aquasomes is generally characterized by the calorimetric anthrone reaction, phenol sulfuric acid methods, and the canavalian A-induced method. The fast and reliable anthrone

Table 11.2 Aquasome structure elucidation employing FT-IR spectroscopy.

FT-IR band	Experimental value (cm^{-1})	Standard value (cm^{-1})
Drug layer (lornoxicam)		
NH bending	1650.85	1550–1640
S = O bending	1301.54	1175–1350
C–Cl	750.96	600–800
Carbohydrate layer (Cellobiose)		
CH_2 stretching asymmetrical	2921.56	2926
CH_2 stretching symmetrical	2879.12	2853
The inner core (Phosphate)		
Phosphate	896	845–725
Phosphate	1178.79	1300–1240

reaction used to determine the inner core coating is based on the principle of ascertaining the residual or unbound carbohydrates left after coating. Residual or unbound carbohydrate is hydrolyzed to a furfural derivative in an acidic medium that reacts with anthrone reagent to give a colored complex (blue or green), which can be measured by a UV-Vis spectrometer at 600 nm. The intensity of the blue or green-colored complex which is a function of the amount of free or residual polyhydroxyl carbohydrate gives the amount of the carbohydrate used up in coating the inner core (Hasanein, 2021; Kossovsky et al., 1996). The phenol sulfuric acid is another rapid and reliable calorimetric method for characterizing carbohydrate coating of inner cores in aquasomes. The principle of the phenol sulfuric acid method is similar to that of the anthrone reagent method. In the phenol sulfuric acid method, unbound or unused carbohydrate is dehydrated to a furfural derivative in the presence of sulfuric acid which reacts with phenol to give a yellow-gold color. The intensity of the obtained gold yellow color is indicative of the amount of unused or used carbohydrates in coating the inner core (Hasanein, 2021; Sachin & Simran, 2020). Whilst, the canavalian A-induced method is a non-calorimetric method of characterizing coated inner cores of aquasomes, the other two hitherto mentioned methods are not. The canavalian A-induced method also differs from the other mentioned methods in its determination of the bound carbohydrates unlike the unbound carbohydrates for the other methods. In the canavalian A-induced method, the canavalian A reagent is added to the carbohydrate-coated inner cores and the absorptions are recorded. The amount of carbohydrates coated on the inner core is then ascertained by graphical analysis (Goyal et al., 2008; Hasanein, 2021).

11.15 Drug loading of aquasomes

Coated inner cores of aquasomes are loaded with drugs which are in turn released to targeted sites. The quantity of drug released at a targeted site is a function of

the quantity of the drug-loaded onto the coated inner core of the aquasome. It is therefore necessary to be able to characterize drug-loaded inner cores of coated aquasomes. The efficiency of drug loading is determined by exposing a coated inner core to a high concentration of a drug for drug adsorption and subsequent extraction of the adsorbed drug in a suitable solvent and quantification of the extracted drug.

11.15.1 Drug release efficiency of aquasomes

The in vitro drug release efficiency of drug-loaded aquasomes is useful as this is at the center of the formulation of aquasomes. The in vitro drug release efficiency of aquasomes depends on factors like particle size of the inner core, desorption of adsorbed drugs, the solubility of drugs, and diffusion of drugs through the matrix. These factors all relate to the surface chemistry of the formulated aquasomes. Damera et al. have studied the drug release efficiency of coumarin 153, warfarin, and ibuprofen drugs in formulated aquasomes (Damera et al., 2019).

11.16 Zeta potential measurements of aquasomes

Zeta potential measurement is an analysis technique for measuring the surface charge of nanoparticles. It gives a measurement of the electrostatic attraction or repulsion of these particles and hence gives an indication of the stability or otherwise of dispersed particles or emulsions. These measurements are important for aquasomes because there are interactions between the drug and the coated carbohydrates, as well as the aquasomes and the targeted biological unit. The zeta potential measurements which are normally expressed in volts or millivolts vary as a function of the carbohydrates used in formulating aquasomes. Khopade et al. for example employed the zeta potential measurements to study the stability of their hemoglobin-loaded aquasomes before and after the coating of trehalose to hydroxyapatite inner core and the loading of hemoglobin to trehalose coated hydroxyapatite inner core (Khopade et al., 2002).

11.17 Formulation of aquasomes

The formulation of aquasomes is done by the principle of self-assembly which involves the spontaneous arrangement of constituent elements (inner core, polyhydroxyl carbohydrate, and drug layers) in certain prescribed structural orientations in two or three-dimensional space. The principle of self-assembly which is useful even outside the sphere of aquasomes is dependent generally on three physicochemical processes that are discussed below.

The interaction between charged groups within the formulated aquasomes is one of the physicochemical processes at play in the principle of self-assembly. Formulated aquasome nanoparticles are made up of groups like phosphate, amino, carboxyl, and sulfate which are electrostatically charged. The interaction of these charged groups helps in stabilizing these nanoparticles in an aqueous medium (a destabilization force for aquasomes) which is a suitable operating medium for them. These intermolecular interactive forces could be from hydrophilic or hydrophobic groups and can extend to a certain range.

Hydrogen bonding or dehydration effect is yet another physicochemical process that is useful in the principle of self-assembly. Hydrogen bonding in aquasomes arises from the interaction of hydrogen atoms attached to or in the neighborhood of highly electronegative atoms like nitrogen or oxygen (usually from the hydrophilic groups of aquasomes). Hydrogen bonding in aquasome nanoparticles usually occurs between the carbohydrate layer and the drug loaded on the coated inner core and in the aquasome interaction with surrounding water molecules. These hydrogen bonding interactions are countered or repelled by the dehydration effects arising from the hydrophobic part of aquasome particles. This interplay of interaction (hydrogen bonding to dehydration) in aquasome particles and their environment serves the principle of self-assembly (Sachin & Simran, 2020).

The molecular structure of a drug especially proteins is one of the key factors for its biological activity. Factors like variations in temperature, solvent, pH, and pressure can denature or alter the conformational integrity of drugs loaded on aquasome nanoparticles which can also affect the biological activity of such loaded drugs. How then do aquasome particles overcome these biophysical constraints and hence maintain their biological activity? The answer lies in the carbohydrate layer that helps the drug maintain its conformational integrity in keeping it from dehydration. The van der Waals forces between the carbohydrate layer and the drug layer within the particles also play a role in the maintenance of structure conformity of drugs loaded on aquasomes. Structure stability or the preservation of conformational integrity of drugs loaded on aquasomes is also another physicochemical process necessary in the self-assembly principle in aquasome nanoparticles. Interaction of charged particles, hydrogen bonding, dehydration effects, and conformational integrity preservation forces are hence key physicochemical processes that serve the principle of self−assembly.

11.18 Applications of aquasomes as a system for drug delivery

Aquasomes are novel nanocarrier or vehicle systems for drug delivery that have strengths over traditional methods in sensitivity, selectivity, efficacy, efficiency, and safety. They have been explored in different aspects of targeted drug delivery as outlined below.

11.19 Antigen nanocarriers

The human body is a complex but well-organized system designed by almighty God with a natural defensive strategy. One way of boosting the human body's defensive ability is to induce its defensive mechanism (production of antibodies) via the introduction of antigens. Aquasomes have become useful nanocarriers of these antigens to targeted sites of the body.

For example, Goyal et al. formulated aquasomes by the principle of self-assembly of hydroxyapatite inner core, cellobiose and trehalose carbohydrate coatings, and bovine serum albumin (BSA) drug (Goyal et al., 2008). The characterized BSA-loaded aquasomes with 20%−30% BSA loading showed better immunological efficiency compared to BSA alone.

He et al. on the other hand formulated a novel nanoparticulate adjuvant containing calcium phosphate and used it to induce an immune response to virus infections in animal models (He et al., 2000). The results they obtained indicated that adjuvants composed of calcium phosphate have better performance compared to the traditional alum adjuvants. The better performance of calcium phosphate was traced to its natural occurrence in the body and the study concluded that these results indicate a good potential for use of calcium phosphate adjuvants in humans.

Kossovsky et al. prepared a novel nanocarrier composed of a diamond inner core coated with cellobiose and loaded with the antigen muscle adhesive protein. The formulated aquasomes for antigen delivery had better performance compared to the traditional systems (Kossovsky et al., 1994).

Goyal et al. proposed the exploration of nanodecoy systems employing a ceramic core for effective immunization (Goyal et al., 2006). The nanodecoy systems were fabricated by using a hydroxyapatite inner core, coated with cellobiose and loaded with hepatitis B surface antigen (HBsAg). These fabricated systems were characterized by parameters like loading efficiency, size, and shape. SDS-PAGE experiments were used to evaluate the stability and integrity of the loaded antigen which is essential to the antigen efficacy. These fabricated systems were then tested in animals and the results obtained showed they were more efficient than the conventional adjuvant alum systems. The study also showed that the nanodecoy systems could induce Th1 and Th2 immune responses and hold promising potential in vaccine technology compared to conventional methods.

Goyal et al. in another study fabricated hydroxyapatite nanoceramic carriers by co-precipitation method comprising of sintering and spray-drying technique. They characterized the fabricated systems by size, shape, and antigen loading efficiency. These carriers showed slower antigen release and hence prolonged exposure to targeted cells and lymphocytes. There was an intense immunoglobulin G response with MSP-1_{19} nanoceramic adjuvant system unlike MSP-1_{19} alone or the alum system. The study showed that aquasomes are better antigen-delivery vehicles compared to conventional systems (Goyal et al., 2009).

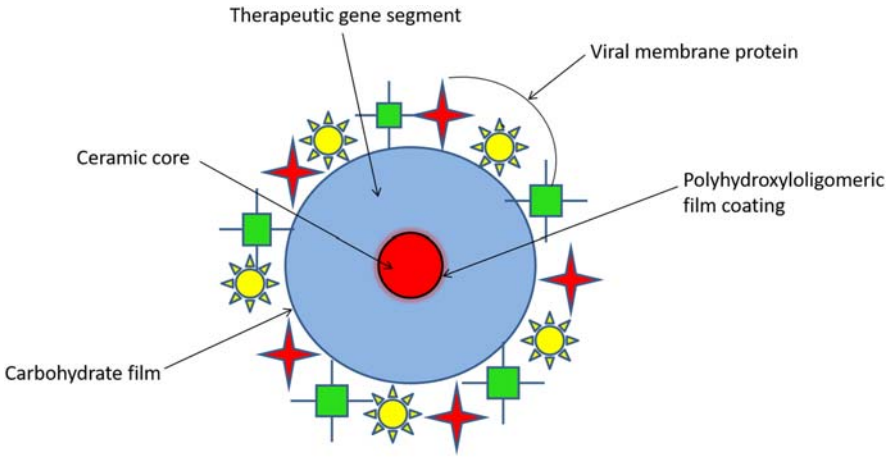

FIGURE 11.3

Aquasome as a vehicle for gene delivery.

Aquasomes have from these results shown potentiality as vehicles that preserve the morphological integrity of proteins and genes for efficient delivery at targeted sites in the body Fig. 11.3.

11.20 Insulin carrier

The destructive interaction between a drug (insulin) and the carrier of the drug is the biggest limiting factor in employing drug carriers for drug delivery in the body. Cherian et al. attempted to circumvent this obstacle by the fabrication of nanocarriers employing calcium phosphate inner core, cellobiose, pyridoxal-5-phosphate and trehalose carbohydrate coatings, and insulin drug. These aquasome self-assembly nanoparticles were characterized by size, shape, size distribution, in vivo performance, and drug loading efficiency. The efficiency of these formulated nanoparticles was compared to standard insulin solutions and observed to have better performance (Cherian et al., 2000).

Paul and Sharma also explored aquasome nanocarriers as vehicles for the delivery of insulin. They formulated hydroxyapatite nanoparticles designed for insulin delivery by oral administration and reported good insulin release at targeted sites (Fig. 11.4).

Deutel, Greindl, Thaurer, and Bernkop-Schnürch (2008) in another study focused on the in vivo potential of insulin-thiomer complex release in a formulated aquasome nanoparticle system. The system was fabricated by preparing poly (vinyl pyrrolidone) and poly(acrylic acid)-cysteine and loaded with insulin. The dissolution efficiency of insulin from the nanofabricated system and the insulin

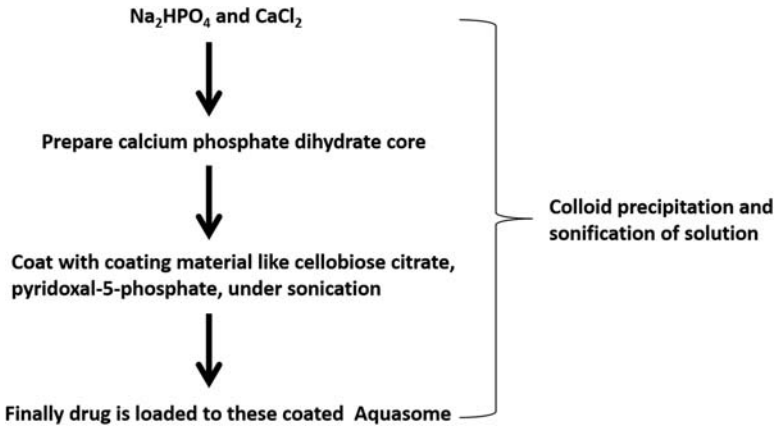

FIGURE 11.4

Schematic illustration for use of aquasome for insulin delivery.

tablets were then evaluated in vitro. The obtained results showed that the nanoparticle system of insulin delivery was better than the tablets medium and hold promising potential in replacing the conventional methods.

Aquasomes have hence proved to be useful vehicles or carriers of insulin and subsequent delivery to the targeted cells. They hold a huge potential in replacing the traditional systems of insulin delivery in medicine.

11.21 Oxygen carrier

Blood is an important connective tissue that transports essential materials like sugars, oxygen, carbon dioxide, and hormones in the body. Emergency cases like accidents and surgery where there could be massive blood loss could necessitate resuscitation which will impose delivery of oxygen artificially to the body. Scientists are therefore exploring resuscitative agents like hemoglobin-based oxygen carriers that could be useful in emergency cases. Khopade et al. (2002) prepared hydroxyapatite inner cores employing poly(amidoamine) as crystal modifiers in line with research to develop oxygen carriers. These inner hydroxyapatite cores were characterized by powder X-ray diffraction, infrared spectroscopy, and transmission electron microscopy. The inner cores that were prepared in spherical shapes depended on factors like phosphate saturation, pH of the body fluid, and crystal growth rate. The hydroxyapatite inner cores were then coated with a carbohydrate (trehalose) and confirmed with zeta potential measurements and colorimetric concanavalin measurements on a UV-Vis spectrophotometer. The trehalose-coated hydroxyapatite cores were then loaded with hemoglobin to form aquasomes which were characterized by hemoglobin loading, size, storage capacity, and size. The formulated aquasomes had

a loading capacity of about 13.7 mg/g of inner core and retained strong oxygen affinity. They were tested in rats and showed good potential to be employed as artificial oxygen carriers in the human body as the need arises.

In line with developing artificial oxygen carriers, Patil et al. formulated a surface-modified nanocrystalline ceramic carbohydrate composite (Shailendra et al., 2008). They are prepared by co- and self-precipitation hydroxyapatite inner cores which they coated with carbohydrates like cellobiose, maltose, trehalose, and sucrose. The concanavalin-induced aggregation technique was employed to characterize the coated inner hydroxyapatite inner cores. These prepared inner cores coated with carbohydrates were then loaded with hemoglobin and confirmed by the benzidine method. The oxygen-carrying capacity of these hemoglobin-loaded aquasomes was then evaluated and found to be similar to fresh blood. These nanoparticulate oxygen carriers were found not to induce blood coagulation or hemolysis of red blood cells and showed stability in hemoglobin content for 30 days. These oxygen carriers showed that they are good oxygen vehicles without side effects in vivo tests in rats.

11.22 Drug carrier

Vengala et al. to enhance the delivery of the poorly soluble drug, Iornoxicam, formulated nanoparticulate aquasomes as test drug vehicles (Vengala et al., 2017). The ceramic inner cores were prepared by co-precipitation and sonication and then coated with cellobiose and subsequently loaded with the Iornoxicam drug. The prepared aquasome was characterized by shape, size, in vitro release, and drug efficiency. The colorimetric phenol sulfuric technique was used to confirm the cellobiose coating of the inner ceramic core. The formulated aquasomes ranged from 60 to 300 nm in particle size with 87 nm media. The Iornoxicam-loaded aquasomes were then tested and found to have a better release in vivo (49%) than the pure drug (34%). In phosphate buffer solutions and pH 6.8, the pure drug had only 51% in 2 h compared to the formulated aquasome delivery of 95% in only 90 min. The study concluded that aquasomes are potential vehicles for improving drug delivery for poorly soluble drugs.

Muzushima et al. in their study in 2006 explored the preparation of aquasomes using the biodegradable hydroxyapatite ($Ca_{10}(PO_4O_6(OH)_2)$) that constitutes a major part of our bones and teeth. These formulated spherical aquasomes prepared by the spray method were tested as carriers of drugs like interferon alpha (IFNalpha), testosterone enanthate (TE), and cyclosporin A (CyA). The biodegradable hydroxyapatite nanoparticle had about 58% porosity and could be subcutaneously injected via a gauge needle. IFNalpha had good loading efficiency to the prepared nanoparticles and was released from the nanoparticles efficiently on testing. The addition of reinforcement like human serum and zinc to IFNalpha-loaded nanoparticles caused the prolongation of drug release in vivo (Mizushima et al., 2006).

Rojas-Oviedo et al. (2007) formulated aquasome nanoparticles loaded with a low-solubility drug Indomethacin. These nanoparticles were formulated by the preparation of calcium phosphate inner cores coated with lactose and indomethacin adsorbed onto it. The prepared aquasomes were evaluated by particle size, powder X-ray diffraction, scanning electron, and transmission electron microscopy. The inner calcium phosphate core and the coated lactose were identified by X-ray diffraction patterns. The indomethacin-loaded aquasomes had an average particle size of 60−120 nm and media of 90 nm. This drug-loaded aquasome has huge potential as a vehicle for indomethacin delivery in vivo.

Basavaraj et al. formulated aquasomes that were loaded with the drug etoposide. The aquasomes were prepared to employ a calcium phosphate inner core and a lactose film carbohydrate layer. Prepared aquasomes were characterized by properties like surface morphology, size, zeta potential, the efficiency of drug release, stability and distribution, and pharmacokinetics. Etoposide-loaded spherical particles prepared were of the size 150−250 nm with a high entrapment efficiency of 88%. Stability studies indicated 4°C as the most suitable temperature for the storage of self-assembled particles. The authors concluded that these etoposide-loaded aquasomes hold a lot of promise and need further investigations (Nanjwade et al., 2013).

Kutlehria et al. similarly investigated aquasomes as vehicles for the delivery of the oral drug Bromelain and the drug's bioavailability. These aquasomes were formulated using a calcium phosphate inner core which was coated with sugars like cellobiose, sucrose, and trehalose. Prepared aquasomes were characterized by the size and distribution of particles, drug loading and release, and zeta potential. The Bromelain-loaded spherically shaped aquasomes in vivo drug release was also investigated and showed a lot of promise (Kutlehria et al., n.d.).

Patali et al. formulated aquasomes loaded with the drug Pimozide to circumvent the problem associated with the poor solubility of the drug. Calcium phosphate was used to fabricate the inner core by precipitation and sonication and then coated with lactose. The lactose coating was confirmed by a colorimetric method. The Pimozide drug-loaded aquasomes showed better solubility compared to ordinary drugs (Pavani, Swetha, & Satya, 2013).

Researchers have reviewed topical drug delivery systems including micellar particulate drugs (Lohani & Verma, 2017; Monisha & Shahid, 2018). These nanoparticulate micellar drugs have good efficiency through skin delivery. The study shows the potentialities of aquasome-loaded drugs as better vehicles of in vivo drug delivery. Table. 11.3 gives a summary of the literature where aquasomes were employed as vehicles for drug delivery.

11.23 Fate of aquasomes

What is the fate of excess of these self-assembled materials in the body since they can concentrate in organs like the liver and muscles (Jain, Jagtap, Dand, Jadhav, & Kadam, 2012).

Table 11.3 A summary of drug applications in aquasomes.

Drug	Application	Preparation method	Notes	References
Iornoxicam	Vehicle for enhancing poorly soluble drug	Precipitation	Inner core: calcium phosphate. Carbohydrate layer: cellobiose	Mizushima et al. (2006)
Interferon alpha (IFNalpha), testosterone enanthate (TE) and cyclosporine A (CyA)	Vehicle for drugs and steady drug release	Spray	Inner core: hydroxyapatite ($Ca_{10}(PO_4O_6(OH)_2)$)	Kutlehria et al. (n.d.)
Indomethacin	To improve drug release	Precipitation	Inner core: calcium phosphate. Carbohydrate layer: lactose	Rojas-Oviedo et al. (2007)
Hemoglobin	Oxygen carrier	Precipitation	Inner core: hydroxyapatite. Carbohydrate layer: trehalose, maltose and sucrose	Shailendra et al. (2008)
Insulin	To treat excess levels of glucose in the blood	Adsorption	Inner core: calcium phosphate. Carbohydrate layer: cellobiose, trehalose, pyridoxal-5-phosphate	Khopade et al. (2002)
Etoposide	Cancer treatment	Precipitation	Inner core: calcium phosphate. Carbohydrate layer: lactose films	Nanjwade et al. (2013)
Bovine serum albumin	Adjuvant and antigen	Precipitation	Inner core: hydroxyapatite. Carbohydrate layer: cellobiose and trehalose	Goyal et al. (2008)
Bromelain	To improve drug bioavailability	Precipitation	Inner core: calcium phosphate. Carbohydrate layer: trehalose, sucrose, and cellobiose	Pavani et al. (2013)
Pimozide	To improve drug solubility	Precipitation and sonication	Inner core: calcium phosphate. Carbohydrate layer: sucrose	Sahoo et al. (n.d.)

11.24 Conclusion

To conclude, aquasomes are three-layer self-assembled particles that are emerging as useful carriers or vehicles for drug delivery. The three layers of this assembly include the inner core, the polyhydroxy carbohydrate layer, and the drug. They have shown strength over the traditional methods of drug delivery in maintaining the drug morphological integrity, sensitivity, specificity, and sustained drug release. They have shown potential as drug carriers for oxygen, insulin, hemoglobin, antigens, and antibodies. Aquasomes have also shown promise in improving drug solubility for poorly soluble drugs and evoking a better immunological response. Further studies of aquasomes are desired to gain more understanding of their toxicity, pharmacokinetics, efficacy, and safety so that they can be widely and commercially made available.

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CHAPTER

Nanostructured lipid carriers: a novel platform for therapeutics

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12.1 Introduction

Recent decades have evidenced the employment of nanocarriers/nanoparticles (NPs) for efficient drug delivery for various drugs/molecules intended for therapeutic and/or diagnosis purposes (Parashar et al., 2018; Parashar, Tripathi, et al., 2019). Various pieces of literature witnessed nanocarriers' capability in the successful delivery of genes, biological molecules, diagnostic agents, protein molecules, phytochemicals, and many more (Kanoujia et al., 2016; Parashar, Mazhar, et al., 2019; Selvamuthukumar & Velmurugan, 2012). The nanocarriers are broadly classified as vesicular systems (niosomes, bilosomes, etc.), lipoidal nanocarriers [nanostructured lipid carriers (NLC) and solid lipid carriers (SLN)], particulate carriers (polymeric NPs, carbon nanotubes, metallic NPs, etc.), emulsified nanocarriers (microemulsion, nanoemulsions, etc.) and micellar systems (micelles, reverse micelles, etc.) (Mishra et al., 2018). Works of research have suggested the drug delivery potential of lipoidal nanocarriers in various therapies. The two kinds of nanoparticulate system comprise NLC and SLN (lipid-based NPs). These were initially developed in the decade of 1990s as an imitation of nanoemulsions (oil-in-water) in which the solid lipid matrix served as the internal phase substituting the oily phase (Khan et al., 2015; Rawat et al., 2015). The benefit of NLC/SLN over other formulations was to avoid nonaqueous solvents solvent that further facilitates scaleup along with protection against degradation and releases during transit (Meraj Anjum et al., 2016; Rawat et al., 2015). Further, SLN holds various limitations such as drug leakage during storage, compromised drug loading, and polymorphic transitions (Müller, Radtke, & Wissing, 2002). SLNs also displayed challenges in the formulation and lead to phase transition of lipids, erratic gelation, crystallization during storage, poor encapsulation, and compromised payload (Rizwanullah, Ahmad, & Amin, 2016). Concomitantly, such challenges were conferred with the formulation of NLCs where a combination of solid lipid and liquid

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lipid was explored to form an amorphous solid matrix that showed improved drug loading, entrapment efficiency and improved microencapsulation of drugs, capability to retain drug without expulsion during storage (Haider, Abdin, Kamal, & Orive, 2020). These improved qualities can be attributed to the formation of amorphous lattice ascribing to liquid lipid and the construction of crystalline solid matrix with imperfections ascribing to solid lipid as shown in Fig. 12.1.

Further, lipoidal nanocarriers are suitable for the delivery of lipophilic drugs and have been reported to deliver various molecules such as carotenoids, flavonoids, polyphenols, phytosterols, tocopherols, fatty acids, oil-soluble vitamins, and nutraceuticals (Babazadeh, Ghanbarzadeh, & Hamishehkar, 2017; Bhise, Kashaw, Sau, & Iyer, 2017; Saez, Souza, & Mansur, 2018; Soleimanian et al., 2020). Also, the nontoxic nature and presence of natural and digestible lipid facilitate bioabsorption of drug/molecules in small intestines ascribing to increased micelles formation (Tamjidi, Shahedi, Varshosaz, & Nasirpour, 2013). The recent decades suggested drug delivery of various drugs employing SLN and NLCs suggesting them to be promising nanocarriers ascribing to their composition and feasible scaleup (Rizwanullah et al., 2016). Ascribing to the aforementioned assets, NLCs have gained significant consideration as nanocarriers for the delivery of drug/xenobiotics and have been explored for oral, parenteral, ocular, pulmonary,

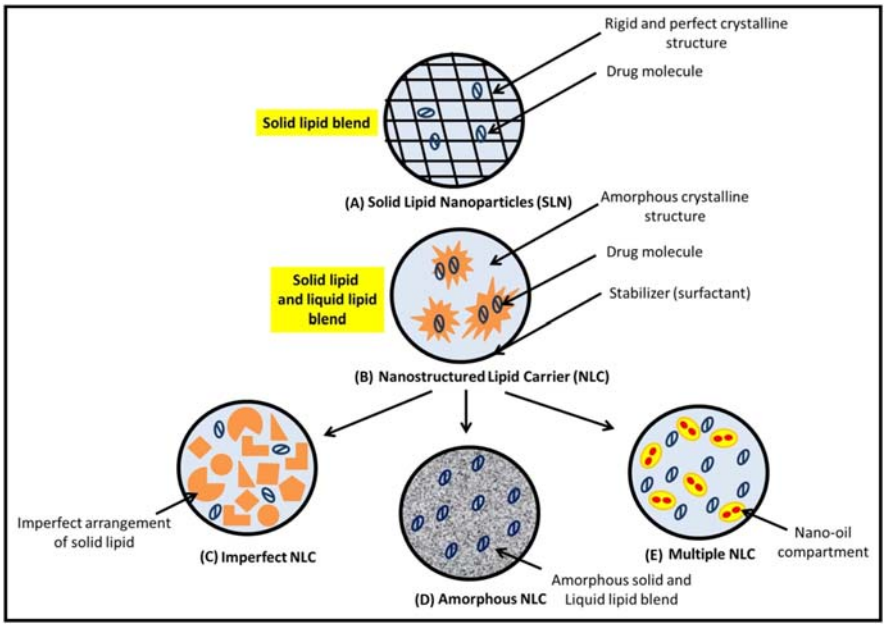

FIGURE 12.1

(A) Structure of solid lipid nanoparticles (B) Structure of nanostructured lipid carriers (NLCs) (C) Classification of NLCs: Imperfect NLC (D) Amorphous NLC (E) Multiple NLC.

topical, and transdermal administration (Khosa, Reddi, & Saha, 2018). Recent studies have suggested the utilization of NLCs in brain targeting (Tripathi et al., 2021), tumor targeting (Tripathi et al., 2020), cosmeceuticals (Rawat et al., 2015), gene therapy (Zhang, Zhang, & Yu, 2017) and nutraceuticals (Babazadeh et al., 2017). The NLCs can be classified as (1) imperfect crystal type; (2) amorphous type and (3) multiple types as shown in Fig. 12.1. The imperfect crystal type NLC comprises an extremely disordered matrix with numerous voids/spaces that can efficiently accommodate the higher amount of drug in amorphous clusters. Such types of NLCs can be formulated using a blend of solid lipids and liquid lipids having variable chain lengths (Chauhan, Yasir, Verma, & Singh, 2020). The amorphous type of NLC is formulated using specific non-crystallizing lipids namely hydroxyl octacosanol, hydroxyl stearate, and isopropyl myristate to avoid drug leakage resulting from crystallization. The lipid matrix exists in a homogenous amorphous state (Chauhan et al., 2020). The third type of NLCs (Multiple types) are oil/lipid/water type NLCs. These are suitable for the loading of highly lipophilic drugs that get entrapped into a liquid lipid matrix (Li, Cai, et al., 2017).

However, the safety and therapeutic potential evaluation of NLCs is compulsory, and thus formulated NLCs are evaluated for various parameters. These parameters comprise particle size distribution, zeta potential (stating stability), surface morphology, compatibility studies, crystal and polymorph formation, drug loading and entrapment efficiency, in vitro drug release studies, in vivo studies (pharmacokinetic & pharmacodynamics behavior), cellular uptake (in case of targeted drug delivery), long term stability studies and most important the toxicity studies (Haider et al., 2020; Kim et al., 2019; Tripathi et al., 2020). Beyond many advantages over other novel delivery systems, NLCs possess some limitations viz. lipid concentration and nature dependent-cytotoxicity and may cause irritation or sensitivity reaction ascribing to the presence of surfactants (Schäfer-Korting, Mehnert, & Korting, 2007). The NLCs can be formulated employing various techniques vis-à-vis. high-pressure homogenization, microemulsion, solvent emulsification-evaporation, melting dispersion method, solvent injection, double emulsion, and many more (Cavalli et al., 2015; Zhao et al., 2010).

The chapter appraises the several intrinsic properties of NLCs and their advantages over other nanocarriers that aid in the enhancing therapeutic efficacy of drugs when delivered through lipoidal nanocarriers. Finally, clinical studies performed on NLCs and translational products are reviewed that validate their regenerative properties and future applications in this area.

12.2 Therapeutic exhibitions of nanostructured lipid carriers

Various pieces of literature have suggested therapeutic applications of NLCs as nanocarriers that have established their potential in drug delivery through various

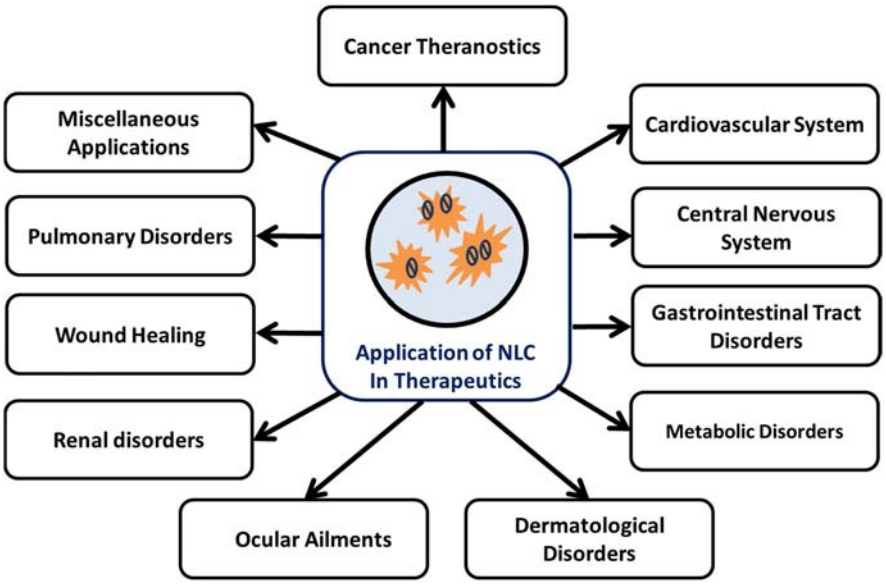

FIGURE 12.2

Therapeutic applications of NLCs.

routes including targeted drug delivery. Further, the drug delivery potential has been evaluated through in vitro and in vivo systematic experiments over simulated conditions, cell cultures, and animal models. These experiments revealed the enhanced therapeutic potential of various drugs delivered as NLCs in confronting various diseases. Also, such systems have been considered nontoxic, well tolerated, and safe for human use. A glance at the therapeutic applications of NLCs is shown in Fig. 12.2.

12.2.1 Nanostructured lipid carriers in cancer theranostics

NLCs have been widely explored in cancer therapy as targeted delivery of drugs and diagnostics, and their theranostics potential has been witnessed through various studies. In the same context, Tripathi et al. formulated biotin-anchored doxorubicin-loaded NLCs employing *Perilla frutescens* oil as liquid lipid (holds anticancer activity) for targeted drug delivery to mammary gland carcinoma. The results revealed enhanced antitumor potential, higher accumulation and retention into tumor vicinity, downregulation of bcl-2, MMP-9 (antiapoptotic proteins), and higher apoptosis when compared with naïve drug and marketed formulations (Tripathi et al., 2020). The enhanced anticancer potential is credited to a synergistic effect of *P. frutescens* oil and drug along with targeted drug delivery through

NLCs. Likewise, Lanna et al. formulated lipid-based artemether and triglycerides of docosahexaenoic acid-loaded NLCs for breast cancer therapy. The findings revealed enhanced cytotoxicity in MDA-MB-231 and MCF-7 cell lines in a dose-dependent manner as well as précised selectivity for these tumor cell lines. Also, rapid cellular internalization and cellular uptake were observed in coloaded formulation treated cells in comparison to other formulations (Lanna et al., 2021). Singh et al. explored topical administration of silymarin-encased NLCs against DMBA- induced (7,12-dimethylbenz[a]anthracene) skin carcinoma. The results demonstrated a decline in proliferation markers viz. ODC, COX-2, and cyclin D1 in an animal model as well as reduced progression and proliferation in cancer cells. The formulation also displayed long-term stability and higher permeation into the cells, crediting to the enhanced solubility of silymarin in the NLC matrix (Singh, Arya, et al., 2016). Parallel to the above studies Singh et al. also explored the anticancer potential of silymarin NLCs in UV-irradiated rat skin and SK-MEL 2 cell line. The results revealed enhanced anticancer potential and apoptosis in Silymarin NLC treated SK-MEL 2 cell lines when compared to marketed formulations (Singh, Singh, et al., 2016). Another study performed by Li et al. demonstrated the formulation of folic acid decorated docetaxel-loaded ultrasmall NLCs (< 30 nm) for cancer therapy (Li et al., 2020). The NLCs demonstrated significant cellular uptake and internalization in HeLa cells. The findings of in vivo experiments suggested that surface-modified ultrasmall NLCs demonstrated higher accumulation and retention in tumor cells and enhanced antitumor effect (xenograft tumor model) when compared with the control group.

NLCs have also proven their potential in drug delivery for colon carcinoma therapy. In this regard, Outuki et al. prepared Pterodon pubescens fruit oil-loaded NLCs (Outuki et al., 2018). The results demonstrated a significantly higher in vitro cytotoxicity in HT-29 cells when compared with free oil suggesting that NLCs can aid in enhancing the therapeutic efficacy of phytopharmaceuticals. Another study by Li et al. demonstrated NLCs having a dual approach that is a combination of drug and photothermal effect and developed AMD3100 loaded photothermal (IR780 photothermal agent) NLCs against breast cancer (Li, Wang, et al., 2017). The combination demonstrated a significant antitumor and metastasis prevention in the syngeneic orthotopic metastatic breast cancer model. Concomitantly, the dual functionalized also displayed theranostic prospective crediting to IR780 (NIR fluorescence sensitive dye) that was encased into the core of NLCs. A representation of this dual approach has been shown in Fig. 12.3. Likewise Ferreira et al. formulated methotrexate-loaded NLCs for cancer therapy. The findings suggested a burst release followed by a controlled release of the drug throughout 24 h suggesting a suitable delivery system for drug delivery to cancer cells. The NLCs displayed noncytotoxic behavior in fibroblast cells after exposure of 24 and 48 h (Ferreira, Chaves, Lima, & Reis, 2015).

All these studies give auxiliary evidence that NLCs can be utilized as a safe and effective delivery system with enhanced potential for cancer therapy.

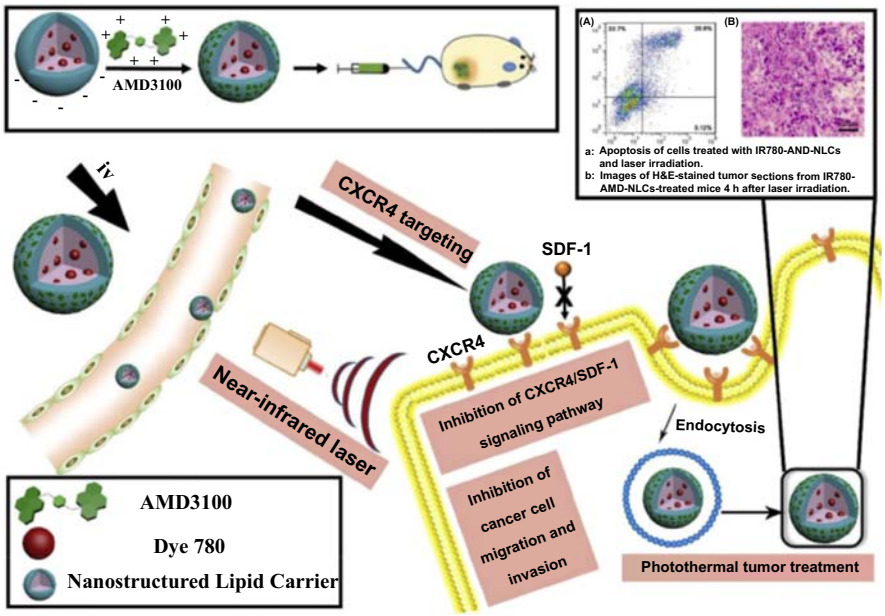

FIGURE 12.3

Schematic design of IR780-AMD3100- (an antagonist of CXCR4) loaded NLCs employing a combination approach for breast cancer therapy.

Reproduced with permission from Li, H., Wang, K., Yang, X., Zhou, Y., Ping, Q., Oupicky, D., et al. Dual-function nanostructured lipid carriers to deliver IR780 for breast cancer treatment: Anti-metastatic and photothermal anti-tumor therapy. Acta Biomaterialia, 53, *399–413.*

12.2.2 Nanostructured lipid carriers in cardiovascular system

Studies have shown the employment of NLC for the delivery of cardiovascular drugs. Soleimanian et al. formulated ß-sitosterol-loaded NLCs for treating hypocholesterolemia. The NLCs displayed stability in variable simulated gastric fluids and controlled release of the drug. The in vivo studies demonstrated significantly reduced LDL and cholesterol plasma levels when compared with drug suspension in an animal model. The improved therapeutic efficacy is credited to enhanced solubility of the drug owing to NLC formulation (Soleimanian et al., 2020). Similar to the above study Raj et al. formulated simvastatin-loaded NLCs for transdermal drug delivery systems (transdermal patches) (Raj, Chandrasekhar, & Reddy, 2019). The pharmacokinetic study revealed 18.40% enhanced bioavailability, 12 h Tmax, and AUC of 163.0397 μg/mL h, which was significantly higher when compared with marked formulation, drug suspension, and plain drug formulated transdermal patch. Another study performed by Li et al. demonstrated the development of rosuvastatin-loaded NLCs for hyperlipidemia therapy (Li, Yang, & Xu, 2018). The pharmacokinetic study demonstrated a 1.65-fold increase

in AUC and significantly higher plasma concentration when compared with rosuvastatin tablets suggesting improved bioavailability. Concomitantly, the pharmacodynamics results exhibited a noteworthy decline in triglyceride levels in NLCs administered group in the poloxamer-induced hyperlipidemia model, suggesting improved anticholesterolemic activity of a drug when formulated as NLCs. Parallel to aforesaid studies Elmowafy et al. developed atorvastatin-loaded NLCs for enhanced solubility and the ability to bypass hepatic metabolism during transit (Elmowafy et al., 2017). The NLCs presented improved pharmacokinetic parameters ascribing to higher entrapment of drugs in the NLC core. There was nearly 3.61-fold enhanced bioavailability when compared with naive atorvastatin and marketed formulation. Alongside a significant reduction in total cholesterol, triglyceride and LDL levels and inversely increased levels of HDL were revealed in NLCs administered group which was supported by histopathological results displaying insignificant hepatic steatosis.

12.2.3 Nanostructured lipid carriers in central nervous system

Literature has reported the inability of most of the drug candidates to cross the blood-brain barrier owing low solubility of the drug, thus a challenge is always offered in the delivery of drugs for CNS-related diseases. However, various reports suggested the capability of NLCs to counter the aforesaid challenges that result in efficient drug delivery that leads to enhanced therapeutic efficacy. Taking into account the NLCs approach Jojo et al. developed pioglitazone-loaded NLCs intranasal formulations for Alzheimer's therapy (Jojo, Kuppusamy, De, & Karri, 2019). The brain-targeted formulation displayed augmented biodistribution of NLCs in rats and direct transport of drug from the nose to the brain ascribing to improved solubility and permeation of drug. Further toxicity studies revealed the biocompatibility of the formulation and thus may be considered safe for human use. Another study performed by Malvajerd et al. demonstrated the formulation of curcumin-loaded NLCs for Alzheimer's disease. The formulation displayed a higher accumulation of curcumin in the brain as well as plasma of rats. Also, a declined level of oxidative markers and amyloid-β peptide in the hippocampal tissue was displayed suggesting enhanced therapeutic efficacy and neuroprotective action of curcumin in Alzheimer's progression when formulated as NLCs (Sadegh Malvajerd et al., 2019).

NLCs have also established their delivery potential in multiple sclerosis, a severe autoimmune disorder of CNS. In the same perspective, Gadhave et al. developed teriflunomide-loaded NLCs for multiple sclerosis therapy to be administered through the intranasal route. The NLCs demonstrated approx. twofold increased permeation and swift remyelination in cuprizone-treated animals and less accumulation in open compartments. Further toxicity studies witnessed the nontoxic nature of the formulation as there was no alteration in hepatic biomarkers (Gadhave & Kokare, 2019).

Various research works have recommended the employment of NLCs in Parkinson's disease, a disease associated with age-related neurodegeneration resulting in dopaminergic neuron damage. A study performed by Mishra et al. evidenced the potential of NLCs in the treatment of Parkinson's disease (Mishra, Sharma, Deshmukh, Kumar, & Sharma, 2019). The researchers formulated selegiline hydrochloride-loaded NLCs for nose-to-brain delivery. The NLCs displayed sustained release behavior throughout 22 h and restoration of behavioral parameters in rotenone-induced rats. Likewise, Hernando et al. prepared a transactivator of transcription peptide conjugated glial cell-derived neurotrophic factor-loaded NLCs for Parkinson's therapy (Hernando et al., 2018). The in vivo outcomes displayed faster motor recovery (evidenced through immunohistochemistry studies) as witnessed by increased levels of tyrosine hydroxylase fibers in the striatum and neuron levels in the substantia nigra. Further, ionizing calcium-binding adaptor molecule 1 immunohistochemistry displayed NLCs as a modulator of microglia activation restoring to normal levels.

Targeting brain tumors remains a formidable challenge for drug delivery. Recent reports proposed employing NLCs to overcome such challenges. One such experiment performed by Emami et al. revealed that NLC facilitated brain targeting of artemisinin (Emami, Yousefian, & Sadeghi, 2018). The researcher's explored transferrin-anchored NLCs for brain tumor targeting. The results revealed considerably higher cytotoxicity in NLC exposed U-87MG cells (brain cancer cell lines) than that with naïve drugs and unmodified NLCs. In the same way, Chen et al. formulated curcumin-loaded NLCs and investigated their cytotoxicity in A172 cell lines and targeting potential in mice (Chen, Pan, Jiang, Li, & Jin, 2016). The results state that curcumin-loaded NLCs exhibited remarkable cellular uptake and cytotoxicity, while a higher payload in the cancer tissue and reduced off targeting were evidenced through in vivo findings.

The challenges associated with schizophrenia are similar as discussed above as it is a kind of CNS disorder. To counter the challenge Jazuli et al. developed brain-targeted NLCs encasing lurasidone hydrochloride for intranasal administration (Jazuli, Nabi, Alam, Baboota, & Ali, 2019). The findings verified a twofold increased concentration of drug in the brain when compared with plain lurasidone subsequent to intranasal administration.

Another CNS applicability of NLCs in epilepsy has been reported by Sharma et al. The investigators prepared brain-targeted embelin-loaded NLCs for the treatment of epilepsy in a rat model (Sharma, Bhandari, Deshmukh, Yadav, & Mishra, 2017). The formulations were administered intranasally and showed potential in reducing glutathione levels while increasing malondialdehyde and nitrite levels in pentylenetetrazole-induced disorders (altered biochemical parameters). Another study by Elmowafy et al. demonstrated an investigation of carbamazepine-loaded NLCs for epilepsy management (Elmowafy et al., 2018). The results indicated enhanced bioavailability by 2.27 fold when compared to a drug suspension. The in vivo findings indicated a significantly higher anticonvulsant activity in terms of seizure expectancy, frequency, and length without showing any hepatotoxicity and testicular toxicity.

All the exemplified studies suggested that NLCs can be employed for treating brain disorders. Also, an improved targeting was achieved when NLCs were administered as nose-to-brain delivery and can be an alternative for the oral route of administration.

12.2.4 Nanostructured lipid carriers in gastrointestinal tract disorders

NLCs have been explored for drug delivery for various applications and their utilization in the management of gastrointestinal tract disorders has been reported in numerous studies (Subramaniam, Siddik, & Nagoor, 2020). In the same context Sinhmar et al. explored NLCs for the management of inflammatory bowel disease, where the site-specific delivery of the drug is the major challenge (Sinhmar et al., 2018). The authors prepared mannosylated NLCs encasing budesonide for targeted delivery to inflamed tissues of the colonic region. The in vivo results demonstrated reduced colonic myeloperoxidase activity and inflammatory cytokines in an oxazolone-induced colitis rat model. Further, the NLCs exhibited noncytotoxic properties in J774A.1 cell line indicating biocompatibility. These findings prove the targeting and superior therapeutic effect of NLCs when compared to plain drugs.

In another study, NLCs were explored for treating nonalcoholic fatty liver disease. Hu et al. formulated naringenin-loaded NLCs and investigated their therapeutic potential in methionine choline deficient diet-induced nonalcoholic fatty liver disease in an animal model (Hu et al., 2021). The findings demonstrated a 3.5-fold increased drug payload. Also, an increase in transepithelial transport and intestinal absorption was perceived through NLC (eightfold low dose) when compared to plain drug ascribing to clathrin pathway mediated intracellular transport evading p-gp efflux. The NLCs exhibited a 1.5-fold enhanced hepatic accumulation and a threefold reduction in diet-induced lipid deposition suggesting prominent inhibitor potential of naringenin-loaded NLCs.

Ahmed et al. formulated pumpkin seed oil-based NLCs where pumpkin seed oil was utilized as liquid lipid and investigated its protective action against NSAID-induced gastric ulcers (Ahmed et al., 2020). The results of the study demonstrated a significantly reduced gastric ulcer occurrence when compared with the raw oil administered group.

Deng et al. explored the drug delivery potential of NLCs in treating ulcerative colitis (Deng et al., 2020). They prepared berberine-loaded NLC and investigated its therapeutic potential in dextran sodium sulfate-induced ulcerative colitis in mice. The NLCs presented substantial cellular uptake of phytopharmaceuticals in RAW 264.7 cells and Caco-2 cells. Further in vivo studies revealed the inhibitory potential of drug-loaded NLCs in terms of augmented colitis symptoms viz. disease activity index, colon length, spleen swelling, and myeloperoxidase activity. The NLCs were capable of inhibiting NF-κB nuclear translocation,

downregulation of pro-inflammatory cytokines (IL-1β, IL-6, TERT, MMP-9, COX-2) expression, and upregulation of ZO-1 expression (tight junction protein). Likewise, Mishra et al. formulated celecoxib entrapped NLCs for treating dextran sodium sulfate-induced ulcerative colitis in an animal model (Mishra et al., 2020). The findings suggested cytocompatible of NLCs in hTERT-BJ cells in a dose of up to 200 μg/mL. Further, a decline in disease activity index and disintegration of goblets cells was observed with a restoration of sulfomucin in the colon. Also, downregulation of COX-2 and iNOS, witnessed enhanced therapeutic efficacy of the drug when formulated as NLC.

Another study by Rouco et al. demonstrated the utilization of NLC in the therapy of Crohn's disease (Rouco et al., 2020). The management of Crohn's disease is challenging owing to the poor aqueous solubility of drugs used for therapy. In the same perspective, Rouco et al. developed rifabutin-loaded NLCs and investigated their potential in the management of Crohn's disease. The formulation displayed payload, stability, and satisfactory permeation across Caco-2 cells. The results suggest that rifabutin NLCs could be a promising approach to improve peroral antimycobacterial therapy in Crohn's disease.

All the aforementioned illustrated studies suggested that NLCs can be employed for treating GIT disorders owing to enhanced solubility and permeation of drugs across the intestinal mucosa.

12.2.5 Nanostructured lipid carriers in diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder that displayed enhanced blood glucose levels medically termed hyperglycemia owing to altered carbohydrate, protein, and fat metabolism (Piazzini et al., 2019). Recently essential oils have been explored for DM therapies. In the same framework, Vieira et al. developed sucupira oil-loaded NLCs and investigated loading capacity, in vitro release, and cytotoxicity in caco-2 cells (Vieira et al., 2020). The prepared NLCs displayed 99.98% entrapment and 9.6% drug loading respectively and no cytotoxicity in caco-2 cells. The release profile exhibited first-order kinetics for 8 h. The studies offer a platform for the utilization of sucupira oil-loaded NLCs as a novel antihyperglycemic formulation for the management of DM. A new study by Piazini et al. displayed the formulation of silymarin-loaded NLCs for DM therapy (Piazzini et al., 2019). Literature has reported the antidiabetic effect of silymarin but its use has been limited owing to poor aqueous solubility and permeability. To overcome these limitations Piazini et al. formulated NLCs of silymarin and were found to be stable in a gastrointestinal environment having variable pH. In vitro experiments revealed enhanced permeation of silymarin through the PAMPA membrane and higher cellular uptake in Caco-2 cells and no cytotoxicity. Further in vivo studies suggested a significantly higher decline in blood glucose levels and triglyceride levels in the streptozotocin-induced DM animal

model when matched with naïve drug. Concomitantly, the formulation also displayed substantial antihyperalgesic outcomes on streptozotocin-induced neuropathy. Similarly, Shi et al. fabricated baicalin encased NLCs for the management of diabetes (Shi, Wei, Zhao, & Xu, 2016). The NLCs displayed a sustained release pattern throughout 24 h and exhibited significantly higher antidiabetic potential when compared with free baicalin.

The above-exemplified studies give auxiliary evidence of improved solubility, permeation, and finally therapeutic efficiency of poorly aqueous soluble drugs when formulated as NLC and can be employed for the management of DM.

12.2.6 Nanostructured lipid carriers in dermatological disorders

Acne is the most common ailment that is encountered by teenagers and young adults. Studies have suggested NLCs dermal application as a topical/transdermal system that had added benefits to the formulation and result in enhanced therapeutic and cosmeceutical potential. One such application demonstrates the utilization of tretinoin (retinoid, limited use owing to skin irritation) NLCs that displayed enhanced antiaging and antiacne potential. Ghate et al. formulated tretinoin-loaded NLCs which were further dispersed into carbopol gel (Ghate, Lewis, Prabhu, Dubey, & Patel, 2016). The NLCs exhibited a sustained release pattern reduced skin irritation and minor erythema within 3 days of application with a prolonged action which was greater than the marketed formulation. A similar study was performed by Kumari et al. where the antiacne compound azelaic acid (poor aqueous solubility) was formulated as NLC for topical application (Kumari, Pandita, Poonia, & Lather, 2015). The ex vivo permeation studies demonstrated initial burst release trailed by a sustained release pattern and a significantly higher flux when compared with a plain drug suspension. Additionally, good retention of 63.96% ± 4.56% indicated the availability of the drug for local action, a prerequisite for topical formulations, and was significantly higher than that of plain drug suspension. The study established enhanced permeation into the stratum corneum at the same time significant retention was observed in the upper layers of skin delivering drugs for local action signifying the vast potential of NLCs for topical application. The results also signify the NLCs' potential in rectifying challenges associated with drugs and avoidance of systemic adverse effects concomitant with their usage. The application and permeation of lipoidal carriers are depicted in Fig. 12.4.

Psoriasis is a chronic disorder with a high relapsing rate of inflammatory skin disorder (Thapa & Yoo, 2014). A satisfactory nonirritating, nontoxic delivery system is needed for its management. Madan et al. investigated apremilast-loaded NLCs carbopol 940 gel for topical application for psoriasis management (Madan, Khobaragade, Dua, & Awasthi, 2020). The in vitro drug release studies revealed sustained release relatively low drug diffusion and higher skin retention of 60%.

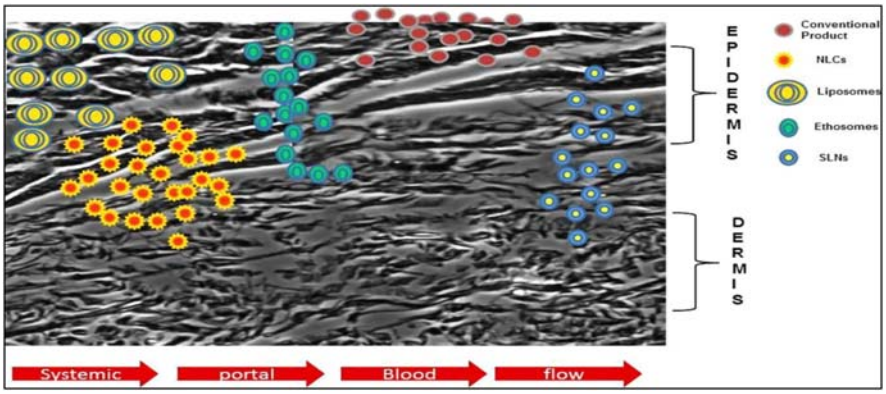

FIGURE 12.4

Representation of permeation potential of lipoidal nanocarriers in various skin layers.

Reproduced with permission Raza, K., Singh, B., Lohan, S., Sharma, G., Negi, P., Yachha, Y., et al. (2013). Nano-lipoidal carriers of tretinoin with enhanced percutaneous absorption, photostability, biocompatibility, and anti-psoriatic activity. International Journal of Pharmaceutics, 456*(1):65–72.*

These findings suggested the NLC potential for improving the therapeutic efficacy of the drug as a topical application. Similarly, Raza et al. prepared tretinoin-loaded NLCs that displayed improved percutaneous absorption, substantial photostability, and biocompatibility leading to significantly enhanced antipsoriatic activity in the mouse tail model when compared with the marketed formulation (Raza et al., 2013). Parallel to the above study Sathe et al. developed dithranol entrapped NLC-loaded gel and investigated its antipsoriasis potential in imiquimod-induced mice psoriatic plaque model (Sathe, Saka, Kommineni, Raza, & Khan, 2019). The findings of the study demonstrated declined psoriasis symptoms supported by the ELISA test as well as reduced levels of cytokines viz. IL-17, IL-22, IL-23, and tumor necrosis factor-α when compared with control and marketed formulation administered to animals. Another study by Pradhan et al. demonstrated the formulation and evaluation of fluocinolone acetonide-loaded NLCs incorporated into a salicylic acid-loaded gel for psoriasis management (Pradhan, Yadav, Singh, & Singh, 2021). The prepared NLCs demonstrated a prolonged release of the drug throughout 24 h, while confocal laser scanning microscopic studies showed substantial retention of the drug in the epidermal and deep dermal layer of skin and negligible systemic absorption. Additionally, ELISA outcomes revealed a significantly reduced concentration of TNF-α, IL-17, and IL-22 (prime pathogenic cytokines) in NLC treated group when compared with the salicylic acid conventional gel treated and control group. All the above-exemplified findings witnessed NLC efficacy in the management of psoriasis in equivalent or reduced drug concentration.

Among skin, cancer melanoma presents the most aggressive form and it is worth developing topical delivery systems for satisfactory clinical outcomes. Various pieces of studies evidenced the utilization of NLCs in melanoma therapy. Taking into account these findings Malta et al. formulated 1-carbaldehyde-3,4-dimethoxyxanthone (TAp73 activator holding antiproliferative effect) loaded NLCs for melanoma therapy via topical administration (Malta et al., 2020). The formulation displayed significantly higher cytotoxicity in the A375 melanoma cell line indicating enhanced efficacy of the drug ascribing to improved solubility than that of the plain drug. Likewise, Iqbal et al. developed silymarin-loaded NLC and investigated its antiproliferative, antioxidant, anti-inflammatory, and antitumor potential in B16 melanoma cell line and animal model (Iqbal, Ali, Ganguli, Mishra, & Baboota, 2019). The prepared NLCs demonstrated substantial cytotoxicity in B16 melanoma cell lines and a significant decline in tumor volume and IL-1α and TNF-α and oxidative stress markers.

Leishmaniasis is an epidemic disease that offers the challenge of drug resistance and requires an efficient treatment strategy (Kar, Chakraborty, De, Ghosh, & Bera, 2017). To encounter such a challenge Kar et al. developed cedrol-loaded NLC and evaluated its antileishmanial potential in drug-resistant l.donovani (wild-type, paromomycin resistant, sodium stibogluconate resistant strains). The results of the experiments demonstrated a significant internalization of the macrophages. Further, cedrol-loaded NLCs exhibited significant antileishmanial activity in all strains of l.donovani and twofold enhanced cytotoxicity in mouse peritoneal macrophage cells. Further, in vivo studies discovered a 2.3 to 3.8-fold enhanced bioactivity in wild-type and 4.9-fold enhanced bioactivity in drug-resistant strains respectively. Likewise, Riaz et al. formulated curcumin-loaded macrophage-targeted NLCs for cutaneous leishmaniasis therapy, where curcumin was taken as a model drug (Riaz et al., 2019). The fluorescence studies revealed that NLCs were targeted into macrophages and deeper skin layers with significant retention when compared with plain curcumin gel. The NLCs also displayed superior in vitro antileishmanial activity in AAL (axenic amastigote-like cells) and promastigotes cells when compared with plain curcumin gel.

Skin damage after sunburns in terms of sunburns is a considerable condition that leads to early skin aging and skin cancer in later stages. The application of sunscreen or protective lotion/creams is a solution to prevent skin aging. However poor aqueous solubility of protective drugs often ends in unsatisfactory outcomes. In the same perspective, Salam et al. developed diflu cortolone-valerate and titanium dioxide-loaded NLC as sunscreen (Abdel-Salam, Ammar, Elkheshen, & Mahmoud, 2017). The formulation exhibited an SPF of 4.94−21.27 that is capable of providing adequate sun protection ascribing to the synergistic effect of cortolone-valerate and titanium dioxide. The formulations also showed acceptable spreadability and no signs of grittiness.

12.2.7 Nanostructured lipid carriers in ocular ailments

The eye is one of the most important organs of the body and any ocular disease needs effective therapy for a swift recovery. Kiss et al. developed dexamethasone-loaded NLCs for ophthalmic use. The prepared NLCs showed acceptable ophthalmic tolerability and nontoxic performance (Kiss et al., 2019). Further, the NLCs deliver a higher payload of the drug over the eye surface forming a depot (corneal) and penetration into the hydrophilic stroma layer with improved bioavailability. Eye dryness is one of the common disorders associated with eyes and is often treated with eye drops which get drained off quickly thus, gels are a better available dosage form. To treat dry eye conditions Tan et al. developed mucoadhesive dexamethasone loaded, boronic acid-conjugated chondroitin sulfate NLCs (Tan et al., 2019). The NLCs displayed extended corneal retention time and improved drug delivery at the corneal site credited to a boronic acid group that owns a high affinity for sialic acids of ocular mucin as well as no signs of ocular irritation in rabbit eye as justified by histological images. Similarly, Salamouni et al. formulated brimonidine encased NLCs for intraocular localization (El-Salamouni et al., 2018). The prepared NLCs exhibited elevated ocular hypotensive effect and prolonged residence time with a 1.2-fold enhanced corneal penetration when compared with SLN and marketed formulation. Further, histological examination supported anterior ocular chamber localization of NLCs and lowered intraocular pressure over an extended period of time with no signs of ocular irritation in rabbit eyes.

12.2.8 Nanostructured lipid carriers in renal disorders

Lupus nephritis is a fatal frequently-occurring inflammatory renal disease. Recent research has reported the utilization of hormones and cyclophosphamide as satisfactory therapy. In the same framework, Li et al. explored the therapeutic outcome of Tripterygium Wilfordii Hook F (TWHF) loaded NLC for lupus nephritis therapy (Li et al., 2021). The in vivo experimental findings revealed that TWHF-NLCs have a recognizable therapeutic outcome in (immune lupus nephritis) mice. Further, histological results indicated dropped collagen content in renal interstitial cells and downregulated MCP-1 expression. Similarly, Dong et al. formulated ergosterol-loaded NLCs for diabetic nephropathy management (Natarajan et al., 2019). The use of ergosterol is limited owing to poor aqueous solubility and poor oral bioavailability which can be probably enhanced through formulating it as NLCs. The NLCs displayed high drug entrapment of $\sim$92% and drug loading of $\sim$6.5%. Further, the pharmacokinetic studies displayed 277.56% higher bioavailability when compared to pure ergosterol and elevated AUC and C_{max} parameters. Concomitantly, pharmacodynamic studies revealed the higher proliferation inhibition of mesangial cells (high-glucose-stimulated) and accumulation of extracellular matrix more effectively when compared with plain ergosterol.

Thus we can conclude that NLCs mediated delivery could be a promising delivery system that can enhance solubility and in turn, the oral bioavailability of therapeutic agents meant for renal therapy.

12.2.9 Nanostructured lipid carriers in wound healing

The diabetic wound is a major concern that needs to be treated at the earliest possible; or else it may lead to amputation. In diabetic patients, the major cause of delayed healing is continued inflammation that blocks the further healing process resulting in wound persistence. Considering such conditions Natarajan et al. formulated a pioglitazone-encased collagen/chitosan scaffold for diabetic wound healing (Natarajan et al., 2019). The scaffolds were bestowed with optimum porosity, slow matrix degradation, biocompatibility, and sustained in vitro release. It also displayed boosted cell proliferation when compared with control and plain NLCs. Further, in vivo studies demonstrated significantly higher wound shrinkage in pioglitazone encased NLCs treated groups when compared with control and plain NLCs. Also, downregulation of matrix metalloproteinases-9 levels was seen in pioglitazone encased NLCs treated groups when compared with control groups. Parallel to the above study Motawea et al. explored phenytoin-loaded NLCs for diabetic foot ulceration treatment (Motawea et al., 2019). In this, a total of 27 patients complaining of neuropathic diabetic foot ulceration were divided into three groups and administered with various dosages for 2 months (Group I: 0.5% w/v phenytoin NLC; Group II: 0.5%w/v phenytoin hydrogel; Group III: blank hydrogel). The patients treated with NLCs formulation showed significantly reduced wound area and faster healing when compared with control groups. Similar to the above study Lee et al. formulated NLC loaded with EGF-conjugated curcumin (epidermal growth factor) for treating diabetic wounds (Lee et al., 2020). The in vitro cell line studies displayed good bioactivity and cell migration in NIH 3T3 fibroblasts and HaCaT keratinocytes imitating accelerated wound healing. Further in vivo studies in the animal models represented faster-wound healing and wound closure and elevated antioxidant enzyme activity when compared to plain curcumin.

These studies indicate that drug-loaded NLCs could be an efficient topical wound dressing that can be explored for fast wound healing without adverse effects.

12.2.10 Nanostructured lipid carriers in pulmonary disorders

Targeting and efficient drug retention in the lungs always remained a challenge to formulation scientists (Parashar et al., 2018). However, studies have suggested the employment of NLCs to overcome such challenges. In the same background, Patil et al. hypothesized the delivery of montelukast via NLC formulations (Patil-Gadhe & Pokharkar, 2014). The drug-loaded NLCs exhibited a sustained release

pattern throughout 24 h and excellent drug entrapment of 96.13%. The NLCs administered peroral showed 143-fold improved bioavailability when compared with montelukast solution indicating NLCs' potential in enhancing bioavailability. Parallel to the above studies Patil et al. repurposed rosuvastatin (an antilipidemic drug) for chronic obstructive pulmonary disease therapy (Patil-Gadhe & Pokharkar, 2016). The NLCs were formulated for lung delivery in form of inhalable powder. The prepared NLC was assessed in vitro for aerosol performance (8-stage cascade impactor) and in vivo pulmokinetics in animal models. The in vitro assessment demonstrated the delivery of particles (<3 μm) via aerosol in the case of rosuvastatin-loaded NLCs. Further, in vivo findings suggested 1.14-fold improved C_{max}, fivefold enhanced $t_{1/2,}$ and about 35-fold greater AUC when compared with plain rosuvastatin giving auxiliary proof of circumventing macrophage clearance and higher drug accumulation in the lung vicinity. Similarly, Maalej et al. formulated beclomethasone-loaded NLCs meant for pulmonary delivery (Jaafar-Maalej et al., 2011). The NLCs displayed high entrapment of 99% and diffusion-controlled release. The formulation when administered as aerosols delivers the drug to the lungs evidencing the delivery potential of NLCs.

12.2.11 Miscellaneous applications

Some studies have presented the potential of NLCs for antimicrobial activity, antiinflammatory activity and antioxidant activity. The research would be advantageous for various types of inflammatory disorders, diseases related to oxidative stress, and reduced microbial load. Shortly, authors expect more applications of modified NLCs with extensive pharmaceutical benefits. The miscellaneous applications illustrating the therapeutic significance of NLCs are recorded in Table 12.1.

12.3 Marketed product of nanostructured lipid carriers

Additionally, there are few marketed products of NLCs that have attained clinical use as shown in Table 12.2. and many NLCs formulations have been patented that could go through clinical trials and conversely establish commercial feasibility (Table 12.3).

12.4 Patents related to nanostructured lipid carrier

NLCs related patents are mainly of NLCs product and method of preparations. The latest patent was from year 2017 based on method of preparation of NLCs.

Table 12.1 The application of NLC in therapeutics.

S.No	Disease state	Effect/disease state	Dosage form	Animal model or cell lines	Outcomes	References
1	Cancer theranostics	Mammary gland carcinoma	Biotin anchored doxorubicin loaded NLCs	Albino Wistar female rats/ MCF-7 cell lines	Biotinylated NLCs cased with Dox are capable of targeting tumors for the treatment of mammary gland carcinoma	Tripathi et al. (2020)
		Skin carcinoma	Silymarin encased NLCs	Female Swiss albino mice	Silymarin NLC possessed activities against progression and proliferation, which were associated with greater permeation into the affected cells	Singh, Arya, et al. (2016)
		Skin carcinoma	Silymarin loaded NLCs	SK-MEL 2 cell line	Enhanced permeation, greater stability with anticancer effect	Singh, Singh, et al. (2016)
		Breast cancer	Docosahexaenoic acid loaded NLCs	MDA-MB-231 and MCF-7	Maximum cellular internalization and cellular uptake	Lanna et al. (2021)
			Folic acid decorated docetaxel loaded ultrasmall NLCs	BALB/c nude mice and New Zealand rabbits/ HeLa cells	NLCs exhibited superior tumor retention and enhanced antitumor effect	Li et al. (2020)
		Colorectal cancer	Pterodon pubescens fruit oil loaded NLCs	HT-29 cells	Effective against colon cancer	Outuki et al. (2018)
		Breast cancer	AMD3100 loaded photothermal (IR780 photothermal agent) NLCs	Animal model	It displayed theranostic prospective for treatment of breast cancer	Li, Wang, et al. (2017)
		–	Methotrexate loaded NLCs	Murine fibroblasts L929	NLCs could be a promising tool for systemic and topical administration	Ferreira et al. (2015)

(*Continued*)

Table 12.1 The application of NLC in therapeutics. *Continued*

S.No	Disease state	Effect/disease state	Dosage form	Animal model or cell lines	Outcomes	References
2	Cardiovascular System	Hypercholesterolemia	ß-sitosterol loaded NLCs	Male mice	Significantly reduced LDL and cholesterol plasma levels	Soleimanian et al. (2020)
		Hypercholesterolemia	Simvastatin loaded NLCs	Albino Wistar rats	Simvastatin NLC packed transdermal patch shows better bioavailability	Raj et al. (2019)
		Hypercholesterolemia	Rosuvastatin NLCs	Sprague Dawley rat	Effective lipid-lowering activity of NLCs	Li et al. (2018)
		Hypercholesterolemia	Atorvastatin NLCs	Male albino rats	NLCs improve oral bioavailability and in vivo performance	Elmowafy et al. (2017)
3	Central nervous system	Alzheimer's disease	NLCs of pioglitazone	SHSY5Y (neuroblastoma) cells lines/male Wistar rats	Improved brain targeting the brain for management of Alzheimer's disease	Jojo et al. (2019)
		Alzheimer's disease	Curcumin loaded NLC	Rat model	An effective strategy for increasing curcumin delivery to the brain and reducing Aβ-induced neurological abnormalities	Sadegh Malvajerd et al. (2019)
		Multiple sclerosis	Teriflunomide loaded NLCs	Male Wistar rats	The optimized formulation was safe and effective in the treatment of multiple sclerosis	Gadhave and Kokare (2019)
		Parkinson's disease	Selegiline hydrochloride loaded NLCs	Animal order	Nano lipid carrier has the potential to be used in the management therapy of Parkinson's disease.	Mishra et al. (2019)
		Brain tumor	Artemisinin loaded NLCs	U-87MG brain cancer cell line	Potential for anticancer and antimalarial drug delivery in brain tumors and malaria.	Emami et al. (2018)
		Schizophrenia	Lurasidone hydrochloride	Albino Wistar rats	Good and efficient approach for delivering the drug directly to the brain	Jazuli et al. (2019)

		Epilepsy	Embelin-loaded nanolipid carriers	–	A beneficial carrier to achieve sustained release and brain targeting for epilepsy therapy	Sharma et al. (2017)
		Epilepsy	Carbamazepine loaded NLCs	Male Wistar rats	Improved targeting was achieved	Elmowafy et al. (2018)
4	Gastrointestinal tract disorders	Inflammatory bowel disease	NLCs encasing budesonide	J774A.1 cell line/rat model	Targeting and superior therapeutic effect of NLCs	Sinhmar et al. (2018)
		Nonalcoholic fatty liver disease	Naringenin loaded NLCs	MDCK cells/ C57BL/6 wild-type mice	Improved drug release rate, transepithelial transport and intestinal absorption, and the elevated oral bioavailability	Hu et al. (2021)
		Peptic ulcer disease	Pumpkin seed oil (PSO)-based nanostructured lipid carriers	Wistar rats	Optimized NLCs formula for better antiulcer.	Ahmed et al. (2020)
		Ulcerative colitis	Berberine loaded NLCs	RAW 264.7 cells and Caco-2 cells/animal model	NLCs improved colitis symptoms and are suggested as a better carrier.	Deng et al. (2020)
		Ulcerative colitis	Celecoxib loaded NLcs	hTERT-BJ cells/ colitis mouse model	Lipid-based colon specific delivery of celecoxib may be used for management of colitis.	Mishra et al. (2020)
		Crohn's disease	Rifabutin loaded NLCs	Caco-2 cells	NLCs could be a promising approach to improve peroral antimycobacterial therapy in crohn's disease.	Rouco et al. (2020)
5	Metabolic disorder	Diabetes mellitus	Sucupira oil-loaded NLCs	Caco-2 cells	The optimized formulation followed a modified release and cytotoxicity studies	Vieira et al. (2020)
		Diabetes mellitus	Silymarin loaded NLCs	Caco-2 cells/ animal model	NLCs displayed substantial antihyperalgesic outcome on streptozotocin—induced neuropathy.	Piazzini et al. (2019)
		Diabetes mellitus	Baicalin encased NLCs	Male Sprague-Dawley	Exhibited significantly higher antidiabetic potential when compared with free baicalin.	Shi et al. (2016)

(*Continued*)

Table 12.1 The application of NLC in therapeutics. *Continued*

S.No	Disease state	Effect/disease state	Dosage form	Animal model or cell lines	Outcomes	References
6	Dermatological disorders	Acne	Tretinoin loaded NLC	Wister rats	The NLCs formulation prolonged drug release by the incorporation into the NLCs.	Ghate et al. (2016)
		Acne	Azelaic acid loaded NLC	Animal model	It improves the penetration of the azelaic acid through the stratum corneum.	Kumari et al. (2015)
		Psoriasis	Apremilast loaded NLCs	–	NLCs confirms potential of the for topical application and increased drug deposition in the skin.	Madan et al. (2020)
		Psoriasis	Tretinoin loaded NLCs	Mouse tail model	Significantly enhanced antipsoriatic activity	Raza et al. (2013)
		Psoriasis	Dithranol entrapped NLC	Mice psoriatic plaque model	Reduced levels of cytokines viz.IL-17, IL-22, IL-23 and tumor necrosis factor-α	Sathe et al. (2019)
		Psoriasis	Fluocinolone acetonide loaded NLCs	–	NLC was effective in the management of psoriasis	Pradhan et al. (2021)
		Melanoma	1-carbaldehyde-3,4-dimethoxyxanthone (TAp73 activator holding antiproliferative effect) loaded NLCs	A375 melanoma cell	Efficacy of drug ascribing to improved solubility.	Malta et al. (2020)
		Skin cancer	Silymarin loaded NLC	B16 melanoma cell line and animal model	Significant decline in tumor volume and IL-1α and TNF-α and oxidative stress markers.	Singh, Arya, et al. (2016)
		Leishmaniasis	Cedrol-loaded NLC	Macrophage cells/animal model	Effective in the treatment of Leishmaniasis.	Kar et al. (2017)

		Leishmaniasis	Curcumin loaded NLCs	Female rats and female mice	Topical application of drug-loaded NLCs can lead to targeted delivery of antileishmanial drugs.	Riaz et al. (2019)
		Discoid lupus erythematosus (sunburn)	Diflucortolone valerate loaded NLCs	–	Enhances the intrinsic sun protection factor	Abdel-Salam et al. (2017)
7	Ocular ailments	Enhanced Ocular delivery	Dexamethasone loaded NLCs	Human cornea cells	Porcine cornea showed a high concentration of nanocarriers in the hydrophilic stroma layer.	El-Salamouni, Farid, El-Kamel, and El-Gamal (2018)
		Dry eye	Dexamethasone loaded NLCs	Human corneal cell	Mucoadhesive NLCs can create a depot on the surface of the cornea, which can predict improved bioavailability.	Kiss et al. (2019)
		Enhanced ocular delivery	Brimonidine encased NLCs	–	Sustained and highest intraocular pressure in rabbits.	El-Salamouni et al. (2018)
8	Renal disorders	Lupus nephritis	Tripterygium Wilfordii Hook F (TWHF) loaded NLC	Immune lupus nephritis animal model	NLCs have a recognizable therapeutic outcome.	Li, Qi, Hamidouche, and Laouid (2021)
		Diabetic nephropathy	Ergosterol-loaded NLCs	Mesangial cells	NLCs mediated delivery could be used as a potential vehicle to enhance solubility, oral bioavailability, and therapeutic efficacy of ergosterol.	Dong, Iqbal, and Zhao (2020)
9	Wound healing	Diabetic wound	Pioglitazone loaded NLCs	Diabetic wound animal model	Use of nanostructured lipid carrier (Pio-NLC-COL-CS) scaffold can prove to be a promising strategy for local treatment for diabetic wounds.	Natarajan et al. (2019)
		Diabetic foot ulceration	Phenytoin loaded NLCs	–	Phenytoin loaded nanostructured lipid carrier dressing was found to be more effective than phenytoin hydrogel.	Motawea, Abd El, Borg, Motawea, and Tarshoby (2019)

(*Continued*)

Table 12.1 The application of NLC in therapeutics. *Continued*

S.No	Disease state	Effect/disease state	Dosage form	Animal model or cell lines	Outcomes	References
		Diabetic wounds	NLC loaded with EGF-conjugated curcumin	NIH 3T3 fibroblasts and HaCaT keratinocytes	These studies indicates that drug loaded NLCs could be an efficient topical wound dressing that can be explored for fast wound healing without adverse effect.	Lee et al. (2020)
10	Pulmonary disorders	Asthma	Montelukast via NLC formualtions	Male Wistar rats	Potential in enhancing bioavailability	Patil-Gadhe and Pokharkar (2014)
		Chronic obstructive pulmonary disease	Rosuvastatin loaded NLCs	Wistar rats	Potential of RNLC-DPI for lung targeting and further for COPD treatment.	Patil-Gadhe and Pokharkar (2016)
		–	Beclomethasone-loaded NLCs	–	The formulation when administered as aerosols delivers the drug to lungs evidencing delivery potential of NLCs.	Jaafar-Maalej, Andrieu, Elaissari, and Fessi (2011)
11	**Miscellaneous**	Antiinflammatory activity	Aceclofenac Nanostructured Lipid Carriers (NLC)	Rat paw edema method	The study showed the promising and stable alternative form for the aceclofenac for topical application.	Phatak and Chaudhari (2013)
		Antimicrobial activity	Dexamethasone acetate	–	NLCs can offer a promising alternative against Gram-negative bacterial infections accompanied by inflammation.	Rocha et al. (2020)

Table 12.2 Some marketed formulations based on NLCs.

S. No	Drug/active	Manufacturer/ trademark	Route of administration	References
1	Ubidecarenone, omega-3, and omega-6 unsaturated fatty acids	Amore Pacific/IOPE Line	Dermal	Montenegro (2017)
2	Olea europaea oil, *Prunus amygdalus* Dulcis oil, hydrolyzed milk protein, tocopheryl acetate, *Rhodiola rosea* root extract, and caffeine	Dr. Theiss/Olivenöl Augenpflegebalsam	Dermal	Montenegro (2017)
3	Lutein	Kemin Industries/ FloraGlo	Oral	Beloqui, Solinís, Rodríguez-Gascón, Almeida, and Préat (2016)
4	Ubidecarenone, highly active oligosaccharides	Beate Johnen /NLC deep effect eye serum	Dermal	Montenegro (2017)
5	Kukui nut oil, Monoi Tiare Tahiti, pseudopeptide, coconut milk, and wild indigo	Isabelle Lancray/ Surmer Elixir du Beauté Nano-Vitalisant	Dermal	Montenegro (2017)

Table 12.3 Some patents related to nanostructured nanocarriers.

S. No	Claimed product and activity	Patent No.	Publication date	Sources
1	Method of producing nanostructures lipid carriers (nlc) and product derives thereof	CN102283809A	21 December 2011	https://patents.google.com/patent/CN102283809A/en
2	Prepare the method for nanostructured lipid carrier (NLC) and prepared product	CN102283809B	14 December 2016	https://patents.google.com/patent/CN102283809B/en
3	Nanostructured lipid carriers and methods for making and using them	WO2017185155A1	2 November 2017	https://patents.google.com/patent/WO2017185155A1/en
4	Nanostructured lipid carriers containing riluzole and pharmaceutical formulations containing said particles	CA2656438A1	3 January 2008	https://patents.google.com/patent/CA2656438A1/en

12.5 Conclusions and future perspectives

Demonstrating reasonable biocompatibility, modulated release behavior noncytotoxicity and stability NLCs have been explored for a variety of applications. NLCs have established themselves as promising nanocarriers for the delivery of a variety of molecules viz. anticancer drugs, antibacterial drugs, antihyperlipidemic drugs, antidiabetic drugs, phytopharmaceuticals, etc. The findings suggested that the therapeutic efficacy, release pattern, and stability were significantly enhanced when therapeutic agents are formulated as NLCs. In addition, they also offer enhanced solubility that results in expressively improved permeation and bioavailability. However, plentiful studies have established NLCs' potential and applicability in various therapies, nevertheless, it is crucial to develop more promising novel systems that can encounter the challenges associated with such formulations. The newer prospects emphasize on development of novel and surface engineering methodologies that holds specificity and selectivity for certain target resulting in the further improved therapeutic efficacy of the delivery system. Through the technology is in improving day by day, NLCs may be explored for broader applications but still, a long way to go. Besides all new technologies and the potential of NLCs, only a few candidates have made it to clinical trials, conversely establishing commercial feasibility. As we reveal the potential of NLC as a delivery system, we may expect improved NLCs that have a probability of clearing clinical trials and finally acceptance for clinical translation.

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CHAPTER

13 Self-assembled protein-drug nanoparticles for enhanced drug delivery and targeting cancer therapeutics

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13.1 Introduction

Nanoparticles are a versatile group of nano-sized materials, offering the advantage of the delivery of therapeutic and diagnostic agents (Murthy, 2007). They are preferred drug delivery systems, owing to their small particle size, which can be internalized and accumulated in diseased tissues, for example, Cancer tissue. The characteristics of the nanoparticles allow their enhanced permeability and retention (EPR) in the tumor cells, by evading the systemic circulation through the fenestrations of the angiogenic blood vessels (Greish, 2010). Subsequently, nanocarriers evade potential off-target toxicity, which may compromise the therapeutic goals of multiple anticancer drugs (De Jong & Borm, 2008). Generally, the nanocarrier systems, that are involved in drug delivery, are categorized into organic and inorganic nanoparticles. Organic nanocarriers are more preferred, due to their less toxic profile, and the capacity of functionalization with targeting materials, in addition to high drug loading capacity (Fang et al., 2020; Khalid et al., 2020). Several organic nanocarriers are investigated. For instance, lipid-based nanoparticles, polymeric nanoparticles, and more importantly, protein nanocarriers.

Protein nano-systems are efficient drug delivery materials, owing to their biocompatible nature. Most importantly, proteins possess the capacity to accommodate hydrophobic cargos (Elzoghby, Samy, & Elgindy, 2012; Elzoghby, Elgohary, & Kamel, 2015). Additionally, proteins are less potentially

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recognized by the reticuloendothelial system, and therefore, evade opsonization and avoid induction of immunogenic reactions (Chen, Remondetto, & Subirade, 2006). Also, proteins could be decorated with multiple materials, provided by the abundance of different functional groups, such as carboxylic and amine groups (Kremer et al., 2000; Mao et al., 2005). On other hand, proteins can inherently target tumor cells at specific sites. For example, albumin can target the overexpressed tumor gp60 and SPARC receptors (Maeda et al., 2000; Rempel, Ge, & Gutiérrez, 1999). Lactoferrin possesses the ability to target the overexpressed low-density lipoprotein-related proteins, and transferrin (Tf) binding receptors (Elzoghby et al., 2020). Collectively, protein nanocarriers have emerged as a successful alternative to conventional drug delivery methods, which are compromised by the application of toxic solvents. For instance, the clinical implementation of paclitaxel-loaded albumin nanoparticles, synthesized via nab-technology, showed better tolerability and negated the anaphylactic reactions exhibited by the patients administered Cremophor EL- solubilized paclitaxel (Fu et al., 2009; Kundranda & Niu, 2015).

Several fabrication methods were studied to synthesize protein nanoparticles. Self-assembly-based techniques are the most reported, due to their preferable implications (Hassanin & Elzoghby, 2020a). The self-assembly process provides many advantages, such as enhanced stability, combined drug loading, the improved pharmacokinetic profile of the encapsulated drugs, the capacity for surface functionalization, and stimuli-responsive drug release. Also, the inherent targeting capabilities of protein nanoparticles ensure their effective localization in the tumor tissue, bypassing drug efflux and uptake mechanisms of cancer resistance. Thereby, we highlight the most significant implications of the self-assembled protein nanocarriers, as well as the fabrication procedures and the factors affecting the self-assembly process.

13.2 Fabrication methods of self-assembled protein nanoparticles

The process of synthesis of self-assembled protein nanoparticles implies several approaches (Table 13.1). The two main categories of the drug-loading are physical or chemical methods (Hassanin & Elzoghby, 2020a). The most reported physical loading method implies the desolvation of the protein, leading to the aggregation of protein molecules into the micellar structure, which is further stabilized by crosslinking agents (Weber et al., 2000). Alternatively, self-assembly can be initiated by inducing the unfolding of the protein structure. A disulfide bond reducing conditions and/ or materials are incorporated to allow the exposure of the hydrophobic binding sites, subsequently, accommodating hydrophobic drugs (Hassanin & Elzoghby, 2020b). On the other hand, a chemical conjugation between a protein and a hydrophobic material can be exploited, to impart amphiphilic characteristics, allowing the spontaneous self-assembly of proteins into nanoparticles (Gong et al., 2009).

Table 13.1 Albumin-based self-assembled nanoparticles.

Protein	Drug	Fabrication technique	Implications	References
BSA	TCS and ABZ	Electrostatic self-assembly	Inhibition of metastasis against A549/T tumor-bearing nude mice	Tang et al. (2017)
BSA	QT and DTX	Desolvation	Enhanced cytotoxicity against drug-resistant MDA-MB-231 cells	Desale et al. (2018)
HSA	Human surviving specific miRNA plasmid	Desolvation	Downregulation of Survivin accompanied by enhanced radiotherapy	Gaca et al. (2012)
HSA	PTX and Ce6	Incubation	Photodynamic and chemotherapy combination and targeting $\alpha v\beta 3$-integrin via RGD functionalization.	Chen et al. (2015)
HSA	DOX and Celecoxib	Incubation	Intra-tumoral accumulation via both its tumor targeting effect and EPR effect.	Shi et al. (2018)
BSA	PTX	Thermal denaturation	Enhanced cytotoxicity compared to Taxol.	Asghar et al. (2014)
Ovalbumin	DOX	Thermal denaturation	Enhanced inhibitory effect parallel with that of free DOX against HeLa cells.	Wen et al. (2018)
HSA	PTX	B-ME induced denaturation	Enhanced cytotoxicity than free PTX or Abraxane.	Ding et al. (2014)
HSA	DOX	B-ME induced denaturation	Elimination of DOX-induced cardiotoxicity.	Yuan et al. (2013)
HSA	IR-780 and DTX	B-ME induced denaturation	Complete tumor eradication in 22VR1 subcutaneous tumor model.	Lian et al. (2017)
HSA	CCM	B-ME induced denaturation	Improved solubility of CCM.	Gong et al. (2015)
HSA	PTX	B-ME induced denaturation	Enhanced cytotoxicity and cellular uptake via Nucleolin targeting aptamer.	Wu et al. (2013)

(*Continued*)

Table 13.1 Albumin-based self-assembled nanoparticles. *Continued*

Protein	Drug	Fabrication technique	Implications	References
HSA	PTX	GSH induced denaturation	Redox-responsive drug release with enhanced antitumor effect against TNBC.	Liu et al. (2017)
HSA	ICG	GSH induced denaturation	Enhanced stability of ICG.	Sheng et al. (2014)
BSA	CCM	Ionic strength-induced assembly	Preserved effect of the loaded CCM.	Safavi et al. (2017)
HSA	PTX and Fenretinide	Urea/NaBH4-mediated denaturation	2.5-fold higher cell permeation via LMWP modification.	Lin et al. (2016)
α-lactalbumin	DOX	Photo synthesis	pH-responsive drug release and enhanced cytotoxicity compared to free DOX in A549 tumor cells.	Xie et al. (2013)

Notes: ABZ, *albendazole;* BSA, *Bovine serum albumin;* CCM, *Curcumin;* DOX, *Doxorubicin;* DTX, *Docetaxel;* EPR, *Enhanced permeability and retention;* HSA, *Human serum albumin;* ICG, *indocyanine green;* LMWP, *Low molecular weight protamine;* PTX, *Paclitaxel;* TCS, *trichosanthin;* TNBC, *Triple-negative breast cancer.*

13.2.1 Desolvation

Desolvation is the most commonly implemented technique in the fabrication of self-assembled protein nanocarriers. The process of desolvation starts with a dehydration step brought by desolvating agents, for example, ethanol or acetone. This leads to dehydration of the protein molecules, inducing their aggregation into nanoparticles. These nanoparticles are further stabilized by applying a crosslinking agent, for example, glutaraldehyde (Fig. 13.1). For instance, it was found that glutaraldehyde can successfully induce the crosslinking of albumin nanoparticles by the interaction with its amine groups, with the lowest effective concentration of 40% (Weber et al., 2000). Doxorubicin (DOX) was loaded in HSA employing the desolvation technique. It is worth mentioning that HSA retained its capacity for functionalization with therapeutic or targeting materials (Bae et al., 2012). Alternatively, a protein copolymer, which can be synthesized from a hydrophobic protein, for example, Zein, and an amphiphilic protein, for example, Lactoferrin, could be self-assembled by implementing the same technique, to

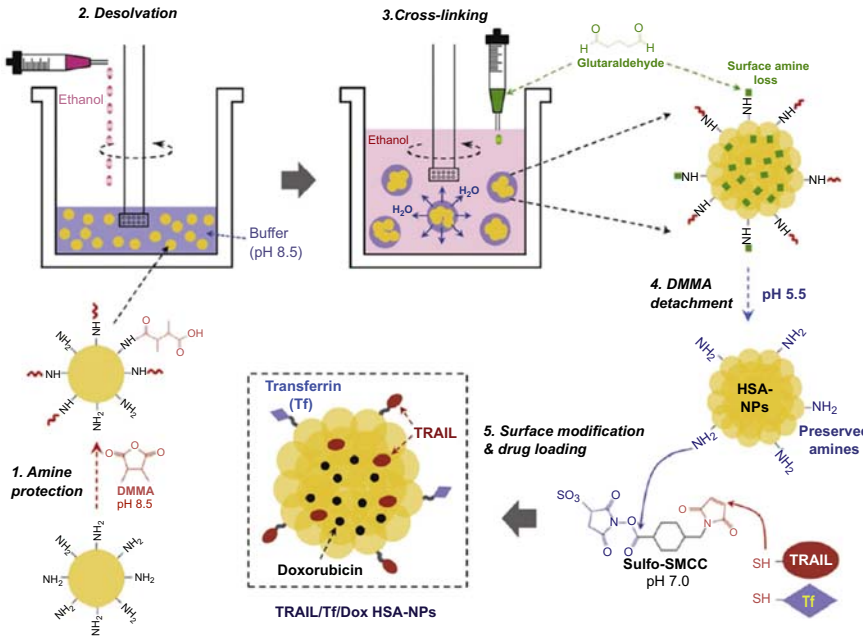

FIGURE 13.1

A representation of the desolvation technique used to prepare DOX-loaded HSA and functionalized with TRAIL and Tf.

Reprinted from Ref. Wang, K., et al., (2015a). Acid denaturation inducing self-assembly of curcumin-loaded hemoglobin nanoparticles. Materials (Basel, Switzerland), *8(12), 8701–8713.*

physically encapsulate hydrophobic drugs (Table 13.2). This technique produces a slightly larger particle size around 200 nm, however, drug loading remains considerably high (Sabra et al., 2018).

13.2.2 Noncovalent self-assembly of protein nanoparticles

The noncovalent self-assembly is reported as an alternative technique for the fabrication of protein-drug nanoparticles, to eliminate the application of considerable amounts of organic solvents or to negate using toxic crosslinking agents (Gong et al., 2011). The mainstay of the proposed method takes into account the inherent ability of the protein molecules to accommodate hydrophobic drugs. Therefore, noncovalent self-assembly can proceed through: the (1) incubation method, where the drug and the protein are simply mixed allowing the self-assembly of the protein by the virtue of the hydrophobic drug. (2) Disulfide bond reducing method, where the disulfide bonds of the protein are exposed by disulfide bond reducing conditions, for example, the addition of a disulfide bond reducing agents,

Table 13.2 Miscellaneous protein-based self-assembled nanoparticles.

Protein	Drug	Technique	Key outcomes	References
Lactoferrin	Shikonin and JQ1 (PD-L1ihnhibitor)	Thermal induced unfolding	Inhibited tumor growth	Wang et al. (2019)
Lactoferrin	Simvastatin and Fenretinide	Thermal induced unfolding	Bypassing BBB in glioma cells.	Mo et al. (2018)
Transferrin	PTX	B-ME induced denaturation	Tumor growth inhibition in H22-tumor-bearing mice.	Wang et al. (2016a)
Transferrin	IR-780	DTT induced denaturation	Higher cellular uptake and enhanced cytotoxicity to cancer cells.	Wang et al. (2016b)
Transferrin	ICG	Incubation	Higher cellular uptake	Zhu et al. (2017)
Hemoglobin	CCM	pH-induced assembly	Enhanced cytotoxicity compared to free CCM.	Wang et al. (2015a)
Bovine Hemoglobin	DOX	pH-induced assembly	pH-responsive drug release.	Wang et al. (2018)
Hexanoyl-modified gelatin	Camptothecin	Conjugation	Improved internalization and accumulation.	Li, Liu, and Chen (2011)
rHG-TOS	17-AAG	Conjugation	10% higher cytotoxicity compared to free 17-AAG.	Won et al. (2011)
Zein	CCM	Incubation/ Centrifugation	The enhanced water solubility of curcumin by 32-folds.	Wang et al. (2015b)
SELP	Doxorubicin	Incubation	Enhanced cytotoxicity.	Xia et al. (2014)

Notes: 17-AAG, *17- allylamino-17-demethoxygeldanamycin;* BBB, *blood brain barrier;* B-ME, *β-mercaptoethanol;* CCM, *Curcumin;* DOX, *Doxorubicin;* DTT, *Dithiothreitol,* ICG, *indocyanine green;* rHG-TOS, *recombinant human gelatin (rHG) modified with alphatocopheryl succinate (TOS);* SELP, *silk and elastin-like protein polymer.*

Thermal- induced unfolding, manipulation of the pH or ionic strength, or UV-induced disulfide bonds breakage. Overall this approach produces smaller particle sizes than other methods, for example, the desolvation method (Hassanin & Elzoghby, 2020b).

13.2.2.1 Incubation

The self-assembly of amphiphilic proteins can be induced by the addition of a hydrophobic drug. The hydrophobic interactions are the major forces affecting

this type of formulation (Yadav, Sharma, & Kumar, 2020). Incubation simply implies the mixing of the drug and the protein, triggering the formation of nanoparticles. The sequence of the addition of the drug may influence the structure of the nanoparticles, as well as the size. It was demonstrated that the simultaneous mixing of PTX and premodified HSA in a single step resulted in smaller particle size (50 nm), compared to a two-step approach where premodified HSA is mixed with PTX forming a core structure onto which more HSA and drug molecules are added to allow the coating of the nanoparticles (~100 nm) (Chen et al., 2015).

13.2.2.2 Thermal-induced unfolding

Noncovalent self-assembly can be approached by inducing protein denaturation by heat treatment. Thermal treatment would expose the buried hydrophobic moieties allowing the interaction between a hydrophobic drug, and the protein molecules (Holm et al., 2007). The temperature used was suggested to be near the melting temperature of the protein. To formulate Bovine Serum Albumin (BSA) nanoparticles using this method, Asghar et al. subjected BSA to 65°C. Therefore, the drug was added to allow the self-assembly of the protein into nanoparticles. It was found that the hydrophobic sites exposed were harboring tryptophan residues (Trp-134), which were responsible for the noncovalent interaction with the added PTX. The forces stabilizing the nanoparticles arise as protein-protein interaction. This interaction was demonstrated to be dependent on hydrogen bonding, electrostatic interactions, and disulfide sulfhydryl interchange reactions (Asghar et al., 2014). Also, to enhance the stability of the nanoparticles, a network of disulfide bonds can be produced following the thermal treatment of proteins, for example, ovalbumin, by adding H_2O_2 as an oxidizing agent for the free thiols groups. This was demonstrated by the complete dissociation of the nanoparticles upon treatment with dithiothreitol (DTT) (a disulfide bond reducing agent), and sodium dodecyl sulfate, which destroys hydrophobic interactions (Wen et al., 2018).

13.2.2.3 Disulfide bond-reducing agents

A "hidden-exposed" molecular switch was proposed by Gong et al., where a disulfide bond reducing agent is employed to induce the exposure of binding regions of the protein, allowing the hydrophobic drugs to incorporate themselves in these sites (Gong et al., 2011, 2012; Wang et al., 2014). Therefore, inducing the self-assembly of the proteins into nano-assemblies (Fig. 13.2). Various disulfide bonds reducing agents were experimented, showing different protein denaturing efficiencies and toxicological profiles.

13.2.2.3.1 B-mercaptoethanol (β-ME)

B-mercaptoethanol is the most commonly employed reducing agent. Gong et al. and Martínez et al. proposed the use of β-mercaptoethanol to induce the exposure of the disulfide bonds and create a "hidden-exposed" molecular switch accommodating the hydrophobic drug (Gong et al., 2012; Martínez et al., 2012). Additionally, the hydrophobic drug act as a bridge to further stabilize the

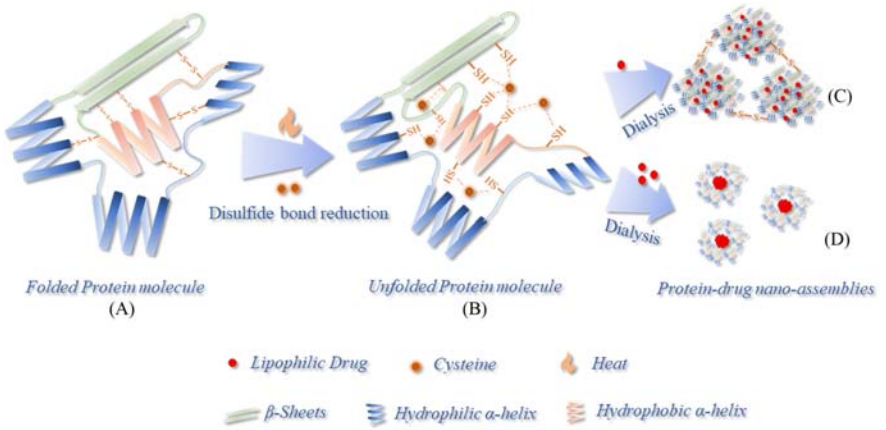

FIGURE 13.2

An illustration of the disulfide bond reducing method for the preparation of self-assembled noncovalent protein nanoparticles.

Reprinted from Ref. Hassanin, I. A., & Elzoghby, A. O. (2020b). Self-assembled non-covalent protein-drug nanoparticles: An emerging delivery platform for anti-cancer drugs. Expert Opinion on Drug Delivery, 17*(10), 1437–1458.*

produced nanoparticle structure (Gong et al., 2012). For instance, Curcumin was encapsulated in HSA nanoparticles using β-ME. The produced nanoparticles showed a particle size of 120 nm. The addition of β-ME allowed the exposure of the subdomain II A of the albumin molecules, allowing the generation of hydrophobic interactions with the albumin molecule, and subsequently, the self-assembly of HSA nanoparticles. The stabilizing forces were elucidated to be comprised of hydrogen bonding and electrostatic interactions, which were confirmed by the addition of urea or ethanol, which induced the precipitation of the nanoparticles (Gong et al., 2015). Moreover, PTX-loaded Tf nanoparticles were prepared using the same approach. The disulfide bond reduction of Tf resulted in a conformation similar to the iron-free Tf, which self-assembled into nanoparticles following the addition of PTX. The amino-acid residues responsible for the hydrophobic interactions were elucidated to be tyrosine and tryptophan (Wang et al., 2016a).

13.2.2.3.2 Dithiothreitol

DTT is a disulfide bond reducing agent, which is larger than β-ME, leading to inefficient disulfide bond reduction inside the protein molecule (Gong et al., 2015; Zheng et al., 2006). The application of DTT as the disulfide bond reducing agent in the fabrication of protein-drug nano-assemblies was shown to be successful, however, the formation process of the nanoparticles was slower than that of β-ME. However, the particle size was comparable. As a complication of the DTT, aggregation of the nanoparticles was exhibited, due to the larger molecular size of

DTT. On the other hand, the interaction of IR-780-loaded Tf nanoparticles with Tf receptors was not impeded by using DTT in the fabrication procedure (Wang et al., 2016b).

13.2.2.3.3 Glutathione

Glutathione is an endogenous antioxidant, which possesses the capability to reduce the disulfide bonds of a protein (van der Vliet et al., 1999). Incorporation of GSH in the formulation of protein nano-assemblies implies the same mechanism of nanoparticle formation and stabilization as demonstrated by β-ME. However, the produced nanoparticles are more stable (Sheng et al., 2014; Wang et al., 2013).

13.2.2.3.4 Cysteine

The weak disulfide bond reducing properties of cysteine require the fabrication method to incorporate high amounts of cysteine or to apply high temperature. Hydrogen bonding, van der Waals forces, and electrostatic interactions are the major forces involved in the stabilization of the nanoparticles (Punith & Seetharamappa, 2012; Zhao et al., 2011). The formation dynamics of the nanoparticles exhibited two phases: (1) a self-assembly process which is accompanied by an increase in both particle size and yield. (2) the nanoparticles are aggregated in the second phase, showing an increase in the particle size with a constant yield. As expected the encapsulation efficiency of the drug-loaded protein nano-assemblies, formulated using cysteine, was lower than that produced using β-ME. However, the particle size remains smaller (Jiang et al., 2013).

13.2.2.3.5 Modulation of the pH and ionic strength

Thermal denaturation of the proteins may compromise the stability of the protein and affect its structure and binding functions. On the other hand, the incorporation of disulfide-bond reducing agents may show some toxicities. Therefore, pH modulation and the addition of electrolytes is an alternative approach to synthesizing protein-drug nano-assemblies. CCM-loaded BSA nanoparticles were formulated using a Tris buffer at certain pH. The particle size of the nanoparticles was influenced by the changing concentrations of the Tris buffer (50−250 mM) and the variations in the pH (6.7 or 8.9). The higher the pH, the smaller the particle size (Safavi et al., 2017). On the contrary, CCM was loaded in Hemoglobin (Hb) nanoparticles. On decreasing the pH, the hydrophobic binding site was more exposed allowing the incorporation of the drug through noncovalent interactions. It is worth noting that the drug loading was enhanced compared to that experienced by β-ME, with smaller particle size distribution (Wang et al., 2015a).

13.2.2.3.6 Urea/$NaBH_4$-induced denaturation

The unfolding of proteins may be achieved using a high concentration of urea, in conjunction with $NaBH_4$. The protein structure is unfolded into the linear structure, which facilitates the lipophilic drugs to be incorporated into self-assembled protein nanoparticles. PTX and Fenretinide (4-HPR) were coloaded into BSA

nanoparticles employing this method. The nanoparticles are stabilized via disulfide bonds, without the involvement of a cross-linking agent. Moreover, the stability of the nanoparticles is enhanced with coloading of the nanoparticles with both drugs rather than a single agent (Lin et al., 2016).

13.2.2.3.7 UV illumination

A new approach, which was proposed by Wang et al., to induce the breakage of disulfide bonds is the application of UV illumination. Based on the illumination time, the size of the resultant nanoparticles can be controlled. Interestingly, light-sensitive drugs were successfully formulated using this approach, with minimal degradation. DOX was incorporated into α-lactalbumin using this method, showing a pH-responsive release. It is worth mentioning that the process is associated with tryptophan exposure, where the drug is incorporated and further stabilized via the generation of intermolecular disulfide bridges (Xie et al., 2013).

13.2.3 Chemical conjugation

The amphiphilic characteristics of proteins are augmented when conjugated with hydrophobic materials (Xu, Fisher, & Juliano, 2011). These conjugation processes will enhance the self-assembly procedure or enhance drug loading potential. For instance, HSA was conjugated to DOX after activation with sulfosuccinimidyl 4-(N-maleimidomethyl)-cyclo-hexane-1-carboxylate (sulfo-SMCC). Then, HSA was further conjugated with octanal, via reductive amination, to enhance the lipophilicity of HSA. By adjusting the pH to 5.5, the conjugate was able to self-assemble into a nanoparticle structure (Choi et al., 2015). The conjugation of two proteins was also achieved. The conjugate of the hydrophobic protein, i.e., zein, was achieved with lactoferrin, which was able to accommodate hydrophobic drugs, following a desolvation step (Sabra et al., 2018). Moreover, amphiphilic characteristics could be imparted to hydrophilic protein, for example, Gelatin. On increasing the hydrophobic substitution, the critical aggregation concentration was decreased. Additionally, the loading of the Camptothecin was significantly enhanced by increasing the hydrophobic substitution, due to enhanced interactions of Camptothecin with the hydrophobic moieties of the self-assembled gelatin nanoparticles (Li et al., 2011).

13.3 Factors affecting the formation of self-assembled protein nanoparticles

13.3.1 Protein concentration

The concentration of a protein solution is a significant factor, which primarily influences the size of the nanoparticles. The higher the protein concentration, the lower the distances between protein molecules, subsequently, affecting the degree

of the cross-linking and formation of protein aggregates (Asghar et al., 2014; Rohanizadeh & Kokabi, 2009). For instance, BSA concentrations up to 10 mg/mL resulted in the formation of gel blocks (Jiang et al., 2013). On the contrary, low concentrations (0.5 mg/mL) were not sufficient for the formation of blank nanoparticles. Instead, such a low concentration requires the addition of hydrophobic drugs to induce the self-assembly process, and subsequent stabilization of the nanoparticles (Gong et al., 2012).

13.3.2 Protein/drug ratio

Particle size, as well as the dynamics of nanoparticle formation, were found to be dependent on the protein-to-drug ratio. At very low concentrations of the drug, the size of the nanoparticles was small (15 nm) (Ding et al., 2014). However, on increasing the ratio to 5%, a significant change in size was observed, indicating the formation of nanoparticles. A steady state was continued with a ratio from 5% to 20%, explained by the abundance of protein molecules in the system undergoing nanoparticle formation. Alternatively, when the drug-to-protein ratio was increased from 20% to 30%, the particle size was increased in response to the excess drug, which may interact with the nanoparticles leading to aggregation and larger particle size (Ding et al., 2014; Gong et al., 2012). Morphological aspects were also associated with the protein-to-drug ratio. Lower drug-to-protein ratios result in the formation of spherical nanoparticles, while higher ratios result in the formation of core-shell structures (Jiang et al., 2013). Furthermore, the encapsulation efficiency is affected by the protein-to-drug ratio (Asghar et al., 2014). HSA NPs were found to exhibit an increasing encapsulation efficiency with an increased protein-to-drug ratio, reaching 98% at a ratio of 15%. However, increasing the ratio of the drug to protein beyond this ratio was associated with decreased encapsulation efficiency. This may be explained by weakened interactions between a drug and protein molecules (Ding et al., 2014).

13.3.3 Temperature

Self-assembly is a thermodynamically controlled process. The formation of nanoparticles requires a high temperature (e.g., 85°C or 95°C). A disulfide bond reducing procedure using cysteine was found to be affected by the temperature involved in the fabrication procedure. For instance, at a lower temperature approaching 55°C, no nanoparticles were formed. On the other hand, higher temperatures were needed to achieve protein unfolding and subsequent incorporation of the drug through noncovalent interactions (Jiang et al., 2013). Additionally, the duration of thermal treatment may affect the degree of crosslinking, and therefore, the protein-protein interactions (Yu et al., 2006). However, the encapsulated drug stability may be compromised. It was shown that DOX was degraded when subjected to a higher temperature (80°C for 60 or 90 min) (Deng et al., 2010).

13.3.4 pH

Based on the protein employed, pH may affect the formation of the nanoparticles in both particle size and yield. The variations in the pH negatively influence the particle size of the formed nanoparticles. This is due to the restriction of the molecular interactions between protein molecules at pH far from the isoelectric point (Asghar et al., 2014). For instance, BSA was not able to self-assemble into nanoparticles at very low pH (2−3). On increasing the pH in the range of 4−6, aggregation was found in the system. On the other hand, higher pH values (7−8) allowed the formation of stable BSA nanoparticles as a result of the conformational isomerization accompanied by the charged BSA molecules available upon the rise in pH away from the isoelectric point (BSA pI 4.7) (Jiang et al., 2013). Contrarily, Hb nanoparticles are formed at lower pH values (4−5.5). However, on decreasing the pH to less than 3, aggregation was achieved, due to a conformational change in the Hb structure, leading to failure in the encapsulation of CCM (Wang et al., 2015a).

13.4 Implications of the self-assembled protein nanoparticles

13.4.1 Targeted drug delivery to tumors and enhanced cellular uptake

Tumor targeting is a crucial step to allow the internalization of the therapeutic cargos into the tumor cells, subsequently, leading to enhanced cytotoxicity. Protein nanoparticles combine the virtue of passive targeting, due to small particle size, which allows tumor infiltration and retention through the phenomenon of EPR (Patel & Patel, 2019). Also, protein nanoparticles possess the capacity to be functionalized with different targeting moieties. In this regard, it was demonstrated that various tumors overexpress multiple receptors. These receptors can be inherently targeted via a wide array of proteins. For instance, albumin-targeting proteins, such as SPARC (secreted protein acidic and rich in cysteine) and gp60 (glycoprotein 60) are overexpressed on various solid tumors (Merlot, Kalinowski, & Richardson, 2014; Rempel et al., 2001; Shi et al., 2007). Therefore, self-assembled BSA nanoparticles loaded with PTX and Fenretinide were successfully delivered to glioma cells. Additionally, functionalization of the BSA nanoparticles with Low molecular weight protamine, allowed an enhanced internalization efficacy compared to BSA nanoparticles (Lin et al., 2016). Active targeting could be also achieved via targeting Tf receptors expressed on colon cancer cells. IR-780 loaded Tf nanoparticles were shown to exhibit less fluorescence following the treatment of the colon cancer cells with free Tf. This explains that the mechanism of cellular uptake is mainly mediated by the interaction of Tf nanoparticles with Tf receptors (Chen et al., 2017; Wang et al., 2016a).

On another avenue, active targeting may enhance the uptake efficiency of the self-assembled protein nanoparticles through the conjugation of targeting materials with the protein molecule. It was found that the uptake of Simvastatin and Fenretinide coloaded lactoferrin nanoparticles, modified with cell-penetrating peptide (TAT) conjugated to TPGS, were augmented by 1.5- folds compared to drug-loaded lactoferrin nanoparticles (Mo et al., 2018). A targeting peptide (Trp-Lys-Tyr-Met- Val- D-Met; Wpeptide, W pep) was implemented to functionalize PTX-loaded HSA nanoparticles, to enhance the targetability of the nanoparticles against triple-negative breast cancer cells (TNBC). TNBCs were shown to overexpress the formyl peptide receptor (FPR). The HSA nanoparticles were decorated with the W peptide, through the amino groups of HSA, to target the FPR receptor, leading to enhanced cytotoxicity (Liu et al., 2017).

13.4.2 Enhanced cytotoxicity

Dose-dependent cytotoxicity was observed for the self-assembled protein nanoparticles. Indeed, the improved cellular uptake contributed to the enhanced cytotoxicity. CCM-loaded Hb nanoparticles showed an enhanced cytotoxic effect on MCF-7 cells ($IC_{50} = 17.75$ μM) compared to free CCM ($IC_{50} = 26.13$ μM). It is worth noting that Hb did not show any toxicity to the cells, even at high concentrations (9 mg/mL) (Wang et al., 2015a). Additionally, PTX loaded HSA nanoparticles ($IC_{50} = 27.7$ ng/mL) were superior to Abraxane ($IC_{50} = 65.4$ ng/mL) against MCF-7 cells (Xie et al., 2013).

13.4.3 Improved stability

The stability of the self-assembled protein nanoparticles is affected by the pH of the solution. Any deviation from the pH away from the isoelectric point improves the stability of the nanoparticles, owing to the charges formed on the protein molecule (Wang et al., 2013). However, the particle size tends to rise, and aggregation occurs. CCM-loaded HSA nanoparticles were found to be stable in the rabbit serum for 24 h, with minimal particle size change (Gong et al., 2015). Also, no change was observed as a result of the incubation of the nanoparticles in a 5% glucose solution. However, it was found that the hydrophobic associations stabilizing HSA nano-assemblies were compromised when incubated in ethanolic solution, evident with an increase in the particle size and swelling of the nanoparticles (Wang et al., 2013).

13.4.4 Enhanced pharmacokinetics

Retention of the nano-formulations in the systemic circulation ensures enhanced tumor accumulation and uptake. Therefore, self-assembled protein nanoparticles showed an enhanced bioavailability. It was shown that DOX and celecoxib coloaded HSA nanoparticles were superior to free DOX, as it was better retained

in the tumor. Furthermore, PTX- loaded albumin nanoparticles and targeted PTX-loaded albumin nanoparticles showed an enhanced AUC 1.2- and 2.5- folds higher than Taxol, respectively. The mean residence time for PTX- and targeted PTX-loaded albumin nanoparticles were longer by 2.3- and 7.5-folds, while the clearance was delayed by 1.1- and 2.3- folds than free Taxol, respectively. Collectively, these data demonstrated the enhanced pharmacokinetic properties of the self-assembled albumin nanoparticles (Liu et al., 2017).

13.4.5 Nanotheranostic applications

Theranostics is a combination between therapeutic approaches and diagnostic procedures. The codelivery of both therapeutic and diagnostic agents can be achieved via self-assembled protein nanoparticles. In this regard, Docetaxel (DTX) and IR-780 were coloaded in HSA nanoparticles. The nanoparticles were internalized and exhibited hyperthermia in response to laser irradiation, where the temperature of the tumor was raised from 26.4°C to 45°C (Wang et al., 2016a). Furthermore, the temperature could be increased to 47°C, which was enough for exhibiting a cancer-killing effect. The improved cellular uptake via HSA through SPARC-mediated endocytosis allowed enhanced retention and better tumoral accumulation. In turn, the retained Indocyanine green dye (ICG)-loaded HSA nanoparticles exhibited a rise in the temperature to 59.4°C upon laser irradiation, with off-target liver accumulation compared to free ICG (Sheng et al., 2014).

13.4.6 Reduced toxicity

Anticancer drugs often suffer from off-target toxicity, which may compromise drug therapy. On the other hand, the enhanced targeting ability of the nanoparticles paves the way for the subsidizing of these adverse effects. It is known that DOX is a cardiotoxic anticancer agent, secondary to the generation of reactive oxygen species (ROS) (Cappetta et al., 2017; Yuan et al., 2013). Mice treated with DOX-loaded HSA nanoparticles showed a more preserved left ventricular ejection fraction (LVEF = 63.7%), compared to the group treated with free DOX (LVEF = 57.1%). In agreement with these results, the free DOX treated group showed an increase in the release of CK-MB and LDH markers, suggesting cardiac damage. Furthermore, an increase in the oxidative stress markers (MDA) and a decrease in the eliminators of ROS (SOD) were more prominent in the free DOX treated group (Yuan et al., 2013).

13.4.7 Stimuli-responsive drug release

The tumor microenvironment possesses various characteristics, which could be exploited to tune the release of drugs from nanoparticles. Self-assembled protein nanoparticles are synthesized such that the forces stabilizing the nanoparticles are sensitive to either an acidic environment or an environment rich in GSH.

The photochemical synthesis approach of DOX-loaded α-lactalbumin showed a pH-responsive drug release at pH 4, where 70% of the loaded DOX was released after 10 h, compared to 10% released at 5 h at pH 7.4 (Fig. 13.3). Additionally,

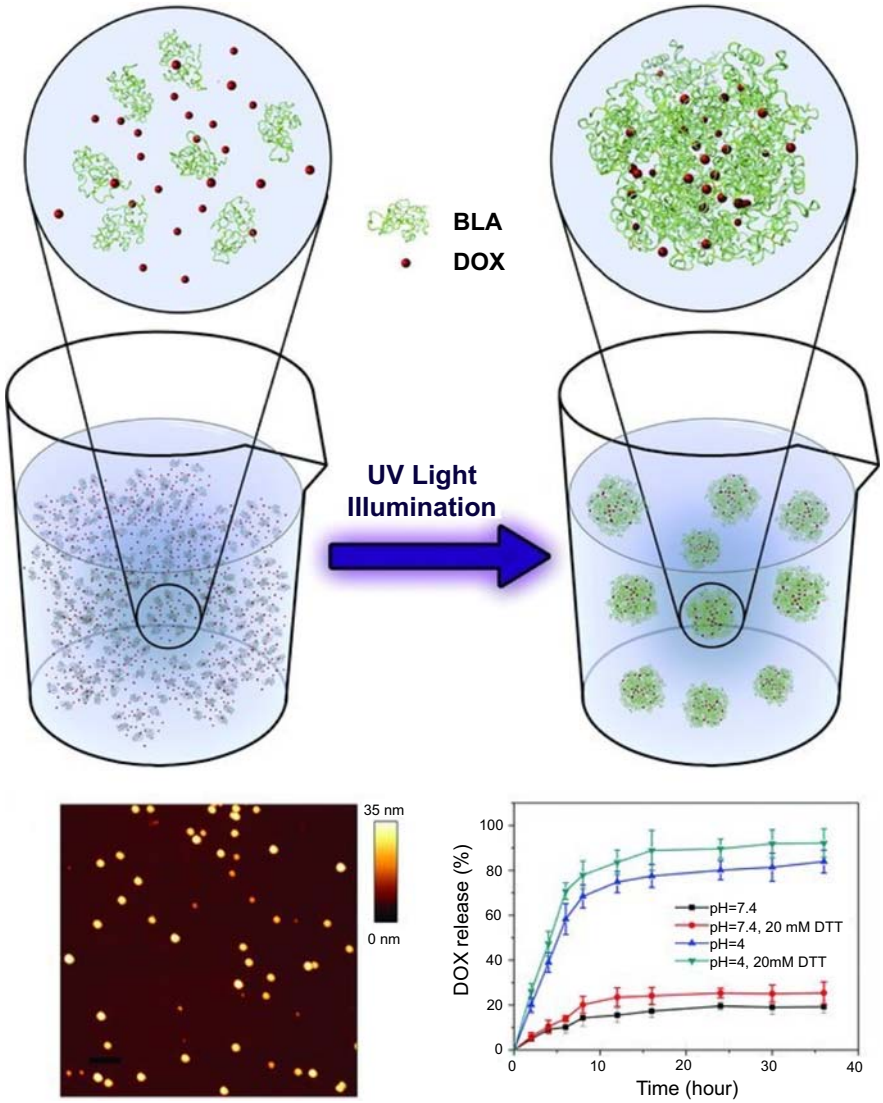

FIGURE 13.3

The formation of protein nano-assemblies via UV- illumination, resulted in a redox-responsive release of the loaded drug.

Reprinted from Ref. Xie, J., et al. (2013). One-step photo synthesis of protein-drug nanoassemblies for drug delivery. Advanced Healthcare Materials, *2(6), 795–799.*

augmenting the release medium with DTT, as a disulfide bond reducing agent, boosted the drug release to 90%. This may be explained by the presence of the intermolecular disulfide bonds acting as the stabilizing forces between protein molecules (Xie et al., 2013). Redox-responsive drug release was attributed to PTX- loaded albumin nanoparticles synthesized using the molecular switch approach. The drug release was enhanced in the presence of GSH compared with no GSH treatment (Liu et al., 2017).

13.4.8 Overcoming cancer drug resistance

Cancer drug resistance is an emerging hurdle facing conventional anticancer therapy. It can be manifested through various pathways and mutations. For instance, one of the major resistance pathways is the efflux of anticancer drugs. Drug efflux could be mediated by the upregulation of the ATP-binding cassette (ABC) (Dvorak et al., 2017). Reversal of the efflux by the ABCB1 (P-glycoprotein; P-gp) may be regulated by Cyclopamine (CYC) (Liu et al., 2016; Taipale et al., 2000). The codelivery of CYC and DOX was achieved via loading both drugs in BSA nanoparticles, implementing a thermal-induced self-assembly procedure. The internalization of the nanoparticle through SPARC- mediated endocytosis was crucial to overcoming cancer drug resistance. It was shown that the expression of P-gp was decreased, the level of DOX accumulation was increased, and only 2% survival was observed in DOX-resistant MDA-MB-231 breast cancer cells (Lu et al., 2019). Furthermore, Quercetin, as a P-gp inhibitor, was coloaded with DTX using the desolvation technique. A 4.27- and 1.87-fold reduction in the IC_{50} was associated with the codelivery of both drugs compared to free DTX or DTX- loaded BSA nanoparticles against MDA-MB-231 breast cancer cells (Desale et al., 2018).

13.5 Conclusion

Protein nanoparticles are biocompatible drug delivery systems, which possess several advantages over other nanomaterials. Less immunogenicity, biodegradability, as well as high drug loading, are attributed to protein nanoparticles. The most facile techniques for the synthesis of protein nanoparticles rely on the self-assembly procedure. The self-assembled protein nanoparticles can be produced by bottom up techniques, for example, desolvation or hydrophobic drug-induced self-assembly. These techniques allow the physical encapsulation of the therapeutic or diagnostic cargo. Alternatively, proteins can be modified to produce amphiphilic structures capable of self-assembly into core-shell structures. These produced systems preserve their capacity for functionalization with targeting materials. Also, they enhanced the physicochemical and pharmacokinetic parameters of the loaded drugs. Furthermore, the forces arising to stabilize the formed protein nanoparticles

impart stimuli-sensitive characteristics, responding to either pH or redox microenvironment. Notably, the inherent nature of protein nanocarriers allows them to internalize into tumor cells, bypassing cancer resistance mechanisms.

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CHAPTER

Stimuli-responsive nanosystems as smart nanotheranostics

14

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14.1 Introduction

In the search for effective approaches to meet the demands of human health, great efforts have been made in the development of novel pharmaceutics. Accordingly, theranostics are promising developments that can be applied to different pathologies in which the formulation is a multifunctional platform that combines therapeutic and diagnostic properties. Ideally, it is also demanded that theranostics achieve targeting modalities for the development of an all-in-one, localized detection and treatment system. Fig. 14.1 schematizes the concept of "theranostics," including some examples of commonly used therapy, diagnosis, and targeting approaches.

Nowadays, in the search for personalized therapies, monitoring the response of specific treatments applied to each patient is desired (Zhao et al., 2019), representing the current goal of theranostics. Thereby, the diagnosis means the ability to define a disease state, whereas theragnosis (or theranostics) means the ability to affect therapy or treatment of a disease state (Adiga & Adiga, 2014), and therefore, improve its efficiency, safety, and economics.

The term *theranostics* (syn: theragnostic) was first used by PharmaNetics president and CEO John Funkhouser in 2002 when described his company's business model as the development of diagnostic tests associated with the application of specific therapies (Funkhouser, 2002; Gilham, 2002). Although the term theranostics begins to appear in the literature at the beginning of the 21st century, some combined developments in diagnosis/therapy of the previous decades could already be defined as the first theranostics. One example is the case of breast cancers that overexpress the Human Epidermal growth factor Receptor-2 (HER-2). Genentech's Herceptin for the treatment of Stage IV breast

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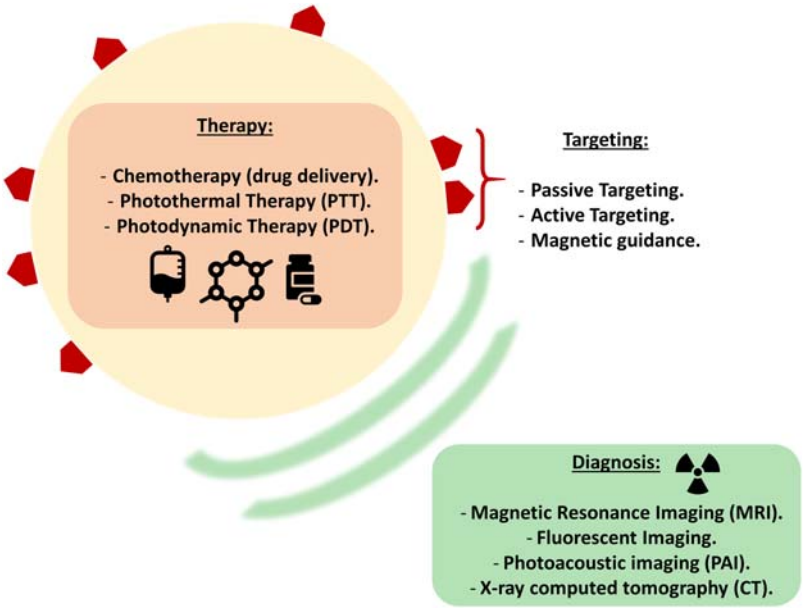

FIGURE 14.1

Representation of the concept of "theranostics".

cancer and Dako's HercepTest for diagnosis of HER-2 overexpression were simultaneously approved by Food and Drug Administration in 1998, changing the treatment strategy of patients with breast cancer who overexpress HER-2 (Adiga & Adiga, 2014).

From that moment to the present, researchers in the field of theranostics have been evaluating a broad variety of drug delivery carriers, imaging contrast agents, and targeting modalities, intending to develop novel all-in-one, detection/treatment systems. In the last few years, more than 1100 articles using this term have been published each year. In addition, the "Theranostics" journal was founded in 2011 (Wiesing, 2019).

Towards the first decade of the 21st century, the concept of *nanotheranostics* started to appear to refer to nanosystems that can diagnose, provide treatment in the target pathological site, and monitor the response to therapy. The number of reports with the word "nanotheranostics" in article title, abstract, and keywords has increased noticeably in the last decade (Fig. 14.2).

Some inherent properties of nanosystems make them ideal candidates for theragnosis. Nanosystems can be designed with the ability to be localized in specific sites and mitigate undesired side effects (Wang, Chuang, & Ho, 2012). In addition, due to their nanometric size, it is possible to achieve extended circulation time in blood.

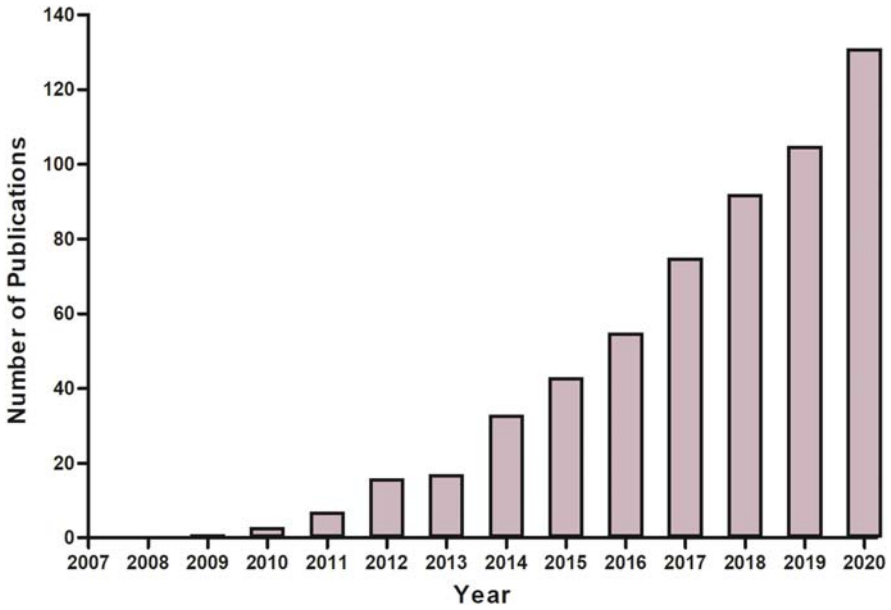

FIGURE 14.2

Number of scientific publications on "nanotheranostics" in article title, abstract and keywords (search performed by Scopus).

The successful development of theranostic nanomedicines requires significant advances in various disciplines ranging from materials science to medicine. Some of the requirements that nanotheranostics must fulfill are (Sumer & Gao, 2008):

- nanometric size: the ideal size range for spherical nanoparticles (NPs) is 10−500* nm (*there is no absolute agreement in the literature on this upper limit),
- high drug-loading densities (in case of chemotherapy): high surface-area-to-volume ratio of nanosystems allows high loading capacity of therapeutic drugs and/or imaging agents,
- efficiency in targeting specific regions with minimal nonspecific uptake,
- ability to provide responsive drug release mechanisms to improve drug bioavailability (in case of chemotherapy), or in general, responsive mechanisms for treatment,
- ability to provide a noninvasive assessment of the carrier biodistribution and quantification of localized accumulation to monitor the response of therapy.

Furthermore, nanotheranostics must fulfill the requirements of any nanomedicine such as being biocompatible, minimizing adverse effects, improving therapeutic efficiency, releasing drugs or performing therapeutic effects in a time-controlled and site-specific manner, and being able to be functionalized with

desired molecules (e.g., targeting moieties) (Baek et al., 2015; Slowing, Vivero-Escoto, Wu, & Lin, 2008).

Another benefit of the use of nanomaterials for theragnosis is that many of them are imaging agents by themselves, for example, magnetic nanoparticles (MNPs) can exhibit magnetic resonance imaging (MRI). Besides, the great surface area of nanosystems can be functionalized with multiple types of molecules such as stabilizing agents, ligands, or stimuli-responsive materials to confer additional properties to the system, for example, reducing systemic toxicity, and selectively targeting diseased tissues, among others.

The alternative designs for nanotheranostics are truly endless. Different synthesis procedures, architectures, configurations, morphologies (shape and size), components (therapeutic, imaging, and targeting agents), functionalities, and therapy/imaging modalities can be chosen for the rational design of nanotheranostics. A schematical representation of possible components and their spatial distributions is illustrated in Fig. 14.3.

In light of the huge variety of nanotheranostics designs, and the real demand to meet multifunctionality, the next generation of nanotheranostics-based research is

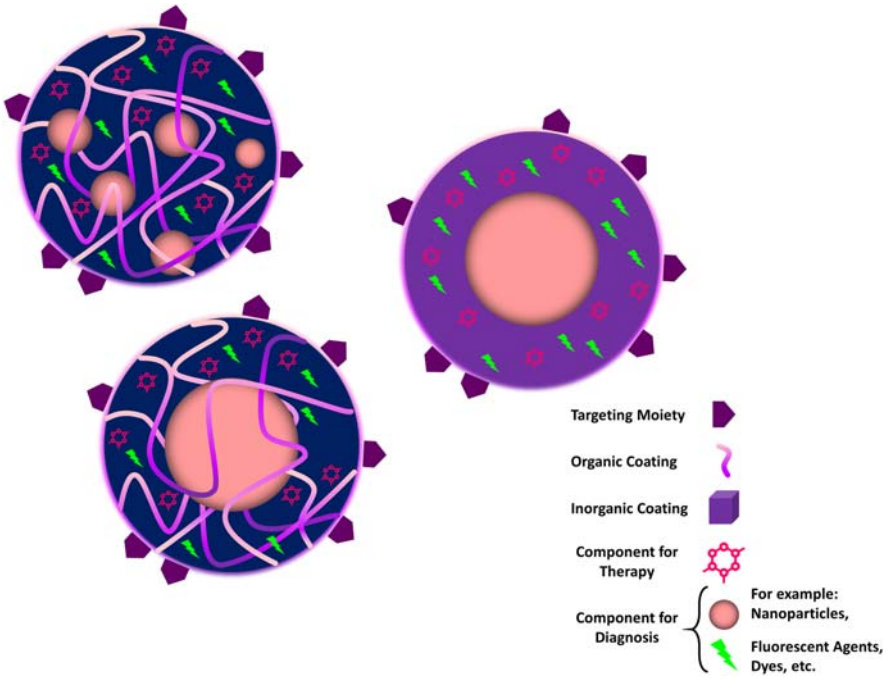

FIGURE 14.3

Schematic representation of several possible components of nanotheranostics and structural configurations with the key elements: targeting moieties, therapeutic agents, and imaging components.

aimed at the consolidation of functions into multifunctional devices, facilitating the realization of diagnosis, targeted therapy, allowing real-time treatment monitoring, and coadministration of multiple drugs for combination therapy (Svenson, 2013).

Hence, nanotheranostics must be designed by integrating different organic and inorganic materials. Some of the organic materials used are polymeric NPs, emulsions, nanocapsules, nanospheres, micelles, liposomes, or dendrimers. Among the inorganic materials, the most frequently used are quantum dots, graphene, carbon nanotubes (CNTs), MNPs, and gold nanoparticles (AuNPs). The combination of both types of materials makes it possible to encapsulate or solubilize chemotherapeutic agents to improve drug delivery in vivo and/or to provide unique optical, magnetic, and electrical properties for imaging. Thus, the concept of "nanocomposites" becomes relevant because it is used to refer to platforms made up of more than one material that has at least one physical dimension in the nanometer range (Mishra, Yadav, Prabhakar, & Gaud, 2018). The adjective "hybrid" is also accurate to describe multicomponent structures.

Among nanomaterials, stimuli-responsive ones possess the special feature of responding to small physical or chemical stimuli that lead to a macroscopic alteration in their structure/properties. This particularity can be exploited in nanotheranostics to enhance therapeutic effects, develop new detection modalities, or guide to the site of interest. Hence, stimuli-responsive nanotheranostics can act in response to both environmental and external stimuli such as pH, redox potential, temperature, magnetic field, light, or specific biomolecules (Caldorera-Moore, Liechty, & Peppas, 2011).

Table 14.1 summarizes the type of stimuli by categorizing endogenous and exogenous stimuli and their applications in the field of theragnosis. Exogenous stimuli are externally applied stimuli, including magnetic fields, ultrasound (US), and light, whereas endogenous stimuli refer to variations in the site of action, such as gradients in pH, temperature, the concentrations of enzymes or specific analytes, and redox potential (Raza et al., 2019).

Within the most fascinating responsive materials, responsive polymers are highlighted due to their versatility and tunable sensitivity. These polymers exhibit physical or chemical conformational changes in response to a stimulus which could range from swelling/contraction to disintegration. Thereby, this property can be used for example, in the release of encapsulated drugs in the field of nanomedicine.

Many diseases can be addressed by the development of stimuli-responsive nanotheranostics. However, due to the significant physiological difference between cancerous tissue and surrounding healthy tissue, the research community is especially interested in the treatment and diagnosis of cancer (Caldorera-Moore et al., 2011).

In this chapter, a brief state of the art in the field of stimuli-responsive nanotheranostics is presented. We summarize the key aspects of the most representative stimuli-responsive nanotheranostics from a chemical point of view. Recent research efforts toward the development of stimuli-responsive nanotheranostics are discussed and addressed herein with a focus on their in vitro and

Table 14.1 Mechanisms and applications of different stimuli.

	Type of stimuli	Applications	Mechanism
Exogenous stimuli	Magnetic fields	Local hyperthermia under an alternating magnetic field for triggering drug release and tumor ablation, tumor imaging by magnetic resonance imaging (MRI) and magnetic-guided accumulation of nanotheranostics	The application of oscillating magnetic fields, which can penetrate in the body tissue in noninvasive way. In the case of hyperthermia, the magnetic field can induce local heat which can be used as a treatment strategy.
	Ultrasound	Triggering controlled drug release, tumor US imaging and enhance accumulation in tumors.	Thermal-induced release mediated by radiation forces and mechanical-induced release mediated by cavitation phenomena Qiao et al. (2019).
	Light	Triggering controlled release of therapeutics, light-activated imaging (e.g., photoacoustic imaging (PAI)) or imaging-guided therapy, generation of singlet oxygen ($^1O_2$) for photodynamic therapy (PDT), and photothermal effect for tumor ablation by photothermal therapy (PTT).	Recommended for light accessible tumors due to limited light penetration. Drug release can be achieved with chromophore which should change its conformation by photoreaction when it is exposed to a specific wavelength, releasing the encapsulated drug. By using photosensitizers, it is possible to achieve PDT and PTT by the production of $^1O_2$ or heat.
Endogenous stimuli	pH	Triggering controlled release of therapeutics or prodrug activation.	The pH in cytoplasm, blood and normal tissues is about 7.0 to 7.4. Inside endosomal/lysosomal organelles the pH is approximately pH 6 to 4, and in tumor microenvironment pH is 6.5 to 6.8. These lower pH values can be exploited to conduce drug release in pathological tissues Mi (2020); Qiao et al. (2019).
	Temperature	Triggering controlled release of therapeutics.	Tumors and inflammation areas typically display abnormal temperature

(*Continued*)

Table 14.1 Mechanisms and applications of different stimuli. *Continued*

	Type of stimuli	Applications	Mechanism
			gradients (40–42ºC) compared to healthy tissues Guo et al. (2014); Hajebi et al. (2019); Karimi et al. (2016); Qiao & Wang (2018); Tsintou et al. (2017). Spontaneous temperature fluctuations also occur during day and night cycles Hoogenboom (2019).
	Enzymes	Triggering enzyme-responsive drug delivery; activation of prodrugs, probes, and ligands.	Enzymes can carry out their functions by cutting the enzyme-sensitive bonds between the bioactive compounds and protective groups to produce: • Degradation of nanocarriers (enzyme triggered cleavage). • Direct cleaving of the conjugation between nanocarriers and drugs.
	Redox	Triggering controlled release of therapeutics.	Tumors exhibit significantly different reduction potentials, for example, the level of glutathione inside cancer cells is remarkable higher than in normal regions.

in vivo applications and advances in both therapeutic and diagnostic aspects. Some relevant topics such as combinational treatments, imaging-guided tumor regression, deep tissue visualization, targeting strategies, some general features of the tumor microenvironment, and so on are also reviewed. The following sections are categorized according to the inorganic material involved.

14.2 Plasmonic-based nanotheranostics

Plasmonic materials are metals that exhibit localized plasmonic excitations when they are interacting with electromagnetic radiation. When a metallic nanoparticle

interacts with an oscillating magnetic field, coherent oscillations of conduction electrons are produced (Maier & Atwater, 2005). This phenomenon is called localized surface plasmon resonance. This type of excitation results in resonance-enhanced local fields and, accordingly, in enhanced optical phenomena such as absorption, Mie scattering, Raman scattering, and various nonlinear effects (Khlebtsov et al., 2013). Different functionalities of plasmon modes can emerge through manipulation of the architectures and morphologies of these materials on the nanoscale (Murray & Barnes, 2007). To provide an overview of plasmonic-based nanotheranostics, developments involving gold and silver NPs are described below.

Gold nanostructures have been intensively studied for simultaneous therapy and diagnosis due to their biocompatibility, well-established strategies for surface modification (e.g., gold-thiol bonding), and unique optical and photothermal characteristics (Wang et al., 2012).

In terms of therapy, AuNPs could be used as photothermal therapeutic agents themselves due to their ability to convert adsorbed near-infrared (NIR) and visible light into heat (Jin, Ovais, & Chen, 2018). This property is exploited to use laser light−generated heat to damage surrounding tissues and cellular components undergoing apoptosis.

For example, Zhao et al. designed a dual-stimuli-responsive and reversibly activatable theranostic platform for in vivo tumor-targeted precision fluorescence-guided PTT (Zhao, Yang, Chen, & Yan, 2017). The nanoprobe was formed by asymmetric cyanine and glycosyl-functionalized gold nanorods (AuNRs) with matrix metalloproteinases (MMPs)-specific peptide as a linker to achieve MMPs/pH synergistic and pH reversible activation. In this case, asymmetric cyanine serves as both a tumor-specific imaging probe through NIR fluorescence signal and an auxiliary photothermal agent. AuNRs act as both PTT agents and ultra-efficient quenchers. Finally, the conjugation of glycosyl endows the nanoprobe with the active tumor-targeting ability and good biocompatibility. Another study combined PTT and chemotherapy in novel theranostic NPs based on AuNPs and HS−poly(ε-caprolactone)-block-poly(N-isopropyl acrylamide)-block-poly(acrylic acid) (HS-PCL-b-PNIPAM-b-PAA), which is a pH and temperature-sensitive material (Mahmoodzadeh, Abbasian, Jaymand, Salehi, & Bagherzadeh-Khajehmarjan, 2018). This gold/polymer conjugate was loaded with doxorubicin hydrochloride (DOX) as an anticancer drug through electrostatic interactions. The results showed that these stimuli-responsive nanotheranostics maintain low drug release values in physiological conditions (pH 7.4 and temperature of 37°C) and high drug release profiles in cancerous conditions (pH 5.4 and 4.0, as well as temperature values higher than 37°C). Furthermore, a pulsed laser NIR beam was used to induce PTT. Thus, the viability of cells treated with chemo-PTT is significantly lower than chemotherapy alone or PTT alone. It is important to highlight that the generated heat by AuNPs upon laser irradiation can also accelerate the drug release due to the collapse of PNIPAM segments according to in vitro drug release studies.

On the other hand, gold nanostructures could simultaneously serve as contrast probes in photoacoustic (PA) tomography, two-photon fluorescence imaging, optical coherence tomography, and X-ray computed tomography (CT), mainly due to their high NIR optical, as well as X-ray absorbance characteristics (Jin et al., 2018).

X-ray CT is a noninvasive imaging technique that provides 3D anatomical information on specific tissues and organs (Dong et al., 2019). This clinical imaging modality is based on the difference in mass attenuation between two tissues; materials with a high atomic number or density absorb more X-rays, making them detectable (Tabish et al., 2020). Gold colloids can be used as contrast agents allowing higher energy and safer scans with high contrast.

Stimuli-responsive materials combined with gold can be excellent platforms for chemotherapy and X-ray CT imaging. As an example of that, multifunctional dendrimer-entrapped AuNPs conjugated with DOX were developed for pH-responsive drug delivery and targeted CT imaging (Zhu et al., 2018). DOX has been covalently attached to the 5th generation partially acetylated poly(amidoamine) dendrimers functionalized with folic acid (FA) through acid-sensitive cis-aconityl linkage to form dendrimer-FA-DOX conjugates to which AuNPs were entrapped. The final conjugates exhibited a pH-responsive release profile of DOX because of the cis-aconityl linkage, having a faster DOX release rate under a slightly acidic pH condition than under a physiological pH. In addition, a much better X-ray attenuation property compared with a clinical iodine-based CT contrast agent, Omnipaque, was observed.

As an example of a multifunctionality platform, AuNRs@polyacrylic acid/calcium phosphate yolk-shell NPs were employed for synergic dual-mode CT/PA imaging and chemo-photothermal cancer therapy (Li, Chen, et al., 2018). These nanotheranostics exhibited high loading content of DOX, superior photothermal conversion property, and pH/NIR dual-responsive drug delivery performance. The pH-responsive drug release of DOX results from the dissolution of calcium phosphate in a mildly acidic environment (pH 5.0), which is similar to the extracellular pH of tumors. While an extremely slow dissolution of calcium phosphate was observed in pH 7.4. When DOX-loaded composites were exposed to NIR irradiation, drug release is significantly increased due to the heat generated by the AuNRs.

Stimuli-responsive nanosystems are also interesting for enhanced imaging of cancer cells in vitro. Considering this, Shi and collaborators designed technetium-99m (99mTc) labeled poly(ethylenimine) (PEI)—entrapped AuNPs (Au PENs) with pH-responsive charge conversion property for enhanced single photon emission computed tomography (SPECT) and CT dual-mode imaging of cancer cells (Zhu et al., 2019). Amine functional groups of PEI were successively modified with monomethyl ether and carboxyl functionalized poly(ethylene glycol) (PEG-COOH), maleimide and succinimidyl valerate functionalized PEG (MAL-PEG-SVA), diethylenetriaminepenta-acetic dianhydride (DTPA), and fluorescein isothiocyanate. AuNPs were entrapped inside, followed by conjugation with the alkoxy phenyl acyl sulfonamide (APAS) through the PEG maleimide, acetylation of the PEI leftover surface amines, and 99mTc labeling. The negatively charged

sulfamide group and the positively charged quaternary ammonium group of APAS ensure that it stays neutral in a physiological environment (pH 7.4) and turns positively charged in the tumor microenvironment (pH 6.0) due to the protonation of the sulfamide group. Thereby, APAS-modified NPs could have limited accumulation in normal tissues and improved accumulation in cancer cells and tumors due to the electrostatic interaction between positively charged particles and negatively charged cell membranes. Due to the enhanced permeability and retention (EPR) effect, the designed APAS-99mTc − Au PENs may be used as promising contrast agents for enhanced dual mode SPECT/CT imaging of different types of cancer cells in vitro and tumors in vivo.

Silver nanoparticles (AgNPs) are another type of plasmonic material that can be used as theranostic agents. This kind of NPs has intrinsic properties of biomedical interest: AgNPs have shown considerable antimicrobial, antiviral, antifungal, antiprotozoal, antiinflammatory activity, and anticancer effects themselves (Barabadi et al., 2020; Li, Chang, et al., 2018). In addition, AgNPs exhibit high efficiency of plasmon excitation, and the wavelength of their resonance can be easily and widely tunable in the visible spectrum which can be beneficial for the monitoring of disease progression (Li, Chang, et al., 2018). Therefore, AgNPs can also be used as photothermal agents. Recently, Bose et al. developed targeted plasmonic AgNPs for light-activated chemo-PTT against breast cancer (Bose, Pattanayak, & Priyam, 2020). In this example, pentagonal plasmonic AgNPs were conjugated with folate receptor and then, a potent coumarin derivative, Herniarin, was encapsulated. These silver nanoformulations act as targeted light responsive nano-delivery platforms for Herniarin to improve its therapeutic proficiency. Besides, they resulted capable of transforming light into heat energy when exposed to laser (800 nm) allowing PTT to complement the chemotherapeutic potential of Herniarin.

For the obtention of nanotheranostics, different strategies to produce AgNPs were investigated in the literature. A novel example of green synthesis of AgNPs is the work of Mukherjee et al. who developed a 4-in-1 multifunctional system from colloidal AgNPs by the reduction of silver nitrate solution using *Olax scandens* leaf extract (Mukherjee et al., 2014). These nanotheranostics displayed enhanced antibacterial activity compared to chemically synthesize AgNPs, anticancer activities to different cancer cells, biocompatibility to rat cardiomyoblast normal cell line (H9C2), human umbilical vein endothelial cells and Chinese hamster ovary cells, and bright red fluorescence inside the cells. The biocompatible nature to normal cells is a good indication for future application as a drug delivery vehicle for cancer therapeutics. Additionally, due to the fluorescent molecules present in the plant, the silver nanoconjugates exhibited red fluorescence that could be utilized to detect their localization inside the cancer cells (a diagnostic approach). Therefore, this nanosystem is 4-in-1 because it demonstrated multifunctional biological activities: (1) antibacterial, (2) anticancer agent, (3) drug delivery vehicle, and (4) imaging facilitator. Another example is the green synthesis process of AgNPs coated with porcine skin gelatin by thermal

reduction of silver nitrate (Vedelago, Gomez, Valente, & Mattea, 2018). These NPs found application in gel dosimetry and as a fluorescent agent in X-ray beam irradiations.

Among stimuli, the US is widely investigated for remote release of a payload. US-based biomedicine is emerging thanks to its advantages such as poor invasiveness, deep penetration, high spatial resolution, and excellent endogenous contrast. Tissue-penetrating depth can be regulated by changing US frequencies, cycles, power densities, irradiation durations, and mechanical indexes. Additionally, the US can increase the accumulation of NPs into the tumor tissue because it is capable of accelerating the extravasation of NPs through the blood capillaries and enhancing the cell-membrane permeability (Zhou, Han, Jing, & Chen, 2017).

Multifunctional silver alginate hydrogel microcapsules were synthesized for US-induced release, whereas the detection capabilities were achieved by surface-enhanced Raman scattering (Lengert et al., 2017). Sodium alginate was immobilized into the pores of calcium carbonate particles of different sizes followed by cross-linking via the addition of silver ions, which also allows the formation of AgNPs and improves the sensitivity to the US. Reduction of AgNPs on the alginate matrix and simultaneous removal of calcium carbonate cores was achieved by the addition of ascorbic acid solution. The encapsulation and stimulated remote release of fluorescently labeled bovine serum albumin by low-power US (allowed in medicine) were performed. Finally, this formulation demonstrated the possibility to enhance the Raman signal in the range for applications in theranostics.

Stable PA properties were achieved by hybrid nanoplatforms based on hybrid silica, eumelanin, and silver nanostructures (Silvestri et al., 2019). Eumelanins hold huge promise as a biocompatible and endogenous PA contrast agent. Furthermore, conjugation with rhodamine isothiocyanate allowed particle detection through fluorescent imaging demonstrating their multifunctional potentialities.

Finally, considering the fluorescent character of camptothecin (CPT), a CPT-based polymeric prodrug attached to the surface of AgNPs was developed as a theranostic system (Li, Qiu, et al., 2018). The variation of fluorescence intensity of CPT due to the presence of AgNPs makes it possible to trace the intracellular drug release process and observe the distribution of released CPT in cells. In this case, a branched star copolymer PEG-star-prodrug was synthesized through reversible addition − fragmentation chain-transfer polymerization and attached to the surface of AgNPs via the interaction between AgNPs and the disulfide of lipoic acid moiety in the polymer.

14.3 Silica and zeolite-based nanotheranostics

Interestingly, in contrast to many other nanomaterials, the physical properties of nanosized silica remain constant compared to bulk material except for the corresponding increase in surface area (Wang et al., 2012). This material prevails in

the field of nanomedicine because it offers the possibility to design well-defined tunable structures (i.e., size, morphology, and porosity) by applying well-established siloxane chemistry. Therefore, silica surface can be easily modified post-synthesis with numerous functionalities (i.e., ionic or covalent ligands) to endow these particles with diagnostic and therapeutic capabilities (Vivero-Escoto, Huxford-Phillips, & Lin, 2012). Additionally, silica NPs are inexpensive, easy to prepare, relatively chemically inert, biocompatible, and water dispersible. Their good biocompatibility is because they degrade to nontoxic silicic acid in vivo, which can be excreted from the body through urine (Chen, Hableel, Zhao, & Jokerst, 2018). Furthermore, silica NPs are intrinsically stable, and their degradability and circulation time can be tuned. Silica-based NPs can be either solid silica nanoparticles or mesoporous silica nanoparticles (MSNs).

MSNs exhibit many unique properties such as intrinsically large surface area, excellent biocompatibility, stable and rigid frameworks, tunable size and pore diameter, and large pore volumes (Chen, Cheng, et al., 2013). Furthermore, MSNs have topologically distinct domains that can be individually functionalized: the silica framework, hexagonal nanochannels/pores, and particle exterior.

Finally, pure silica has intrinsic theranostic potentials due to its high acoustic mismatch with most soft tissues (Chen et al., 2018). Because of this and their photophysical stability, biocompatibility, and favorable colloidal properties, silica have been extensively utilized as an optical imaging contrast agent.

Silica NPs are an excellent scaffold for facile loading of a wide variety of imaging and therapeutic moieties, making them promising candidates for theranostic applications (Vivero-Escoto et al., 2012). For instance, MSNs-supported red-blood-cell-mimetic with deep-red light-activated tumor imaging and drug release were reported (Su et al., 2017). DOX and a NIR photosensitizer chlorin e6 (Ce6) were coloaded achieving Ce6-based PDT and DOX-based chemotherapy. These theranostic NPs could realize in vivo tumor imaging with a long tumor accumulation lifetime of over 24 h, and laser-activated tumor-specific DOX accumulation.

One very exploited strategy is the use of gadolinium chelates (with Gd(III) ions) doped into MSNs as contrast agents for MRI. Theranostic nanoplatforms as effective PDT agents were constructed by coating tirapazamine (TPZ)-loaded MSNs with layer-by-layer assembled multilayer and by chelation with paramagnetic Gd^{3+} with magnetic resonance contrast ability (Chen et al., 2017). TPZ is a bioreductive prodrug that is only cytotoxic to hypoxic cells allowing cancer treatment due to its exacerbated hypoxic environment. These nanotheranostics can be specifically uptaken by the CD44 receptor overexpressed in tumor cells and respond to hyaluronidase to trigger the release of therapeutics. Therefore, they showed preferential accumulation in the tumor site and significantly inhibited the tumor progression by PDT because, upon laser irradiation, NPs were excited to generate toxic $^1O_2$, and by bioreductive chemotherapy under NIR fluorescence/MRI guidance. A schematic illustration of this tumor-targeted versatile theranostic nanoplatform is presented Fig. 14.4.

Moreover, Sun et al. developed a NIR irradiation-triggered, triple-modal imaging-guided nanoplatform based on DOX@Gd-doped MSNs, which is conjugated to indocyanine green (ICG)-loaded thermosensitive liposomes (designated as DOX@GdMSNs-ICG-TSLs) (Sun et al., 2018). ICG contributed to both PDT and PTT effects. NIR fluorescence imaging and PA imaging from ICG combined with the MRI function of Gd allow achieving successful triple-modal imaging. Moreover, FA-modified thermosensitive liposomes were employed to coat the surface of DOX@GdMSNs to prevent DOX leakage, as well as to improve cellular uptake. Under NIR irradiation, ICG could generate heat, thus leading to the rupture of ICG-TSLs and the consequent release of DOX. Similarly, Zhang et al. synthesized theranostics platforms by doping Gd ions to MSNs via a coassemble process. Iopamidol and DOX were incorporated to combine the bifunctional diagnosis of MRI and CT imaging, and sustained drug release (Zhang et al., 2019). The obtained multifunctional NPs highlighted because they show MRI contrast due to their outstanding T1-weighted MRI signal in vivo, excellent performance

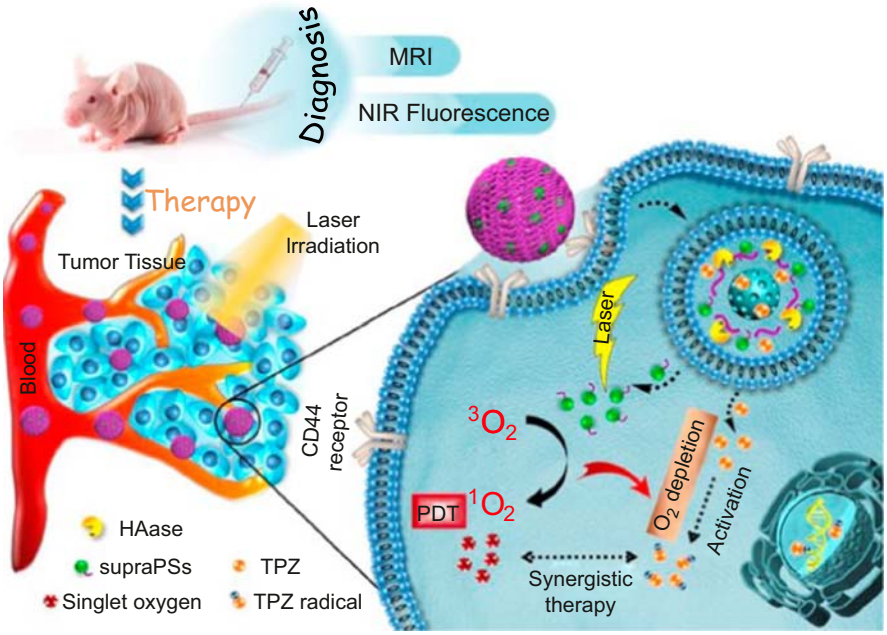

FIGURE 14.4

Schematic illustration of tumor-targeted versatile theranostic nanoplatform to achieve superior antitumor efficacy by PDT and bioreductive chemotherapy.

Reproduced with permission from Chen, W. H., Luo, G. F., Qiu, W. X., Lei, Q., Liu, L. H., Wang, S.B., et al. Mesoporous silica-based versatile theranostic nanoplatform constructed by layer-by-layer assembly for excellent photodynamic/chemo therapy. Biomaterials, 117, 54–65. https://doi.org/10.1016/j.biomaterials.2016.11.057.

in inhibiting lymphatic tumor growth in mice, and high efficiency of lymphatic targeting.

Regarding zeolites, they are natural or synthetic porous crystalline aluminosilicates that are composed of a tetrahedral structure (TO4) in which T denotes either aluminum (Al) or silicon (Si) (Přech, Pizarro, Serrano, & Áejka, 2018). Among the main advantages of these materials feature their inorganic nature with porous structure, high specific surface area, ion-exchange capabilities, thermal stability, adsorption features, low toxicity, stability, and surface modification capabilities (Khodadadi Yazdi et al., 2020). The porous structure of zeolites is determined by channels and cavities of different sizes, shapes, and electric charges. The Si/Al ratio determines the cation exchange capacity and hydrophilicity of the zeolite (Li et al., 2019).

As nanometric platforms, they can be used to encapsulate and transport various substances such as therapeutic agents, enzymes, radiopharmaceuticals, photosensitizers, fluorescent dyes, or contrast agents through pore encapsulation, surface adsorption, or simple ion-exchange method, and also carry them to desired sites (Khodadadi Yazdi et al., 2020). In other words, zeolites provide a robust theranostic platform for addressing various diseases, especially cancers.

By way of illustration, Zheng et al. presented a novel composite material consisting of upconversion Er^{3+} and Yb^{3+} ions and targeted/therapeutic drugs anchored on biosafe Linde Type A zeolite nanocarriers with unique advantages of thermal stability, PTT and PDT simultaneously induced by the US and NIR light dual-sensitive triggering mechanism (Zheng et al., 2020). DOX and FA-hydrophilic PEG (FA-PEG)-loaded NPs were prepared to achieve a good tumor therapeutic effect.

Gd(III) ions are also incorporated into zeolites to develop contrast agents for MRI. For example, aqueous suspensions of nanometric Gd-loaded zeolites with high pH responsiveness were tested as MRI contrast agents (Zhang, Peters, Mayer, Helm, & Djanashvili, 2015). In this case, a large increase in relaxivities was observed upon decreasing the pH below 8, which results in beneficial for in vivo applications due to the lower extracellular pH of tumors. Therefore, the presence of these NPs should result in bright spots near tumors in T1 weighted images and dark spots in T2 weighted images. In addition, pH-dependent magnetic properties of the complexes $[Fe(bipy)_3]^{2+}$ and $[Fe(bpp)_2]^{2+}$, both in solution and composite materials encapsulated in a zeolite matrix, were reported as pH-responsive contrast agents in MRI (Nowak et al., 2017). These results have demonstrated that the composite materials prepared show excellent long-term stability.

14.4 Magnetic-based nanotheranostics

Magnetic-based theranostics present MNPs which are advantageous due to their inherent biocompatibility and cost-effectiveness, and their response to a noninvasive stimulus, the external magnetic field (Behrens & Appel, 2016). This

magnetic property means that they can be used in MRI. Moreover, they can display magnetic guidance toward the target tumor or organ (Castro, Sarmento, Madureira, & Pintado, 2019). In the field of therapy, this kind of composites is employed in magnetic hyperthermia, which is a type of thermal treatment based on the local heat generated when the MNPs are subjected to an oscillating magnetic field (Bañobre-López, Teijeiro, & Rivas, 2013). Thereby, when exposed to the alternative magnetic field of appropriate amplitude and frequency, MNPs produce heat that induces a decrease in cancer cell viability through apoptotic or necrotic pathways (Cazares-Cortes et al., 2019). Several designs of electromagnetically activated NPs designed for cancer treatment are already in clinical trials.

Superparamagnetic iron oxide nanoparticles (SPIONs), mainly magnetite (Fe_3O_4) and maghemite (Fe_2O_3), are the most used magnetic materials in the development of nanotheranostics because they have been deeply investigated and successfully synthesized. In addition to their magnetic properties, they have good chemical stability and low toxicity, while they can be easily obtained by coprecipitation methodology (Zhao et al., 2019).

As it was mentioned above, magnetic hyperthermia takes advantage of the ability of MNPs to convert electromagnetic energy into thermal energy (heat) in the presence of an alternating magnetic field (Lorkowski, Atukorale, Ghaghada, & Karathanasis, 2021). In the context of cancer, hyperthermia arises as a complementary treatment in combination with chemotherapy or radiation (Kakwere et al., 2015; Najafipour, Gharieh, Fassihi, Sadeghi-Aliabadi, & Mahdavian, 2021; Tapeinos et al., 2019). At present, the only clinically available magnetic hyperthermia device is the NanoTherm system (MagForce AG, Germany), approved in Europe for use in brain cancer since 2011 and recently authorized for clinical trials of prostate cancer in the US (Lorkowski et al., 2021).

Some recent developments of SPIONs combined with different materials including polymers, clays, silica, carbon, or metal-organic frameworks that display theranostic behaviors are described below.

A dual responsive polymer, poly(N-isopropyl acrylamide)-*co*-tyrosine unit, modified with gadolinium-doped iron oxide nanoparticle (poly@Gd-MNPs) has been reported as a cancer theranostic agent (Roy, Patra, Madhuri, & Sharma, 2016). The polymeric component exhibited excellent loading for the anticancer drug (methotrexate) and stimuli-dependent release (changes in pH and temperature). In vitro assays revealed that the poly@Gd-MNPs displayed T1-weighted MRI capability with good in vitro hyperthermia response. Furthermore, NPs were shown not to affect normal human fibroblast viability even in the presence of a magnetic field, while efficiently killing MCF7 cancer cells by combining high temperature (hyperthermia), as well as targeted and stimuli-responsive delivery of MTX (chemotherapy) in the presence of an external magnetic field. Finally, a blood clearance study of the poly@Gd-MNPs after in vivo administration in rats indicated that after 5 h injection the complete clearance of poly@Gd-MNPs from the body was confirmed. Another relevant example is the magnetic pH-sensitive theranostic nanocomposite for DOX release reported by Delavari et al. (2019). In

this case, Fe_3O_4 NPs were coaggregated with α-lactalbumin during cross-linked enzyme aggregate formation. α-lactalbumin was precipitated and cross-linked using PEG and glutaraldehyde. Moreover, PEI was used to increase the number of amine groups during cross-linking between α-lactalbumin and MNPs. The formation of imine bonds between glutaraldehyde and amine groups on α-lactalbumin and PEI allows higher release of DOX at acidic pHs. The loaded nanocomposite displayed higher cytotoxicity, apoptosis induction, and DOX uptake in cancer cells, as well as more suppression of tumor growth in vivo than free DOX. Moreover, in vitro and in vivo MRI studies presented a higher r2/r1 ratio and comparable contrast to the commercially available DOTAREM, respectively.

Likewise, pH/laser dual-stimuli responsive nanotheranostics composed of melanin coated MNPs and Wnt signaling inhibitor obatoclax (OBX) were reported for multimodality imaging guided mild hyperthermia-enhanced chemotherapy (Feng et al., 2020). The antitumor compound OBX was used for a novel Wnt/β-catenin signaling inhibitor. This system could be used as a contrast agent for MRI and PAI-guided PTT. OBX release can be triggered as a response to both pH changes and NIR light illumination. Finally, the authors demonstrated that mild hyperthermia could dramatically enhance drug accumulation in tumor cells/tissues.

Hybrid theranostic systems that display magnetic hyperthermia, MRI, and magnetic drug delivery were recently developed (Szczęch et al., 2020). Magnetically responsive means that when an external magnetic field is applied, delivery of the drug to the specific site of action can be achieved. In this example, a model hydrophobic drug (paclitaxel) was encapsulated into a polymeric core of poly(caprolactone) (PCL) while SPIONs were embedded into a multilayer shell of polyglutamic acid (PGA). The formation of the hybrid multilayer consisted of electrostatic interaction between polyelectrolyte PGA and magnetic NPs. The obtained NPs exhibited size below 200 nm, low polydispersity index, and surface modification to prolong blood circulation. Furthermore, they can be monitored by MRI and are capable of locally increasing temperature (local hyperthermia). In brief, the authors demonstrated the synthesis and properties of magnetically responsive NPs as an MRI-detectable drug delivery system.

Another combination of magnetic hyperthermia and MRI was recently reported by Sánchez-Cabezas, Montes-Robles, Gallo, Sancenón, and Martínez-Máñez (2019). Ultrasmall SPIONs were obtained by the one-step coprecipitation method under mild reaction conditions. The NPs were then coated with a highly packed monolayer of oleate molecules, providing long-term stability. These theranostic nanodevices exhibited significant T1/T2 dual signal enhancement and good heating efficiency in magnetic hyperthermia experiments.

14.5 Carbon-based nanotheranostics

Among the numerous types of nanomaterials developed in recent years, research on carbon-based systems which include fullerenes, CNTs, graphene

and its derivatives, graphene oxide (GO), nanodiamonds, and carbon quantum dots (CQDs) has become a booming area, especially for the development of nanotheranostics.

This group of materials attracts attention in the field of theragnosis mainly due to their unique optical properties that is, intrinsic fluorescence, tunable narrow emission spectrum, high photostability, and intrinsic theranostic properties (Patel, Singh, & Kim, 2019). Furthermore, they have other interesting aspects such as unique structural dimensions, high surface area, chemical stability, excellent mechanical, electrical, thermal, and chemical properties, and surface tunability which allows for optimizing their properties.

CQDs are clusters of carbon atoms with traces of nitrogen and considerable fractions of oxygen and hydrogen, whose diameters range from 2 to 10 nm (Boakye-Yiadom et al., 2019). They are biocompatible, nontoxic, photostable, can be easily functionalized, and possess good photoluminescence and water solubility. CQDs are distinguished from traditional metal-based semiconductor quantum dots because their precursors are more abundant, synthesis procedures are easier, and they involve lower costs. Moreover, they exhibit higher biocompatibility, strong photo-responsiveness, tunability, and stability (Hassan, Gomes, Dehghani, & Ardekani, 2018).

A functionalized CQD-based theranostic nanoplatform with microenvironment-driven cascaded responsiveness and imaging-tracking capability has been successfully developed for tumor-specific TRAIL gene precise delivery with enhanced therapeutic efficiency (Zhao et al., 2018). In this example, CQD cores were functionalized with PEI end-capped disulfide-bond-bearing hyperbranched poly(amido amine) (HPAP) which endows them with higher fluorescent quantum yield, glutathione triggered degradability, and efficient gene delivery capability. The shell was fabricated by modifying PEG-PEI copolymer with dimethyl maleic acid (DMMA) which could be reversed to positively charged in a mildly acidic tumor microenvironment via hydrolysis of DMMA, leading to a cleavable PEGylated shell. Briefly, the main properties of this nanoplatform are prolonged blood circulation due to PEGylated shielding, and effective accumulation at tumor tissues induced by the elevated EPR effect resulting from the microenvironment-driven cleavable PEGylated shell, and finally controlled gene release in tumor cell cytosol facilitated by glutathione-triggered HPAP degradability. TRAIL-mediated apoptosis, as well as excellent biocompatibility, were also demonstrated. Similarly, another CQD-based phototheranostic agent was developed, which displays both efficient fluorescences for bioimaging and PDT (Wen et al., 2019). PEGylation strategy was used to improve the water solubility of these CQDs. The hydrophilic CQDs assembly showed bright red fluorescence in the cytoplasm of 4T1 cells and exhibited high singlet oxygen ($^1O_2$) generation. After NIR laser irradiation, cells incubated with 250 mg/mL of CQD assembly exhibited approximately 95% of mortality rates. In vivo experiments in mice showed that the tumor was completely inhibited, indicating successful accumulation in tumor tissue and production of $^1O_2$ after irradiation.

Imaging-tracking capability via fluorescence and chemotherapy is another interesting combination. Theranostic nanogels for optical imaging-directed chemotherapy based on hyaluronic acid (HA) and CQDs were obtained by crosslinking the folate-terminated PEG-modified HA with CQDs (Jia et al., 2016). DOX was encapsulated via electrostatic interaction with HA. Therefore, nanogels could be used for real-time and noninvasive location tracking of cancer cells through fluorescence and DOX release in a weak acid environment, while DOX release was restricted in neutral media, demonstrating a controlled release performance that responds to the tumor microenvironment.

Graphene is a single or few-layered two-dimensional sp^2-bonded carbon sheet that is intensively employed in a variety of applications in the biomedical field (Chen, Huang, et al., 2013; Patel et al., 2019). Graphene can be economically obtained on a large scale from graphite (Chen, Huang, et al., 2013). Unlike pristine graphene, GO exhibits high water dispersibility and pH-dependent negative surface charge to maintain high colloidal stability (Patel et al., 2019). Regarding drug delivery applications, GO has a poly-aromatic surface structure like graphene that allows efficient loading of aromatic drug molecules via $\pi-\pi$ stacking. Thereby, many anticancer drugs with an aromatic structure such as DOX can be effectively loaded. Additionally, GO can release drug molecules upon stimuli such as NIR light, potentiating its use as a delivery carrier.

Moving on to the examples in theragnosis, fluorinated graphene oxide (FGO) is a good material to combine the spatial resolution of MRI, drug loading, and NIR hyperthermia (Razaghi et al., 2020). FGO was employed for simultaneous pH-sensitive release of Linoleic acid-curcumin conjugate (FGO-Lino-CUR), an anticancer formulation, and MRI agent. In vitro and in vivo studies revealed higher tumor suppression capability of FGO-Lino-CUR compared with free Lino-CUR. Besides, in vivo MRI in tumor-bearing BALB/c mice was demonstrated.

On the other hand, a combination of PDT and PTT against breast cancer was presented by using nanosized PEGylated GO coloaded with photosensitizer and a two-photon compound (Liu et al., 2020). The photoactivity of both compounds is quenched on the graphene sheet, whereas it is activated with the release of the drug in a physiological medium. Particularly, PTT and PDT were simultaneously stimulated in vivo by 980 nm laser which allows the high depth of light penetration and limits damage to normal tissue such as skin (Fig. 14.5). This theranostic formulation was shown to effectively accumulate at the tumor site due to its nanosized dimensions and induced large population of cancer cell apoptosis and considerable suppression of tumor growth. In vivo thermographic images demonstrated its diagnostic capacity.

Recently, a multifunctional nanotheranostic for in vivo highly efficient NIR fluorescence/PAI tumor-targeted PTT was reported (Jun et al., 2020). The formulation was based on FA conjugated with chitosan-functionalized GO (FA-CS-GO); FA allows efficient tumor targeting. CS-functionalized GO was formed by a strong interaction between CS and GO via hydrogen bonding and electrostatic interaction, whereas carboxyl groups of FA molecules were covalently bonded to

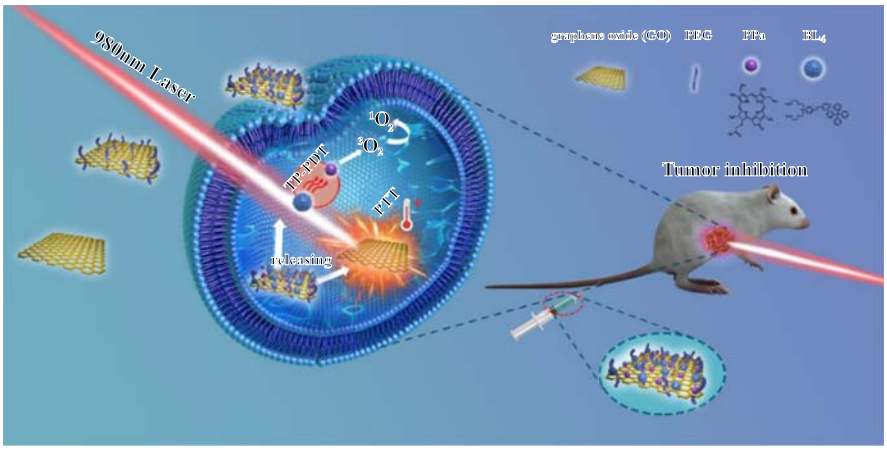

FIGURE 14.5

Schematic illustration showing the synthesis of GO-PEG to facilitate the combination of nanomaterial-mediated photodynamic and photothermal therapeutic destruction of tumors.

Reproduced with permission from Liu, J., Yuan, X., Deng, L., Yin, Z., Tian, X., Bhattacharyya, S., et al. (2020). Graphene oxide activated by 980 nm laser for cascading two-photon photodynamic therapy and photothermal therapy against breast cancer. Applied Materials Today, 20, *100665. https://doi.org/10.1016/j.apmt.2020.100665.*

free amino groups of CS. Favorably, in vivo experiments showed that in the presence of targeted FA-CS-GO with laser irradiation photothermal effect, tumors were completely inhibited. The detection of high PA signal in the tumor area of mice 24 h after the injection of FA-CS-GO indicated its ability as a PAI contrast agent.

Finally, CNTs are tubular structures made of graphene sheets rolled up into a cylinder with unique intrinsic properties (Negri, Pacheco-Torres, Calle, & López-Larrubia, 2020). They are classified according to the number of graphene layers present in the cylindrical tubes: single-wall carbon nanotubes (SWCNTs) and multiwall carbon nanotubes (MWCNTs). SWCNTs exhibit diameters ranging from 1 to 2 nm, whereas MWCNTs show increasing diameters ranging from 3 to 30 nm (Mohanta, Patnaik, Sood, & Das, 2019). Their length can reach several hundred micrometers or, in some cases, millimeters (Kinloch, Suhr, Lou, Young, & Ajayan, 2018).

The carbon atoms are bonded with sp^2 hybridization, which is stronger than the sp^3 bonds in diamond, providing this type of material with outstanding mechanical, electrical, optical, and thermal properties (Negri et al., 2020). However, the poor dispersibility of CNTs is one of the main drawbacks of their use in biomedical applications and makes the use of functionalization pathways essential to improve their biocompatibility (Patel et al., 2019).

To highlight some remarkable and recent examples of the research developments using CNTs in theragnosis, we present the versatile nanoplatform based on indocyanine green (ICG)-loaded FA-modified MWCNTs for targeted dual-mode fluorescence/PA imaging, and in vivo tumor PTT/PDT (Hu et al., 2018). Firstly, regarding the synthesis protocol, the acid-treated MWCNTs with carboxyl residues were first covalently conjugated with PEI. The product was then functionalized with PEGylated FA, fluorescein dye isothiocyanate, and succinic anhydride. Advantageously, these nanoplatforms possess exceptional colloidal stability, good hemocompatibility/cytocompatibility in a studied concentration range, and high affinity for cancer cells that overexpress the FA receptor, that is targeting specificity. The ICG loaded via π-π stacking interactions displays good photostability and can be used for fluorescence imaging/PDT of tumors because ICG is able to generate $^1O_2$ after exposed to an NIR laser. Moreover, the NIR absorption character of ICG also gives the ability for simultaneous PA imaging and PTT of tumors. Concerning stimuli-responsive behavior, the release of ICG from the ICG-loaded MWCNTs is pH-dependent due to the pH-dependent π-π stacking interaction. On the other hand, agglomerated SWCNTs were shown to be promising theranostic agents that enable photoactivated "cold" destruction of cancer cells by picosecond laser irradiation keeping their environment alive (Golubewa et al., 2020). Authors developed a theoretical model which allows them to recognize photothermal, PA, and photothermoacoustic regimes of cancer cell destruction. Consequently, the destruction of cancer cells is produced by a mechanical effect (due to the PA effect) rather than photothermal. Simultaneous fast cancer cell visualization was performed by coherent anti-Stokes Raman spectroscopic (CARS) imaging technique with the same laser source. Therefore, CARS allows the visualization of the cancer cell and its photo-induced SWCNT-conditioned destruction. In this example, it is worth noting that only cells that have accumulated SWCNTs as agglomerates are destroyed because individual nanotubes in the extracellular environment are photoacoustically inactive.

14.6 Multicomponent nanotheranostics

Throughout this chapter, the examples shown demonstrated that the feature of "multifunctionality" (including targeting, imaging (diagnosis), and therapy) is crucial in the design and development of efficient theranostic agents. Nevertheless, from all the above, it is also possible to conclude that most types of nanomaterials exhibit some special properties that make them suitable for either therapeutic or diagnostic applications, being less common than a single nanomaterial can be used for both applications simultaneously. Consequently, the development of efficient nanotheranostics demands the integration of different materials in a single nanosystem. Achieving multifunctional devices confined within such a small size

range is a challenging task in the development of theranostic nanomedicine (Gharatape & Salehi, 2017).

From a synthetic point of view, we refer to these nanosystems as nanocomposites, hybrid nanomaterials, or multicomponent nanotheranostics. To perform the integration of different materials to produce devices for biomedical applications, it is essential to achieve suitable interactions between components. In other words, it is imperative to attain significant chemical or physical affinity (e.g., electrostatic, hydrophobic, etc.) (Chen et al., 2018). Furthermore, the rational planning of a theranostic nanoplatform should be done considering structure/properties relationships by carefully choosing the materials and combining them into an appropriate design, hierarchical architecture, and morphology. Only in this way, it is feasible to integrate multiple diagnostic/therapeutic modalities into one platform for the next generation of personalized nanomedicines. Finally, it is important to note that the multicomponent nature provides theranostics with resulting physicochemical properties that are at least an additive combination of the properties of the individual constituents separately but, even a synergic addition of individual properties can take place resulting in outstanding formulations.

In this section, we describe some combinations of materials commonly used by the theranostic research community. In the text, we list the main features of some types of multicomponent nanotheranostics while, in Table 14.2, some recent efforts in this area are summarized.

First, one type of multicomponent nanomaterials typically employed as nanotheranostics is core/silica shell structures (Chen et al., 2018). In this case, cores made of gold, carbon, and iron oxide NPs are coated by growing a layer of silica on their surfaces. This design consisting of a silica shell provides many advantageous features to the final structure that are listed below:

1. Easy functionalization of NPs surface.
2. Amplified PA effect.
3. Reservoir for drugs, dye molecules, or other imaging agents.
4. Enhanced colloidal and chemical stability of the core.
5. Decreased cytotoxicity of the core.

The combination of magnetic materials and silica, mostly mesoporous silica, can integrate both diagnostic and therapeutic methods into one single system for better cancer theranostics. Typically, SPIONs are coated with mesoporous silica material or embedded in a mesoporous silica matrix.

The inclusion of silica shells into AuNPs is another commonly used strategy for the development of theranostics. In this combined platform, AuNPs can efficiently convert the NIR light to heat and induce a photothermal effect, since the silica shell does not compromise their photothermal therapeutic ability (Feng, Panwar, Jian, Tng, & Tjin, 2016). As we mentioned before, the silica coating can also enhance the colloidal stability of AuNPs, as well as allow the encapsulation of drugs, dye molecules, or other imaging agents either via physical adsorption or covalent attachment (Moreira, Rodrigues, Reis, Costa, & Correia, 2018). Hence,

Table 14.2 Structural features and properties of different multicomponent nanotheranostics.

Type	Structural features	Properties	Additional notes	References
Magnetite + mesoporous silica	SPIONs embedded in mesoporous silica matrix coated with thermosensitive poly(NIPAM-*co*-hydroxymethyl acrylamide)	Hyperthermia and chemotherapy triggered by alternating magnetic field application	The presence of magnetic cores combines the stimuli-responsive capacity with imaging applications	Guisasola et al. (2015, 2018)
	Core-shell magnetic MSNs modified with P(NIPAM)	Triple-responsive DOX release: magnetic, reductive, thermoresponsive	Further research can be extended to MRI	Hegazy et al. (2017)
	Electrostatic assembly of AuNRs and silica-coated iron oxide NPs	Photothermal ablation of tumor tissue and magnetic guidance of the nanocomposites	Accumulation via external magnetic field solves inefficient tissue heating issues	Redolfi Riva et al. (2017)
Magnetite + zeolite	Magnetic zeolite nanocomposite	T2-MRI contrast enhancers in MRI	The encapsulation properties of zeolite and T2-MRI enhancement suggests its theranostic properties	Vilaça et al. (2019)
Gold + mesoporous silica	Gold nanostar core and a DOX-loaded mesoporous silica shell coated with FA-modified thermosensitively supported lipid bilayer as a gatekeeper	Targeted chemo – photothermal synergistic therapy and PA/CT imaging of tumors	Targeting to overexpressed FA receptors on tumor cells, PAI and CT, and NIR activated controlled release (photothermal effect of Au) were achieved	An et al. (2017)
	Dual-stimuli responsive (pH and NIR light irradiation) mesoporous silica-coated AuNRs, Indocyanine Green (ICG), and 5-fluorouracil (5-FU)	Multimodal imaging guided synergistic therapy. NIR light irradiation and acidic environment accelerated the 5-FU release. Upon light irradiation, heat generation and $^1O_2$ production were induced	The cancer theranostic capability was evaluated both in vitro and in vivo	Fang et al. (2017)
Magnetite + plasmonic materials	PEI-modified MNPs (inner cores) with gold shells	Remarkable biostability, rapid cellular uptake, and excellent in vitro and in vivo antitumor activity. NIR PTT, magnetic tumor-targeted therapy, and accurate dual-mode imaging (MRI/X-ray)	Epigallocatechin gallate was used as both an anticancer drug and as reducing agent for the construction of core–shell NPs	Yin et al. (2017)

Magnetite + carbon materials	SPIONs/CNTs nanoplatform: CNTs modified with dendrimer with PEG liner and four phenylboronic acid and coloaded with porphyrin and SPIONs	Significant T2 relaxation rate (MRI), synergetic PTT, binding ability on boronic acid group with sialic acid overexpressed on tumors (targeting ability)	This system could selectively identify solid tumors and micro-sized metastatic tumor (1 mm) in the liver	Lu et al. (2020)
	Precipitation of iron oxide NPs on GO nanosheets	MRI-guided chemo-PTT therapy for cancer: loading of DOX and in vivo T1/T2 MRI	Good remote photothermal effect, which can damage the dense shell of solid tumor tissue, facilitating the delivery of the drug into tumor cells	Li et al. (2020)
	Magnetic graphene nanosheets functionalized with chitosan	Magnetic behavior and pH-sensitiveness and biocompatibility of chitosan	GO decreased the contrast efficacy of the MNPs, but chitosan grafting enhanced the contrast efficacy	Baktash et al. (2021)
Inorganic NPs + smart polymers	MSNs as cores, gel network of N,N-dimethylacrylamide and a redox responsive crosslinker with disulfide groups	Chemotherapy and US imaging: DOX loaded within nanoshells and perfluorohexane loaded within MSNs mesoporous channels for US imaging	Redox stimuli-responsive degradation of gel shells produces an increased DOX release and enhanced US imaging	Qiao et al. (2016)
	MNPs were used as cross-linkers in the polymerization of oligo ethylene glycol monomers resulting in magnetic hybrid thermoresponsive nanogels	DOX release profile could be controlled by either pH or external triggering by NIR light. Magnetic nanogels demonstrated their properties to be used as PTT and MRI agents	In vivo NIR triggered chemotherapeutic effect produces tumor volume reduction at mid and long-term	Biglione et al. (2020)

chemotherapy, PTT, and multimodal imaging integrate into a single system simultaneously.

Other highlighted nanocomposites are the so-called magneto-plasmonic nanomaterials. Particularly, the plasmonic properties can be controlled by an external magnetic field (Armelles, Cebollada, García-Martín, & González, 2013). Indeed, this modulation of the optical signal can be used to significantly increase the imaging contrast of cancer cells (Armelles et al., 2013; Wu, Cook, Emelianov, & Sokolov, 2014). In Table 14.2, some recent examples of nanotheranostics based on AuNPs and magnetic materials are exposed.

The combination of SPIONs and carbon-based nanostructures such as CNTs or GO stands out for their use in biomedical MRI applications. The comparison in MRI performance between SPIONs and this type of composites is not fully examined, but some studies indicate that these conjugates are an effective strategy to enhance the relaxation rate for detection (Lu et al., 2020).

In the development of the next generation of nanotheranostics, polymeric materials play a very important role given their advantageous properties such as their great versatility, biocompatibility, and affordable costs. Moreover, the look towards a more promising future is focused on the use of intelligent or smart polymers because they can respond to different stimuli. These polymers exhibit a nonlinear response to a specific stimulus that could range from swelling/shrinkage to disintegration (Bedoya, Figueroa, Macchione, & Strumia, 2020). Moreover, the wide polymer sources and versatile synthesis strategies available allow the sensitivity of the polymeric material to be adjusted to the desired stimulus within a narrow range. This enables more precise and programmable drug delivery. It is important to note that the selection of the polymer that will have an intelligent behavior depends on several factors, such as (1) the ability of the components to form stable colloidal systems in biological fluids; (2) the requirements for adequate functionality of the monomers (charges, hydrophilicity, presence of functional groups); (3) quick and reversible responses.

Beyond the promising properties of this type of material, such as their versatility, safety, and others that have already been described, they have the advantage that they can be combined with other materials, such as inorganic NPs (Baktash, Zarrabi, Avazverdi, & Reis, 2021; Vijayan, Beeran, Shenoy, Muthu, & Thomas, 2019), or synthesized with different comonomers to confer the final structure with dual or multiple responses (Wang, Ju, et al., 2019). Therefore, these smart nanosystems offer the possibility of designing personalized or patient-based therapies, which could represent an especially important advance in the treatment of diseases.

In this way, starting from the advantages of smart polymers, there is a special interest in the integration of stimuli-responsive polymers into smart nanotheranostics since these systems allow the administration of the drugs specified in the appropriate site, giving special protection to the drug in circulation, and allow to fulfill the function of diagnosis and monitoring. Success in achieving this specificity is crucial for the efficiency of the nanotheranostics.

One strategy is the combination of inorganic MSNs with smart polymers to achieve the encapsulation of different cargos. An example is hydrophobic imaging agents within MSNs mesoporous channels and chemotherapeutic drugs in the polymeric matrix. Additionally, MNPs conjugated with thermoresponsive polymers can be used for guided therapy, MRI, and triggered release of encapsulated cargoes. Indeed, magnetic heating can induce conformational changes (swelling/collapse) in the polymer that produce drug release (Louguet et al., 2012).

14.7 Conclusion and perspectives

The advanced knowledge in nanotechnology has offered a great opportunity to encourage the design and development of innovative nanotheranostics. The improvements found in these theranostic platforms are increasingly promising, which could allow their application in specific medicine and biomedical research areas soon.

Throughout this chapter, we have highlighted the prominence of stimuli-responsive nanotheranostics as fascinating platforms for medical applications. Particularly, when their design allows achieving inherent properties that respond to chemical/physical stimuli, giving rise to a "smart" behavior, the result is more attractive for clinical translation.

The reported examples demonstrated that the development of efficient nanotheranostics demands the integration of different materials in a single nanosystem. However, regarding the multicomponent nature of theranostics, we must be clear in saying that greater complexity or sophistication in their synthesis, not only hinders their production, but also increases the polydispersity of the systems, raises costs, and complicates evaluation and regulatory approval. In addition, there will be a greater probability of having negative biological results considering the multiple demands to which they are exposed.

Consequently, toxicity (i.e., cytotoxicity, genotoxicity, and immunotoxicity) is one of the current concerns in the development of nanotheranostics, since not much is yet known about how nanostructured entities behave in human systems. Certainly, more in vivo experiments will be required to fully investigate the safety and efficacy of these new theranostic platforms before clinical application. In this sense, new suitable test methodologies are demanded to evaluate their performance in vivo.

Currently, the scientific community is tirelessly working to develop new formulations of nanotheranostics, solve the previously mentioned problems, and analyze the information collected, which will allow to short the gap for their prompt application in patients, offering high social impact. Moreover, multidisciplinary cooperation is completely beneficial in this advancement process. Additionally, considerable advances already achieved in nanotheranostics applied to cancer therapy will make possible their applications in other fields such as cardiology,

tissue engineering, and microbiology. This exciting new field of nanotheranostics has a bright future ahead, offering the unique capability of more personalized and predictive medical treatment with simultaneous monitoring of several disorders and diseases.

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CHAPTER

15 Nanoconjugates and nanoconjugate formulations for improving drug delivery and therapeutic efficacy

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15.1 Introduction

There is still a crescent interest in the discovery, development, and study of new drugs possessing suitable characteristics to be applied in the biomedical field. It is, however, common to find some drawbacks in the researched drugs such as their toxicity, adverse effects, and low solubility, which limit their application in the clinical context. More than half of the drugs currently available in the pharmaceutical market present poor water solubility or ineffective targeting delivery (Tran & Tran, 2017). In this sense, scientists have investigated several approaches to increase the therapeutic outcomes mediated by the delivery of poorly water-soluble drugs. These include the design and development of emulsions and dispersions or the conception of nanoparticles (Maincet & Williams, 2018; Tuong, Van-Thanh, Wei, Phuong, & Tran, 2017). Nanotechnology has grown immensely in the past decade and this field of research has greatly contributed to improvements concerning drug solubility and bioavailability, leading to increased therapeutic efficacy (Costa, Valente, Queiroz, & Sousa, 2018). Innovative strategies have been applied to drug delivery systems to promote drug loading, stimuli responsiveness, or specific targeting (Costa et al., 2018; Mi, 2020). In this way, relevant signs of progress have been reported in the development of drug-based vectors that can be biocompatible, able to target cancer cells, or pH-sensitive (Costa et al., 2018; Men et al., 2020; Thagipour-Sabzevar, Sharifi, & Moghaddam, 2019).

Since 1960, a strategy using hydrophilic polymers in solid dispersion has been explored to increase both drug solubility and dissolution. However, with the crescent evolution of nanotechnology, this procedure has been overtaken by hydrophilic-hydrophobic polymer nanoconjugates and hydrophobic drug-hydrophilic polymer nanoconjugates as a convenient way of drug loading into nanoparticles (Hoshikawa et al., 2018; Kopeček, 2013). Nanoconjugation has been revealed to be a suitable method to reduce the size of drug-based systems, and increase drug dissolution and

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solubility while enhancing bioavailability and, consequently, increasing therapeutic outcomes. Despite this, conventional conjugations involving hydrophilic and hydrophobic molecules may present some disadvantages such as the large particle size or short half-life, with clear and direct repercussions on treatment efficacy. In line with this, researchers have devoted full attention to novel conjugation strategies for nanoparticulate vectors, providing advanced methods for higher performance and opening new routes for specific drug applications in the biomedical area.

15.1.1 Nanoconjugation

Common nanoconjugation involves polymeric amphiphiles that display a tendency to self-assemble in the aqueous environment, resulting in the formation of nanoparticles with the hydrophobic segments positioned in the inner core and the hydrophilic ones directed to the aqueous environment (Salimi, Zadeh, & Kazemi, 2019; Tran, Tran, & Vo, 2014). The nanoconjugates induced a size reduction in the particles and promoted drug dissolution and solubility. Amphiphilic polymers have found application mostly in cancer therapy due to a favorable accumulation of the loaded drug into the tumor region. In addition, the resulting nanoparticles may increase the drug's half-life in blood circulation; also the functionalization of their surfaces contributes to specific tumor targeting (Abramović, Mandić, Savić-Radojević, & Simić, 2020; Wan, Li, & Pan, 2020). To conceive an amphiphilic polymer from the attachment of hydrophobic and hydrophilic segments, researchers have deeply explored several strategies. For the development of an amphiphilic polymer, hydrophobic interactions, electrostatic ones, hydrogen bonding or host-guest interaction have all been the subject of study (Cheng et al., 2020; Tu et al., 2011). The conceived amphiphilic polymer can thus self-assemble, in an aqueous environment, into nanoparticles from interactions between the hydrophobic segments. Following this, the hydrophobic segments play a crucial role in determining the properties of the formed nanoconjugates such as drug-loading efficiency and stability (Hussein & Youssry, 2018). Drugs exhibiting low water solubility can significantly benefit from their loading into hydrophobic moieties as this procedure can enhance their solubility and circulation time and, even, promote cancer cell targeting.

The construction of self-assembled polymer nanomaterials is a topic of intense research and has been focused on different polymer types, spacers, ratios, and self-assembly mechanisms giving rise to a broad variety of developed polymer systems with astonishing applications (Araste et al., 2021; Eskandari, Guerin, Toth, & Stephenson, 2017; Pan, Chen, Metavarayuth, Su, & Wang, 2018). Peptides and proteins have been investigated and a wide range of self-assembly methods have been tested such as the layer-by-layer (LbL) assembly technique, spin coating technique, deposition method including hybridization and crosslinking, and grafted polymer surface modification approach (Ata, Banerjee, & Singha, 2016; Fleury et al., 2019; Kobayashi & Arai, 2017). LbL technique is a simple and versatile procedure for the conception of polymer nanocomposites providing a tool for the production of

new nanomaterials with tailored composition and structure, having utility in several biomedical applications (Amasya, Bakar-Ates, Wintgens, & Amiel, 2021; Campbell & Vikulina, 2020; Xuan et al., 2017). The polymer grafting surface technique was revealed to be a suitable procedure to disperse nanoparticles in a polymer. Radical polymerization methods, such as atom transfer radical polymerization (ATRP), allow the creation of materials with end-group functionality. Following this, surface-initiated ATRP from a solid surface provides a way for the preparation of nanocomposite materials being particularly adequate for the conception of polymer-based ones (Lathwal et al., 2021; Rodrigues & Vieira, 2019). This method offers control over the properties of these systems allowing them to tailor and fine-tune the functionalities displayed by the formed polymer materials. Scientific literature provides several examples of the application of surface-initiated ATRP for a diverse range of nanomaterials, including clay, silica, magnesium hydroxide, or gold (Han et al., 2020; Mao, Lee, Shin, & Yoo, 2020; Vo et al., 2016). Fig. 15.1 illustrates the LbL assembly technique and the grafted polymer surface modification strategy in the conception of polymer nanomaterials.

15.1.1.1 Clay

Clay nanomaterials have been developed by the LbL technique and their properties have been researched for numerous biotechnological applications. Ziminska et al. designed and created a foam coated with clay and polyethyleneimine (PEI) which provides the route for tailored/controlled properties (Ziminska, Dunne, & Hamilton, 2016). Other authors studied the effect of nanoclay orientation on the properties of nanocomposite-coated films. In this work, multilayer coatings with hydrogen bonding interactions were deposited, by LbL technique, on a substrate with alternate deposition of a layer constituted by nanoclay and different polymer

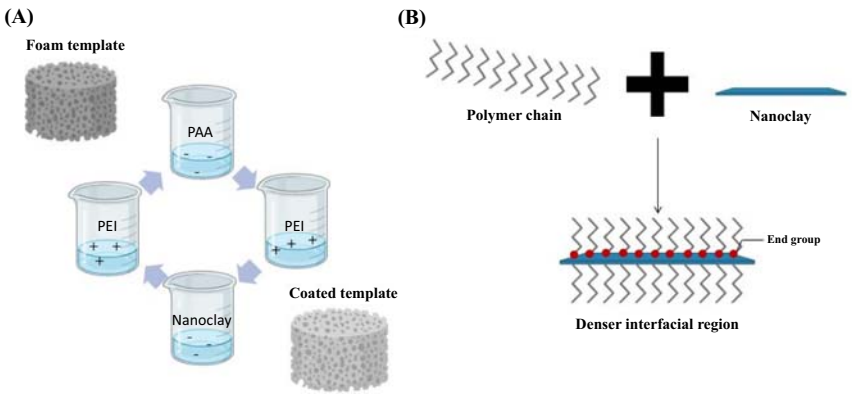

FIGURE 15.1

The layer-by-layer assembly technique (A) and the grafted polymer surface modification strategy (B) in the conception of polymer nanomaterials.

layers, such as chitosan, polyvinyl alcohol or polyvinylpyrrolidone (Dhieb, Dil, Tabatabaei, Mighri, & Ajji, 2019). They found that the dispersion level and the orientation of clay nanoparticles vary with both the molecular structure of polymers and their interactions with the nanoclay (Dhieb et al., 2019). Polymer-clay nanocomposites of vinyl monomers have been created by the surface-initiated ATRP technique. The grafted polymer nanomaterials were characterized using several techniques, for instance, by X-ray diffraction and differential scanning calorimetry. The majority of the developed nanocomposites presented semiexfoliated to exfoliated structures (Amin, Sarkar, Moorefield, & Newkome, 2013). Vo et al. reported on in situ photoinduced surface-initiated ATRP for the preparation of nanoclay fillers displaying a clickable tool. They promoted the growth of poly (propargyl methacrylate) into the clay interlayer after silanization and grafting of bromine ATRP initiator (Vo et al., 2016).

15.1.1.2 Silica

Silica nanoparticles have been prepared by the LbL assembly method and revealed to be useful in the release of anticancer drugs, namely doxorubicin (DOX) (Li et al., 2014). The coating process involved bis-aminated poly(glycerol methacrylate)s (BA-PGOHMAs) and cucurbit[7]uril (CB[7]), with CB[7] operating as a molecular bridge between two different polymeric layers associated by host-guest interactions. The developed system has been designed to release DOX in acidic conditions or by the addition of adamantaneamine hydrochloride (Li et al., 2014). Su et al. conceived high chroma-colored silica-based nanoparticles (SiNPs) by the LbL technique (Su, Zhao, & Dou, 2020). Poly(sodium-p-styrenesulfonate) and Poly(ethylene terephthalate) were employed as polyanion and polycationic electrolytes, respectively, and silica nanoparticles were considered as the matrix. Poly(ethylene terephthalate) and a dye were electrostatically adsorbed on the surface of SiNPs and the film-coated high chroma SiNPs were developed by repetition of this procedure. Parameters such as the concentration of polyelectrolyte, salt (NaCl), amount of dye, and deposition time on the dye coupling rate were investigated (Su et al., 2020). Alswieleh et al. synthesized 2-(tert-butylamino)ethyl methacrylate-b-poly(ethylene glycol) methyl ether methacrylate diblock copolymer, which subsequently has been grafted onto mesoporous SiNPs by ATRP (Alswieleh et al., 2020). Following this method, the authors achieved a high polymer surface grafting density. The pH sensitivity of the produced materials was explored to investigate the performance of the vectors to release loaded doxycycline (Alswieleh et al., 2020).

15.1.1.3 Magnesium hydroxide

Magnesium hydroxide materials have been prepared by surface-initiated ATRP and this approach was revealed to be convenient to optimize their properties. Zhang et al. prepared polystyrene grafted magnesium hydroxide nanoparticles by two different procedures (Zhang, Lei, Su, & Zhang, 2008). A study of the characteristics displayed by the $Mg(OH)_{2\text{-based}}$ biomaterial was adequately

characterized and revealed that the graft polymerizations are consistent with the properties of tunable/"living" polymerizations. Moreover, it was found that the grafting polymerization of polystyrene onto the surface of $Mg(OH)_2$ nanoparticles improved the dispersibility of these vectors in organic solvents which may broaden their applications in several areas (Zhang et al., 2008). Liu et al. enhanced the compatibility of magnesium hydroxide particles by testing two different strategies for the immobilization of the ATRP initiator on the particles' surface. The method of introducing more hydroxyl groups via ATRP of 2-hydroxyethyl methacrylate on the surface increased the initiator density (Liu, Feng, Chang, & Kang, 2012). The percentage of grafting on $Mg(OH)_2$ particles was close to 120% and the initial decomposition temperature of the prepared $Mg(OH)_2$ material is much lower than the one corresponding to magnesium hydroxide (Liu et al., 2012).

15.1.1.4 Gold

LbL assembly has been used to generate gold-based multilayer materials and coatings at the nanoscale. Several researchers have followed this method to conceive gold nanomaterials to operate therapeutically. For example, Labala et al. focused their work on LbL polymer-coated gold nanosystems as a delivery system for the topical iontophoretic release of imatinin mesylate. The gold nanoparticles were formulated by the Turkevich approach and were functionalized with polyvinylpyrrolidone and polyethyleneimine. The particles were then coated with poly (styrenesulfonate) and polyethyleneimine and loaded with IM (Labala, Mandapalli, Kurumaddali, & Venuganti, 2015). O'Neal et al. were pioneers in revealing the spray-assisted LbL assembly of hydrogen-bonding polymer nanocomposites of poly(ethylene oxide) and poly(methacrylic acid) (PMAA) containing discrete regions of AuNPs vertically positioned throughout the film structure. They reported that the AuNP regions are separated by "empty" regions of polymer with no drift or aggregation of AuNPs (O'Neal, Bolen, Dai, & Lutkenhaus, 2017). Other scientists prepared LbL films of graphene-gold nanoparticles (Gr-AuNPs) and molecularly imprinted polymers modified glassy carbon electrodefor electrochemical detection of trans-resveratrol (Yang et al., 2021). To create an electrochemical sensor suitable for trans-resveratrol, films of Gr-AuNPs were developed step by step via in situ and controllable electrodeposition and polymerization processes (Yang et al., 2021). Wei et al. prepared nanosized near-infrared nanohybrids by surface-initiated ATRP of *N*-isopropylacrylamide on gold nanorods (Wei, Ji, & Shen, 2008). The novel materials demonstrate to possess a defined core/shell structure. Moreover, norvancomycin has been loaded into the nanohybrids and a release study demonstrated that the drug release rate can be tailored by near-infrared irradiation (Wei et al., 2008). Mao et al. reported on surface-initiated ATRP atom transfer radical as a suitable method to grow a dense polymer layer on a gold surface. This strategy has been followed to promote the release of anticancer agents from AuNPs as they are bioinert and biocompatible (Mao et al., 2020). In this study, AuNPs were polymerized with sulfoethyl

methacrylate to provide an anionic corona to the particles, and PEI and siRNA were then layered onto this corona of AuNPs by electrostatic interactions (Mao et al., 2020).

15.2 Drug delivery

The development of nanoconjugates and nanoconjugate formulations as drug delivery systems has been a subject attracting great attention in many research areas, ranging from the biomaterials field to nanomedicine. This fact paves the way for a new opportunity for biologists, chemists, and material scientists to gather knowledge and create original and innovative drug-based vectors as an appropriate answer to current biomedical demands. This perspective marks a profound departure from the synthesis of conventional drug delivery systems only focused on the ability of a certain nanocarrier for drug loading/encapsulation. In addition, it highlights the need for the conception of advanced and high-performance vectors exhibiting, for instance, the capacity to respond to external stimuli, convert external signals into desired effects or provide synergistic profiles for combinatorial therapies. In this regard, a powerful tool can be developed for many biomedical applications and, particularly, for the most serious and deadly diseases.

15.2.1 Strategies to improve drug solubility

Nanoconjugates and nanoconjugate formulations have been designed and prepared, among other goals, to improve drug solubility. The interest relies on the fact that this parameter can greatly affect the properties displayed by the drug delivery system with repercussions on toxicity and therapeutic efficacy. Nanoconjugates are developed to present small sizes and morphologies adequate to favor their cellular uptake, transport/diffusion, and, ultimately, therapeutic action. However, the low size of nanoconjugates may pose a relevant drawback concerning drug loading and solubility, as a significant reduction in the dimensions of the nanosystems may decrease their encapsulation efficiency (EE). Therefore, researchers worldwide have addressed this problem and interesting solutions have been presented (Alshehri et al., 2020; Kumar, Dalvi, & Siril, 2020; Larson & Ghandehari, 2012; Tran & Tran, 2019). Zhang and coworkers developed an X-shaped four-armed Gemini-like pegylated distearylglycerol bearing two hydrophilic polyethylene glycol (PEG) heads and two hydrophobic stearic acid tails as a micellar vector for DOX delivery (Zhang et al., 2015). The prepared nanomicelles present a DOX EE close to 95% and revealed stability in the blood circulation. Moreover, the DOX release was pH-dependent (Zhang et al., 2015). Another team focused on a micelle self-assembled DOX-arabinoxylan (AX) conjugate as a tool to enhance DOX solubility and promote its controlled release (Wang et al., 2017). The solubility of DOX is increased due to the formed

hydrogen bond with AX which originates the amphiphilic DOX-AX. Self-assembly of AX-DOX with DOX core and AX shell led to the formation of micelles displaying a pH-cleavable bond. Following this, at low pH, the drug can be easily released from the nanomicelles (Wang et al., 2017). Liu et al. formulated a self-assembled pectin-eight-arm PEG-drug nanoconjugate for anticancer drug loading and release (Liu, Liu, et al., 2018). The prepared delivery system exhibited a size below 100 nm and demonstrated high drug EE, with quite low toxicity. The presence of the eight-arm PEG offer control over the ability for drug loading/encapsulation (Liu, Liu, et al., 2018). Gu et al. conceived nanocarriers by conjugating DOX to PEG polymers of various molecular weights (5, 10, 20, and 40 kDa) and architectures (linear, four-arm, and eight-arm), in an attempt to increase drug solubility while reducing cytotoxicity (Gu et al., 2018). The authors found that, in comparison with free DOX administration, DOX-polymer vectors induced significantly less toxicity and were revealed to be promising for sustained drug release (Gu et al., 2018). Another group of scientists focused on the encapsulation of cytarabine (Ara-C), an anticancer drug commonly used in the treatment of acute myeloblastic leukemia (Liu, Zhang, Zhang, Wang, & Luan, 2018). To overcome its main disadvantages of low lipophilicity and fast plasma degradation, they synthesized a novel Ara-C prodrug DTA-Ara through the conjugation of 2-decyltetradecanoic acid (DTA) with 24 carbons with Ara-C. The intrinsic ability of DTA-Ara molecules to self-assemble was explored to prepare nanoparticles presenting high drug loading. The DTA-Ara nanosystems had average sizes below 150 nm and negative surface charges. Additionally, these vectors were stable in deionized water or phosphate buffer solution for, at least, 8 days, and the hemolysis rate was found to be $<10\%$, therefore, demonstrating that the studied nanoparticles could be administrated intravenously (Liu, Zhang, et al., 2018). To overcome the poor solubility of the anticancer drug paclitaxel (PTX), Xu et al. designed and created a simple conjugation method involving this drug and succinic acid, which can be self-assembled into fibers at the nanoscale (Xu et al., 2017). Following this approach, the authors were able to efficiently load and encapsulate PTX, with a loading content near 90%. Furthermore, they demonstrated that the developed delivery system can be easily uptake by A549 cells. More recently, Liu et al. conceived stable polymeric nanoparticles with exceptionally high drug loading by an innovative precipitation method (Liu et al., 2020). A simple and sequential precipitation procedure is employed to generate stable drug-core polymer-shell nanosystems exhibiting high drug-loading capacity. The developed method includes the use of a solvent system, constituted of multiple organic solvents, to help control and tune the precipitation time of drugs and polymers. This original technology enabled the preparation of drug-based nanoparticles followed by immediate precipitation of one or two polymers. These researchers found that this approach provided a novel tool for the conception of polymeric nanoparticles possessing high drug loading, while, offering stability and tailored release (Liu et al., 2020). Another interesting strategy includes the formation of nanocrystals for insoluble drug loading (Zhang, Li, et al., 2020). The

poor solubility of the sedative drug Midazolam (MDZ) limits its clinical application. The loading of MDZ into nanocrystals proved to be a convenient solution to improve its solubility and bioavailability. The formed nanovectors present a size of around 285 nm and it was verified that the crystalline state of MDZ remains unaltered in the size reduction process. Moreover, this study suggested that the nanocrystals deeply influenced the pharmacokinetic properties and the neuroprotective effects of MDZ, and thus, enhanced their efficacy in vitro and in vivo (Zhang, Li, et al., 2020).

15.2.2 Responsive drug release

The development of responsive drug delivery systems (pH-, redox-, light or magnetic-field-responsive) has gained special attention in the last decades due to their crescent applicability in the biomedical/nanomedicine field (Fig. 15.2) (Deirram, Zhang, Kermaniyan, Johnston, & Such, 2019; Guo et al., 2018; Liu, Du, et al., 2018; Mura, Nicolas, & Couvreur, 2013; Wu et al., 2019). The intense research on this topic gave rise to relevant findings and progresses. For instance, Liu et al. prepared redox/enzyme-responsive chondroitin sulfate-ss-deoxycholic acid conjugates using cystamine as the linkage, due to its self-assembly property, to develop self-assembled nanoparticles in an aqueous environment (Liu, Du, et al., 2018). The drug docetaxel (DTX) has been loaded, to a great extent, into the produced nanosystems. The nanoparticles were found to be sensitive to both hyaluronidase-1 and glutathione, and authors took advantage of this dual-sensitive property to enhance drug delivery (Liu, Du, et al., 2018). A relevant review on redox-responsive drug delivery systems highlighted the link between nanotechnology and the

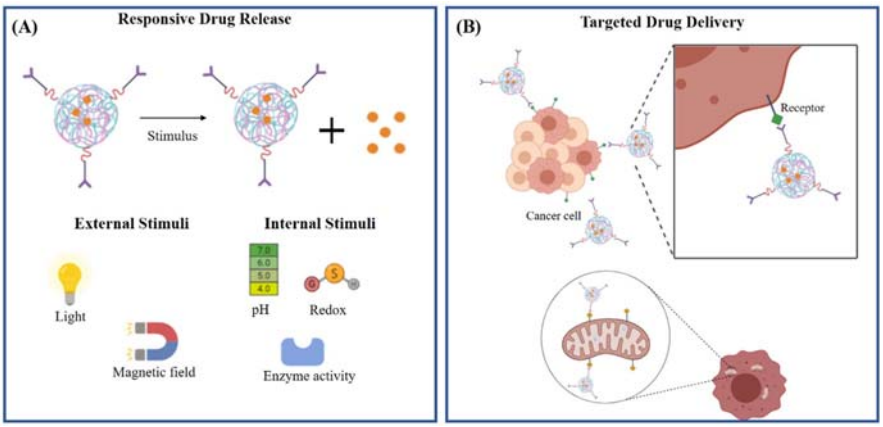

FIGURE 15.2

Illustration of the development of responsive (A) and targeted (B) drug delivery systems for improved therapeutic efficacy.

development of advanced drug-based vectors in the improvement of practical applications in biomedicine (Fig. 15.2A) (Guo et al., 2018). The publication showed that redox-responsive nanoconjugates can respond to a high content of intracellular glutathione and deliver loaded payloads, exclusively when they reach specific and/or targeted cells. Furthermore, these stimuli-responsive carriers are commonly employed to increase the concentration of the drug in targeted cells, therefore enhancing the efficacy of therapeutic action and reducing cytotoxicity and side effects of primary drugs (Guo et al., 2018). Duo et al. successfully prepared a degradable pH and redox dual-responsive nanosystem for drug delivery purposes (Du, Choi, Liu, Feng, & Thang, 2019). To accomplish this, the drug PTX was modified into a polymerizable monomer and thereafter it was copolymerized with pH-sensitive monomers and redox-sensitive disulfide-based cyclic monomers. These scientists found that the formed diblock copolymer can self-assemble giving rise to vesicles where hydrophobic PTX can be incorporated. The created nanoparticles are pH-sensitive, and by decreasing this parameter the size of the vesicles significantly varied. Moreover, by adding reducing agents, the authors were able to promote the disassembly of the complexes and, therefore, release the drug (Du et al., 2019). Other teams of researchers developed redox-responsive drug delivery vectors by binding the anticarbonic anhydrase IX antibody (A-CAIX Ab) on the surface of mesoporous SiNPs via disulfide linkages (Chen et al., 2020). The procedure for the preparation of these nanoparticles involved the synthesis and surface functionalization with thiol groups, 2,2′-dipyridyl disulfide, and CAIX antibody. DOX was loaded into these nanocarriers and the redox property was explored to promote drug release in the presence of glutathione owing to the cleavage of the disulfide bond. The authors demonstrated that the conceived DOX-based system was internalized into mouse breast cancer cells by receptor mediation (Chen et al., 2020). Andrgie and coworkers created a redox-responsive heparin−chlorambucil conjugate polymeric prodrug delivery system to overcome the limitations encountered by a single drug administration (Andrgie et al., 2020). In this way, redox-responsive disulfide bond-containing amphiphilic heparin−chlorambucil nanoconjugates loading prodrugs were designed and prepared to improve the anticancer effect of chlorambucil. From the self-assembly of the prodrug, spherical vesicles with grafted chlorambucil were obtained. Moreover, this team showed that prodrugs can be internalized into cervical cancer cells, and this cellular uptake increased with time (Andrgie et al., 2020). Another group of scientists considered redox-responsive dipeptide nanostructures to load/release anticancer drugs (Chibh et al., 2020). The redox-responsive nanocarriers were formulated from disulfide-linked oxidized cysteine-phenylalanine and were then conjugated with folic acid. The disulfide bonds allowed the disintegration of the developed vectors in the presence of glutathione in cancer cells. From this fact, loaded DOX into the particles was able to be released in the presence of high levels of glutathione (Chibh et al., 2020). Zhou et al. developed DOX-loaded protein-polymer nanoconjugates for enhanced drug delivery and therapeutic index (Zhou et al., 2019). In this work, DOX was conjugated to bovine serum albumin (BSA) and the resulting complex was treated with lactobionic acid and folic acid to facilitate drug endocytosis. The functionalized BSA was conjugated with

phenylboronic acid and poly(N-isopropyl acrylamide) (PNIPAAm) forming a pH-sensitive borate ester bond to give the functionalized protein-polymer nanoconjugates prodrugs. Studies on cancer cells confirmed a fast accumulation of the conceived nanoconjugates during the first 30 min and reached a maximum at 24 h (Zhou et al., 2019). Kong et al. investigated polymeric nanoconjugates with tunable sizes for drug delivery (Kong et al., 2020). These researchers designed and developed amphiphilic cellulose derivatives *via* Schiff base reactions between 2,3-dialdehyde cellulose and amino compounds. The self-assembly property of these amphiphilic molecules was explored to produce polymeric nanoparticles. They found that the size distribution of the particles can be efficiently tailored by varying the amount and length of grafted hydrophobic side chains. DOX has been encapsulated into these vectors and, in acid conditions, they degraded allowing the drug to be delivered (Kong et al., 2020). In this context, other research teams synthesized two types of mesoporous carbon nanoparticles presenting different sizes, morphology, and structure to investigate the use of pH as a tailoring parameter in drug delivery (Gisbert-Garzarán et al., 2020). For this, the developed nanosystems have been functionalized with a pH-sensitive polymer. A released study based on a model dye and performed at different pH values proved that the vectors are responsive to pH changes, demonstrating that at pH 5 self-immolation occurred and high levels of dye can be released (Gisbert-Garzarán et al., 2020). Steiert et al. recognized the suitable properties of proteins for the conception of drug delivery systems and formulated pH-responsive protein nanoparticles based on cytochrome *c* (Steiert et al., 2020). In addition, the surface of the nanocarriers has been modified with PEG (PEGylation) and degradation at acidic pH has been acquired from vinyl ether moieties located in the polyether backbone. PEGylation allowed cytochrome *c* to exhibit different solubility profiles in organic solvents. This tool led to the formation of nanoparticles through an emulsion-based solvent evaporation technique. In consequence, the developed delivery systems degrade at low pH values and the release of encapsulated dextran, in an acidic environment, was observed (Steiert et al., 2020). Recently, Gannimani and coauthors published an interesting review article focused on the design and production of several acetal-based polymers as pH-sensitive drug delivery systems in the form of nanoplexes, micelles, polymeric and lipid-based nanoparticles, among other delivery systems, adequate to be applied in the biomedicine field. The publication highlighted the main assets of the vectors, the most relevant challenges, and the future demands of acetal-based pH-responsive drug nanocarriers (Gannimani, Walvekar, Naidu, Aminabhavi, & Govender, 2020).

15.2.3 Targeting

Targeting drugs to a specific location to enhance their performance and achieved therapeutic effect has been an emergent topic in recent years (Fig. 15.2B) (Fu et al., 2020; Nag & Delehanty, 2019; Rosenblum, Joshi, Tao, Karp, & Peer, 2018; Yetisgin, Cetinel, Zuvin, Kosar, & Kutlu, 2020; Zhao, Ukidve, Kim, & Mitragotri, 2020). In line with this, numerous strategies have been developed.

Angelopoulou et al. synthesized folic acid functionalized iron oxide condensed colloidal clusters for the selective release of DOX to tumor cells overexpressing the folate receptor (Angelopoulou, Ntoukas, Fytas, & Avgoustakis, 2019). Magnetic nanoparticles coated with alginate (co-MIONs) were prepared by following an alkaline precipitation procedure. PEG was conjugated with alginate and folic acid to generate folate-pegylated co-MIONs. An anticancer drug has been loaded into the formulated delivery systems and internalization into tumor cells expressing and not expressing the folate receptor was investigated. The obtained data revealed the high drug content in cancer cells expressing the folate receptor (Angelopoulou et al., 2019). The group of Vinothini K. also centered attention on folate receptors to target breast cancer cells and deliver PTX (Vinothini, Rajendran, Ramu, Elumalai, & Rajan, 2019). These authors reported research based on graphene family compounds as an adequate drug delivery platform to target tumor cells. They developed modified graphene oxide-methyl acrylate (GO-g-MA) nanoparticles containing folic acid to ensure the effective targeting of cancer cells. Release experiments, on breast cancer cells, examined the PTX delivery from the folic acid/GO-g-MA vectors at different pH values and confirmed the targeting ability of the conceived nanoparticles (Vinothini et al., 2019). Mahalunkar et al. developed a nanoconjugate constituted of folic acid and gold−polyvinylpyrrolidone where curcumin has been encapsulated for targeted release in cancer models (Mahalunkar et al., 2019).

These folic acid−curcumin−gold-based nanoparticles were fully characterized using several techniques and their properties were considered suitable for drug delivery purposes. The authors verified that, by using the LbL assembly method, both the size and surface charge of the developed vectors can be gradually increased. Furthermore, the nanoparticles were able to target cancer cells and ensure the release of approximately 80% of curcumin (Mahalunkar et al., 2019). Pursuing the goal of cancer cell targeting, another team of scientists created a dual-targeting drug delivery system, ensuring both magnetic targeting and active targeting (Du et al., 2020). This approach revealed to not only enhance targeting ability but also promote drug loading efficiency of the magnetic nanocarriers. Spectroscopy and flow cytometry techniques were applied to monitor drug loading, targeting, and release performance and the results revealed that the formulated dual-targeting vector can target folate receptors located on the surface of cancer cells and release an anticancer drug (Du et al., 2020). Kumar et al. also bet on targeted dual delivery regarding the treatment of colon cancer (Kumar, Thangam, et al., 2020). To meet this goal, they conceived chitosan-coated-trans-resveratrol and ferulic acid loaded solid lipid nanoparticles that were conjugated with folic acid following the encapsulation method of stearic acid. In vitro studies performed on HT-29 cells demonstrated that the folic acid-nanoconjugates possess convenient stability and can target cancer cells, which is considered a promising nano-platform for further anticancer therapy studies (Kumar, Thangam, et al., 2020). Yan et al. followed the hypothesis of targeting both the folate receptor and integrin $\alpha_v\beta_3$ that are highly expressed on the surface of human breast cancer cells MCF-7

(Yan, You, et al., 2020). In this sense, they focused on the use of both folic acid and arginine-glycine-aspartate (Arg-Gly-Asp, RGD) tripeptide sequence due to their high affinity for folate receptor and integrin $\alpha_v\beta_3$, respectively, to construct a delivery vector for PTX. The formed dual-targeting nanocarrier was based on mesoporous SiNPs and showed to be able to cancer cells targeting (Yan, You, et al., 2020). Bose et al. developed quercetin (QRC) loaded plasmonic silver nanoparticles also aiming for folate receptor targeting (Bose, Priyam, Kar, & Pattanayak, 2020). In the reported work, these researchers optimized a simple method for preparing folate-receptor-targeted-plasmonic silver nanoparticles to act as nanoscaled delivery vectors to address the targeted delivery of QRC while inducing photothermal therapy. These vectors presented a robust plasmon tunability in the near infrared (>800 nm) region and in vitro studies revealed their great targeting ability (Bose et al., 2020).

Targeting mitochondria offers the possibility to overcome the multidrug resistance problem found in the use of many chemotherapeutic drugs (Fig. 15.2B) (Li et al., 2017; Tomşa, Picos, Picos, & Răchişan, 2020; Wang, Zhang, et al., 2020). Searching for new antiresistant drugs, Wang et al. developed triphenylphosphonium-Pluronic F127-hyaluronic acid (HA) (TPH), with a mitochondria-targeting triphenylphosphine (TPP) head group, to load/encapsulate PTX (Wang, Zhang, et al., 2020). This team formed nanomicelles that displayed suitable physical properties and proved to be efficient in the inhibition of A549/ADR cells. The formulated TPH/PTX nanomicelles entered lysosomes through micropinocytosis and were able to lysosomal escape at 12 h, reaching the site of mitochondria for 24 h in A549/ADR cells (Wang, Zhang, et al., 2020). Shi et al. synthesized a mitochondrial-targeted nanosystem for DOX encapsulation based on HER-2 peptide-mediated multifunctional pH-sensitive DQAsomes (Shi et al., 2018). In this work, HER-2 peptide-PEG_{2000}-Schiff base-cholesterol (HPSC) derivate was prepared and incorporated on the surface of the DOX-dequalinium (DQA) chloride vesicle (HPS-DQAsomes) for the treatment of breast cancer cells. In vitro experiments demonstrated that the constructed HPS-DQAsomes could target mitochondria and deliver the anticancer drug to this organelle, leading to apoptosis (Shi et al., 2018). In another study, scientists have combined the enzyme-responsive property of nanoconjugates with their mitochondrial-targeting capacity to improve drug delivery and therapeutic effects (Naz et al., 2019). For this, triphenylphosphine was grafted onto the surface of mesoporous SiNPs. DOX was loaded into these particles followed by capping with HA through electrostatic interactions to obtain a nanoconjugate constituted by DOX, TPP attached, and HA mesoporous SiNPs. The developed nanocarrier was taken up by cancer cells via CD44 receptor-mediated endocytosis and accumulated into mitochondria. The degradation of HA, promoted by the enzymatic action of HAase led to DOX release in tumor cells (Naz et al., 2019). Czupiel et al. investigated an innovative approach toward multidrug resistance (Czupiel, Delplace, & Shoichet, 2020). These researchers developed pH-sensitive nanoparticles comprising DOX and a mitochondrial targeting VES-H_8R_8 to synergistically eliminate multidrug resistant breast tumor cells. They focused on the hypothesis that the effective cell internalization, efflux inhibition, and mitochondrial depolarization capacity of VES-H_8R_8

would synergistically improve the toxicity of a pH-sensitive prodrug of DOX when loaded into nanoparticles. The accomplished synergistic action of DOX and VES-H_8R_8 was observed, in vitro, against MDR breast cancer cells (Czupiel et al., 2020). Yu et al. recently published an article reporting the development of ROS-responsive drug delivery nanoconjugates combining mitochondria-targeting ceria vectors with atorvastatin for acute kidney injury (Yu, Jin, et al., 2020). For this aim, ceria particles were modified with triphenylphosphine and subsequently coated with ROS-responsive organic polymer and encapsulated atorvastatin. The properties exhibited by the conceived nanocarriers were investigated and the drug release pattern, mitochondria-targeting performance, and antiapoptotic capacity were examined. The obtained results showed that the ROS-responsive drug delivery vector combining mitochondrial-targeting ability with atorvastatin can be classified as promising for the treatment of sepsis-induced acute kidney injury (Yu, Jin, et al., 2020). Dhanasekaran et al. revised the recent advances on both issues of mitochondria-specific targeting and drug delivery approaches and their impact on therapeutic outcomes. This team addressed the main advantages of nanoparticles as targeted delivery systems and correlated their assets with new opportunities to evolve in anticancer therapies (Dhanasekaran, Venugopal, Al-Dayan, Ravinayagam, & Mohammed, 2020).

15.2.4 Codelivery

The combination therapy through the codelivery of two or more drugs already proved to be powerful in improving the efficacy of therapeutic outcomes, in reducing individual dosages and toxicity (Afsharzadeh, Hashemi, Mokhtarzadeh, Abnous, & Ramezani, 2018; Kushwah, Katiyar, Agrawal, Gupta, & Jain, 2018; Nair, 2019; Pan, Rostamizadeh, Filipczak, & Torchilin, 2019). In this way, one hydrophobic drug and one hydrophilic can be simultaneously encapsulated by using a spacer, normally a hydrophilic vector, ensuring a convenient hydrophobic-hydrophilic balance in the resulting carrier. Following this approach, a team of researchers has conjugated two drugs to PEG to create an amphiphilic molecule with the capacity to self-assemble (Kushwah et al., 2018). The amphiphilic molecule self-assembled and formed nanoparticles with sizes around 125 nm. A fluorescence study suggested cellular uptake via clathrin-mediated endocytosis. Additionally, the formulated particles showed low hepato- and nephrotoxicity (Kushwah et al., 2018). Xu et al. formulated pH-sensitive core-shell nanosystems to target tumor cells of glioma (Xu et al., 2018). To accomplish this, a cationic micellar core (Cur-M) was prepared from D-α-tocopherol-grafted-ε-polylysine polymer to load the hydrophobic curcumin, followed by dopamine-modified-poly-γ-glutamic acid polymer further deposited on its surface, through pH-sensitive linkage, to encapsulate DOX. By tailoring the Cur/DOX ratio, they formed core-shell nanoparticles to target both the tumor cells and the cancer stem cells. In vitro and in vivo experiments confirmed the codelivery ability of the developed carrier (Xu et al., 2018). Also pursuing the aim of targeting cancer

cells and stem cells, Gao and coworkers formulated polylactide-*co*-glycolide/D-alpha-tocopherol PEG succinate (PLGA/TPGS) nanoparticles to release both DTX and salinomycin (SAL), an antibreast cancer stem cells (Gao et al., 2019). These systems were prepared by a precipitation method and the molar ratio of SAL/DTX was optimized to obtain a convenient synergistic effect. The cytotoxicity of the formed nanoparticles against both MCF-7 cells and MCF-7-Ms was higher when compared with the one achieved with the individual use of each drug in vitro. In vivo studies demonstrated that these vectors were able to prolong the circulation time and keep the synergistic SAL/DOX ratio for 24 h (Gao et al., 2019). Lei et al. also explored the self-assembly strategy to develop a nanoparticulate system to load and release PTX and gemcitabine (Lei et al., 2019). They searched for a multifunctional and biocompatible nanocarrier by loading folic acid, PTX, and gemcitabine, via self-assembly, to address cancer therapy. The developed nanoparticles displayed a spherical morphology and are stable in an aqueous solution. Moreover, they presented comparable proliferation inhibition to breast cancer cell 4T1 in comparison with the individual drug application. In vivo antitumor analysis demonstrated the inhibition of tumor growth and proliferation mediated by the formulated nanoparticles (Lei et al., 2019). Another group progressed on the combinatorial therapy strategy by combining the codelivery of chemodrugs and plasmonic-induced heat, for cancer treatment, by using protein nanocapsules (Villar-Alvarez et al., 2019). For this, they formulated a hybrid nanocarrier based on human serum albumin/chitosan nanoparticles able to encapsulate free DTX and DOX-modified gold nanorods. These authors tried to link the anticancer activities of both drugs to the plasmonic optical characteristics of the embedded gold nanorods to promote plasmonic-based photothermal therapy (PPTT) (Villar-Alvarez et al., 2019). In vitro studies on triple-negative breast MDA-MB-231 cancer cells revealed that the fabricated vectors induced a strong synergistic toxic effect on these cells compared to the administration of the combined free drugs. Furthermore, PPTT increases cytostatic efficacy and promoted apoptosis (Villar-Alvarez et al., 2019). A recent review article, from Gao's team, discussed the main developments in the rational design and consequent progress on nanoparticle-based systems loaded with two or more therapeutic molecules toward the elimination of breast cancer stem (-like) cells (Gao et al., 2020). Therapies including the combination of anticancer drugs and specific inhibitors, phytochemical agents, or ribonucleic acid-based therapy are presented. A perspective on clinical translation through the identification of some of the hardest obstacles to overcome was included in this publication (Gao et al., 2020). Zhang et al. focused on the versatility of gold nanomaterials to develop delivery systems for the release of multiple agents (Zhang, Zhang, et al., 2020). They produced gold particles with novel affibody-DNA hybrid strands for the coencapsulation of nucleoside analog (5-fluorodeoxyuridine, FUdR) and DOX. This innovative drug carrier led to a higher inhibition in HER2 overexpressing breast cancer cells and demonstrated a better synergistic antitumor effect than the one accomplished with the mixture of the two drugs (Zhang, Zhang, et al., 2020). Yan et al. conceived a

pH/redox-triggered delivery system with microfluidics for the release of both DOX and PTX (Yan, Xu, et al., 2020). In this study, PTX was covalently attached to the surface of mesoporous SiNPs, where DOX was encapsulated, via a linker with a disulfide bond. The direct attachment of PTX to the nanoparticles led to a higher drug loading and ensured the optimum PTX/DOX loading ratio which led to an improved synergistic effect. Moreover, PTX and the linker showed redox sensitivity, allowing control over drug codelivery to cancer cells (Yan, Xu, et al., 2020). Thereafter, polystyrene sulfonate was coated to DOX-nanoparticles-PTX in microfluidics, via electrostatic interactions, and pH-responsive behavior was observed. In vitro studies in cancer cells, BT549, and healthy breast cells demonstrated that the developed particles selectively released both drugs, eliminating cancer cells while negligible effects were observed in healthy ones (Yan, Xu, et al., 2020). Costa et al. also conceived a nanosystem for the corelease of DOX and epirubicin (EPI) based on poly-(lactic-*co*-glycolic) acid nanoparticles conjugated with PEI (unpublished work). The conceived PEI-modified vectors remained stable over time (at least for two months), presented a spherical morphology revealed by scanning electron microscopy experiments, and possessed positive charges. The two drugs were efficiently loaded into the conceived vectors, with the EE increasing when considering the higher initial drug loading amount into the nanoparticles. Table 15.1 shows the obtained EE values for the developed dual drug systems. Additionally, release studies mimicking intracellular environments suggested the effective codelivery of both DOX and EPI at acidic pH. Moreover, cytotoxicity studies using MTT colorimetric assay performed on HeLa cells demonstrated the potential anticancer effect of the formulated codelivery nanoparticles. Yu et al. developed an original strategy for the combined delivery of PTX and DOX to enhance the pharmacokinetics behavior of a prodrug PTX-S-DOX and increase its accumulation in tumor region (Yu, Wang, et al., 2020). This involves the coordination of copper ions (Cu^{2+}) with the anthracene nucleus of DOX and the loading of the drugs into liposomes by Cu^{2+} gradient. The liposome vector was revealed to be more promising as a nano-platform for

Table 15.1 Encapsulation efficiency of doxorubicin (DOX)-epirubicin (EPI) loaded PEI-modified poly-(lactic-*co*-glycolic) acid nanoparticles for DOX and EPI drugs.

Initial drug loading (μg)	EE % DOX EPI
1	67 ± 3.0 63 ± 2.1
2	74 ± 1.9 68 ± 1.4
4	81 ± 3.3 77 ± 2.8
5	83 ± 3.6 78 ± 2.3
10	91 ± 2.9 84 ± 3.1

The values are calculated with the data obtained from three independent measurements (mean ± SD, n = 3).
EE, *Encapsulation efficiency;* PEI, *polyethyleneimine.*

cancer treatment when compared with the corresponding nanoparticulate system. The DOX/PTX-loaded liposomes displayed longer blood circulation, lower cytotoxicity levels, and enhanced antitumor activity (Yu, Wang, et al., 2020). Nanotechnology has also been employed in the search for an alternative approach to overcome physiological barriers during antitumor treatment (Li, Xiong, Ji, & Yan, 2019). To surpass the main limitations related to the action of tumor-targeted drug delivery systems, such as blood circulation, tumor penetration, or specific drug release/response, scientists have studied suitable alternatives. The codelivery of active constituents of plants and anticancer drugs using nanocarriers appears as a valuable strategy to deal with the mentioned obstacles. The latest findings on this subject were recently revised and discussed (Li et al., 2019).

15.3 Biomedical applications

Nanoconjugates and nanoconjugate formulations have both been widely applied for drug delivery in biomedical applications. In this context, a variety of diseases and pathologies have benefited from recent progress in nanotechnology. In diabetes, for example, current advances in the design/formulation of drug delivery vectors have instigated the delivery of small therapeutic molecules, improving the quality of life of diabetic patients (Fig. 15.3A). As diabetes can be seen as a chronic disease, nanotechnology-based strategies revealed to be quite promising to ensure the site-specific release of drugs with a higher bioavailability and lower dosage regimens (Uppal, Italiya, Chitkara, & Mittal, 2018). In particular, diabetes mellitus, a metabolic and incurable disease affecting approximately 500 million individuals to date, can be controlled through the administration of oral hypoglycemic agents (Souto, Souto, et al., 2019). In this context, nanoparticles from different materials, including polymeric or lipid carriers, dendrimers, micelles, or emulsions, have been investigated for insulin delivery via more convenient methods than the conventional injection, that is, via oral or nasal routes (Fig. 15.3A) (Souto, Souto, et al., 2019). In this context, Mansoor et al. revised the strategies for insulin delivery from polymeric nanoparticles (Mansoor, Kondhia, Choonara, & Pillay, 2019). Given increasing patient compliance, several novel drug delivery carriers and alternative routes for insulin administration have both been studied. New polymer-based vectors have been designed and produced as insulin delivery systems, for diabetes mellitus, aiming to improve bioavailability, controlled/tailored release, safety, and therapeutic efficacy (Mansoor et al., 2019). Trinh et al. developed an injectable pH-temperature sensitive hydrogel containing chitosan-insulin-loaded nanospheres for diabetes treatment (Trinh et al., 2020). The novel composite system contains a pH- and temperature-sensitive hydrogel, made of an oligomer serine-*b*-poly(lactide)-*b*-poly(ethylene glycol)-*b*-poly(lactide)-*b*-oligomer serine pentablock copolymer, as matrix and chitosan−insulin electrosprayed nanospheres as constituent materials. The chitosan-insulin nanospheres were

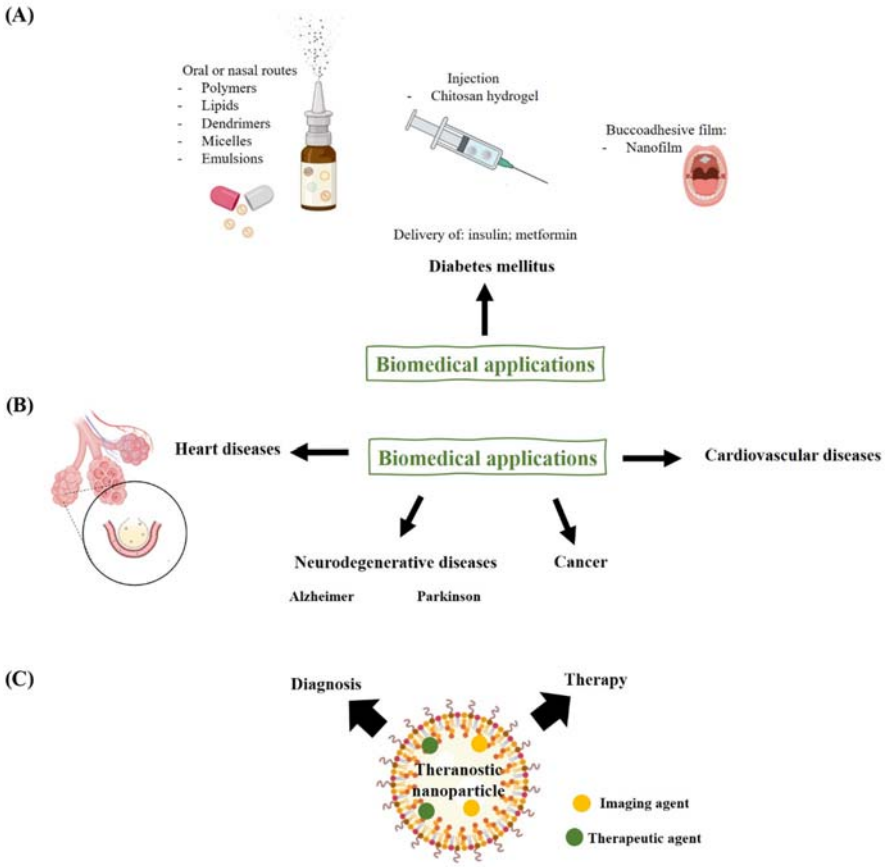

FIGURE 15.3

Schematic representation of the application of drug delivery systems on diabetes mellitus (A), and on heart, cardiovascular and neurodegenerative diseases (B) and a drug-based nanoparticle for theranostic nanomedicine (C).

distributed in the matrix and showed to enhance the mechanical properties of the hydrogel while prolonging its degradation time. In vivo experiments of insulin delivery are promising regarding insulin blood concentrations and blood glucose reduced levels. Therefore, the authors reported on the potential clinical application of the developed pH-temperature sensitive hydrogel containing chitosan-insulin nanosphere composites when dealing with type 1 diabetes (Trinh et al., 2020). Salatin et al. centered their attention on the use of metformin for the treatment of type 2 diabetes (Salatin, Alami-Milani, & Jelvehgari, 2020). These researchers designed metformin-based nanoparticles to load into a mucoadhesive film to increase drug bioavailability (Fig. 15.3A). A Box–Behnken design was

explored to analyze and optimize the formulation parameters. Moreover, drug permeation studies were carried out in sheep buccal mucosa and demonstrated that the nanofilm promoted a high metformin permeation within 6 h (Salatin et al., 2020). Recently, aiming for better management of diabetes mellitus (type 2), Raza et al. developed ethyl cellulose and PEG microparticles for prolonged delivery of metformin (Raza, Javeria, & Rashid, 2020). For the synthesis of microparticles, these scientists used an emulsion solvent evaporation method. All the prepared particles revealed a sustained drug delivery profile at pH 6.8, and up to 91.34%, ± 1.68 metformin was released in 12 h. They found that the produced microparticles may represent a great asset due to the achieved prolonged release, the possibility of lowering the dose frequency, and the promising patient compliance (Raza et al., 2020).

Heart diseases have also been the subject of research in the development of high-performance drug delivery systems (Fig. 15.3B) (Ahmed, Abdelrahman, & Salama, 2017; Ben-Mordechai et al., 2017; Fan et al., 2020; Gorain et al., 2018; Lakshmanan & Maulik, 2018). To illustrate this, we highlight here the work developed by Miragoli and coworkers on the design of calcium phosphate nanoparticles for cargo release (Miragoli et al., 2018). They demonstrated that the inhalation of calcium phosphate-based carriers led to rapid translocation of these vectors from the pulmonary tree to the bloodstream and to the myocardium, where encapsulated therapeutics can be delivered in a fast way. The treatment of a rodent model of cardiomyopathy by inhalation of peptide-loaded calcium phosphate nanosystems resulted in enhanced myocardial contraction instigating the restoration of cardiac function (Miragoli et al., 2018).

Cardiovascular diseases also received considerable attention from the scientific community and important progress has been made in the last decade (Fig. 15.3B) (Deng et al., 2019; Pala, Anju, Dyavaiah, Busi, & Nauli, 2020; Park et al., 2020; Prajnamitra, Chen, Lin, Chen, & Hsieh, 2019). On this matter, Zhang et al. published a relevant review article discussing the future treatment for atherosclerosis with special emphasis on theranostic nanomedicine (Zhang et al., 2019).

Respiratory illnesses are frequent worldwide and treatments based on therapeutics delivery became a valuable approach to ensuring therapy efficacy and limiting adverse secondary effects. In line with this, several publications brought the latest contributions in this area (Alexescu et al., 2019; Anderson, Grimmett, Domalewski, & Cui, 2020; Pontes & Grenha, 2020).

The development of drug-based release systems also found important applications in the diagnosis and treatment of neurodegenerative disorders (Fig. 15.3B) (Asil, Ahlawat, Barroso, & Narayan, 2020; Calzoni et al., 2019; Cano et al., 2020; Zhao, Francis, Calvelli, & Moghe, 2020). For instance, great progress has been made in the treatment of Alzheimer's disease (AD) through nanotechnology (Harilal et al., 2019). Generally, the drugs applied to target the central nervous system have to face many barriers such as the difficulty to surpass the "blood-brain barrier" or the "blood-cerebrospinal fluid barrier." The use of targeted drug

delivery systems in AD added a great contribution to overcoming this obstacle. In this regard, different nanotechnology strategies involving vectors based on lipids, polymers, emulsions, carbon tubes, and metal-based systems have been explored for the treatment of AD. Furthermore, nanotechnology has also helped to improve the early diagnosis of this disease as well as to increase the therapeutic index (Harilal et al., 2019). Radwan et al. also investigated the development of chitosan nanoparticles by gamma radiation method for the encapsulation of memantine towards AD (Radwan, Ghaffar, & Ali, 2019). Parkinson's disease (PD) has also been subject to research with several interesting articles published in the recent literature (Baskin, Jeon, & Lewis, 2020; Chen et al., 2019; Zhao et al., 2016). In this context, Chen et al. prepared carriers for the controlled release of puerarin, aiming to increase its half-life and accumulation in the brain, a promising therapy for PD (Chen et al., 2019). Other neurodegenerative diseases also received attention from scientists and drug delivery-based therapies were developed to deal with these conditions (Godinho, Ogier, Darcy, O'driscoll, & Cryan, 2013; Hsiao et al., 2012; Moradi, Momtaz, Bayrami, Farzaei, & Abdollahi, 2020; Naqvi, Panghal, & Flora, 2020; Niu, Chen, & Gao, 2019; Ojha & Kumar, 2018).

Among all diseases where drug delivery systems have been wisely employed, cancer was perhaps the most investigated of them all. This has contributed to the fact that cancer is the second leading cause of death globally with an estimated 60% rise in new cases worldwide over the next two decades. Therefore, more efforts need to be focused on both cancer detection and treatment. In line with this, cancer therapy management via drug delivery approaches will be fully addressed in a separate subsection of this chapter.

In the field of theranostic, drug-based nanosystems proved to be useful and the intensive research on this topic gave rise to valuable knowledge and advances (Fig. 15.3C) (Bulmahn et al., 2020; Ghitman, Biru, Stan, & Iovu, 2020; Guo et al., 2015; Sk & Kojima, 2015; Tao, Wang, & Xu, 2020).

Due to the enormous potential applications of drug delivery systems mostly in biology and medicine areas, it was not surprising to assist the growing position of these vectors in sectors such as the pharmaceutical, medical or biological.

15.3.1 Cancer therapy

Drug delivery systems appeal to researchers in the areas of cancer diagnosis and treatment. Nanoconjugates and nanoconjugate formulations conceived to present tunable biological properties and operating in a range of settings can be a valuable choice in terms of efficacy and safety to deliver chemotherapeutic drugs. As our understanding of cancer evolves, the development of new and high-performance drug delivery systems for targeted and controlled drug release against tumor cells greatly increases. The recent achievements in this field are fully reported in the literature, with the key challenges and the future directions to follow, given advances in cancer therapy, being highlighted (Colone, Calcabrini, & Stringaro, 2020; Jun et al., 2020; Navya et al., 2019; Tharkar,

Varanasi, Wong, Jin, & Chrzanowski, 2019; Yao et al., 2020; Zhang, Zhou, et al., 2020). In the treatment of cancer, the number of different drugs aiming to eliminate or significantly inhibit tumor cell growth and proliferation is diverse and includes natural products and their derivatives, anthracyclines with anticancer activity, taxanes, antimetabolites, and antineoplastic agents, to refer the most common. To load/encapsulate these therapeutic molecules and promote their controlled release into tumoral cells, different nanomaterials have been explored and different strategies investigated. These have included the conception of responsive, targeted, or codelivery systems. In this matter, multifunctional delivery systems combining two or more of these functionalities were revealed to be the most promising in successful cancer therapy (Fig. 15.4).

Chaudhary and coworkers developed resveratrol (RES)-loaded mesoporous SiNPs for prostate cancer treatment (Fig. 15.4A) (Chaudhary et al., 2019). The encapsulation of RES into silica carriers showed an improvement in RES antiproliferative activity and sensitization of DTX in hypoxia-induced drug resistance in

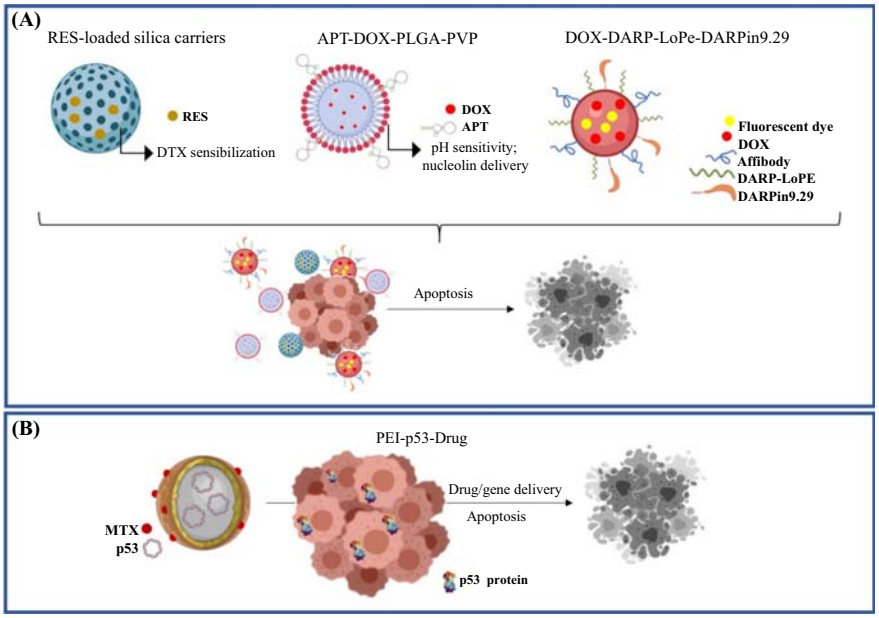

FIGURE 15.4

Representative scheme of the application of drug codelivery nanosystems to address cancer therapy. Illustration of the conception of responsive, targeted or codelivery systems: (A) the formation of RES-loaded silica carriers, APT-DOX-PLGA-PVP nanoparticles for dual stimuli pH and nucleolin-targeted drug delivery, and a nano delivery system for the encapsulation of both an imaging fluorescent dye and DOX and functionalized with an affibody; (B) a codelivery system based on PEI/p53/MTX nanoparticles, as a promising strategy to improve cancer treatment.

prostate cancer. This work demonstrated the applicability of mesoporous SiNPs loaded with polyphenols as suitable delivery vectors to address drug-resistant cancers, namely, prostate cancer (Chaudhary et al., 2019). Another group has researched the utility of polyphenols release in cancer treatment (Souto, Sampaio, et al., 2019). They revised the properties of a series of phenolic compounds with biological properties towards skin cancer, discussed the mechanisms of activity, and presented novel approaches for their release directed to address skin cancer (Souto, Sampaio, et al., 2019). Other authors evolved in cancer treatment by combining the assets of surface functionalization and pH-sensitivity (Fig. 15.4A) (Saravanakumar et al., 2019). For this, aptamer functionalized DOX-encapsulated poly(D,L-lactic-*co*-glycolic acid) (PLGA), poly (*N*-vinylpyrrolidone) (PVP) nanoparticles (APT-DOX-PLGA-PVP NPs) were formulated. The developed system was prepared to aim at dual stimuli pH and nucleolin-targeted drug delivery (Fig. 15.4A). APT-DOX-PLGA-PVP NPs demonstrated enhanced anticancer activity, with less toxicity to normal cells (Saravanakumar et al., 2019). Mortezazadeh et al. innovated with the conception of a targeted theranostic system for cancer chemotherapy and molecular resonance imaging (Mortezazadeh et al., 2020). β-Cyclodextrine-based polyester was coated on the surface of gadolinium oxide nanoparticles and then functionalized with folic acid to generate a pH-responsive targeted theranostic system for DOX release and magnetic resonance imaging (MRI). The dissolution profile of DOX was consistent with a pH-sensitive delivery and drug-loaded vectors were more effective in inhibiting M109 cell viability compared with free DOX. In vivo experiments revealed that the developed delivery system presented significant relaxivity behavior as a contrast agent for MRI, and also enhanced the antitumor efficacy. The obtained data indicated that the developed nanosystem may represent a great contribution to advances in the treatment of solid tumors being a suitable drug delivery nanoplatform for chemotherapy and molecular imaging diagnosis in MRI (Mortezazadeh et al., 2020). Bonferroni et al. published a review discussing the utility of chitosan drug-based carriers for the diagnosis and treatment of hepatocellular carcinoma and liver-targeting (Bonferroni, Gavini, Rassu, Maestri, & Giunchedi, 2020). Recently, Shipunova and colleagues reported a remarkable tool for aggressive tumor treatment (Shipunova et al., 2020). These researchers combined immunotherapy with image-guided anticancer drug delivery for cancer theranostics. They wisely demonstrated the reached synergistic effect with immune/chemotherapy combination considering dual regioselective targeting by the use of two different binding sites of a single oncomarker with theranostic molecules displaying different mechanisms of action. To accomplish this, a nano delivery system for the encapsulation of both an imaging fluorescent dye and DOX, functionalized with an affibody, along with a bifunctional DARP-LoPE immunotoxin comprising a modification of therapeutic Pseudomonas exotoxin A and a scaffold targeting protein, DARPin9.29 were considered, as illustrated in Fig. 15.4A. The authors showed that the dual targeting strategy led to an improved anticancer effect, which turned possible a significant decrease in the

drug concentration employed. Furthermore, the combined therapeutic approach prevented secondary tumor nodes and, therefore, the reported synergistic approach based on targeting the same oncomarker represented a powerful tool against aggressive tumors (Shipunova et al., 2020). Other scientists introduced a considerable novelty in cancer therapy through the addition of targeting to microenvironment modulation (Duan et al., 2019). They focused their investigation on heparanase (HPA), highly expressed in various cancers, namely, triple-negative breast cancer (TNBC). PTX has been loaded into PEGylated PLGA nanoparticles that were functionalized with the HPA aptamers (Apt(S1.5)-PTX-NP). These vectors bonded to the HPA overexpressed on the surface of TNBC cells and were taken up by these cells, which originated an increment in cellular toxicity compared with the corresponding nontargeted PTX-carriers that lack the HPA aptamer (Duan et al., 2019). Additionally, Apt(S1.5)-PTX-NP presented increased antiinvasive and antiangiogenesis activities, in comparison with the ones found for other experiment groups. In vivo studies greatly confirmed the efficacy of the proposed system in reducing tumor mean size. In this way, authors found that HPA is a convenient target for drug release to TNBC cells, and HPA-aptamer nano-bioconjugates may instigate relevant signs of progress in TNBC therapy (Duan et al., 2019).

As discussed above in this chapter, codelivery is a valuable approach in cancer therapy contributing to novel insights and therapeutic outcomes. Regarding this, Nasab's team has designed and formulated a multitargeted drug-delivery system using multifunctional nanoparticles for the codelivery of siRNA and PTX (Nasab, Amani, Ebrahimi, & Hamidi, 2020). The prepared nanosystem displayed high toxicity levels in MCF-7 cells and showed increased uptake of siRNA in the same cells. Moreover, the PTX-siRNA loaded vector presented a high anticancer effect against MCF-7 and BT-474 cell lines (Nasab et al., 2020).

The simultaneous release of anticancer drugs and tumor suppressor genes, such as the p53 gene, have already proved to be an adequate solution to treat cancer cells due to synergistic effect (Faria, Sousa, Neves, Queiroz, & Costa, 2019; Lin et al., 2019; Tamura, lana, Constanzi-Strauss, & Strauss, 2020; Wang, Guan, et al., 2020). Cancer gene therapy can be used as an adjuvant to chemotherapy since perturbations of certain genes can sensitize tumor cells to drugs enhancing the effect of the clinical approach. In line with this finding, Costa's research group has developed drug/gene delivery systems for targeted and controlled/tailored release of chemotherapeutics and the p53 gene to cancer cells (Costa et al., 2018; Costa, Valente, & Queiroz, 2015; Faria et al., 2019). PEI (25 kDa) was used to form complexes, by a coprecipitation method, with a p53 encoding plasmid DNA (pDNA) at various nitrogen-to-phosphate groups ratios (N/P). Cancer therapeutics namely, DOX, EPI, PTX, or methotrexate (MTX) were also loaded into these systems (Fig. 15.4B). The properties of the formed nanoparticles at an N/P ratio of 4, such as the drug and p53 gene encapsulation efficiencies and the mean size diameter of the formed vectors are presented in Table 15.2. All formulated codelivery carriers exhibited high drug and gene EE values and showed

Table 15.2 Drug and pDNA encapsulation efficiency (EE) %, mean size of PEI/pDNA/drug, p53 protein levels, and cellular viability of HeLa cells, analyzed by MTT assay, after 5 days of treatment with PEI/pDNA/drug complexes formulated at N/P of 4.

System	Drug EE	pDNA EE	Size (nm)	p53 (ng/mL)	Viability (%)
PEI/pDNA/DOX	79 ± 2.2	88 ± 3.1	156 ± 5.0	410 ± 4.8	36 ± 2.8
PEI/pDNA/EPI	76 ± 1.7	87 ± 3.3	179 ± 4.7	401 ± 5.2	39 ± 3.9
PEI/pDNA/PTX	78 ± 3.3	88 ± 4.0	148 ± 2.8	414 ± 4.6	32 ± 3.6
PEI/pDNA/MTX	70 ± 3.9	86 ± 2.7	182 ± 3.6	462 ± 2.5	22 ± 1.9

The values were calculated with the data obtained from three independent measurements (mean ± SD, n = 3). Data were analyzed by one-way ANOVA followed by the Bonferroni test, P <.05 was considered statistically significant.
PEI, *Polyethyleneimine.*

mean sizes below 200 nm which favored cellular uptake. Moreover, the delivery systems were able to successfully transfect HeLa cells and promoted gene expression. The levels of p53 protein were quantified by a p53 Elisa kit assay; the obtained results are shown in Table 15.2. The protein was produced at high levels which led to a significant reduction in the viability of cancer cells, in comparison with untreated cells (which corresponds to 100% cellular viability). The PEI/pDNA/MTX-based nanoparticles seemed to be the most efficient system in producing high p53 content and, therefore, in inhibiting the proliferation of HeLa cells (Fig. 15.4B). This effect can contribute to the targeting ability displayed by the drug MTX towards the folate receptors overexpressed in cancer cells (Faria et al., 2019). This feature turned the transfection process mediated by the MTX/p53-loaded formulations more efficient, with a consequent increment in p53 gene expression and, thus, direct repercussions on the significant decrease of cancer cell viability (Table 15.2). Therefore, targeting drug/gene codelivery systems was revealed to be a suitable approach to improve the efficacy of cancer treatment.

15.4 Concluding remarks

In the last decades, the research on nanoconjugates and nanoconjugate formulations for drug delivery has experienced a crescent increase. This fact has contributed to the design/development of advanced and high-performance drug-based carriers which found several applications in the biomedical field. Improvements in nanoconjugation methods and techniques and the exploration of the intrinsic properties of materials gave rise to the formation of nanoconjugates that can display tailored, targeted, and specific characteristics. The gathering of scientists with physics, chemistry, biology, and medical backgrounds instigated continuous progress in the development of more sophisticated and multifunctional nanoconjugates. Following this, stimuli-responsive, cancer cell-targeting, or codelivery,

drug-based systems have been created and optimized for biomedical applications. In this way, a great number of diseases and pathologies have deeply benefited from the new knowledge and innovation, with novel therapy approaches being introduced in the clinics. Cancer therapy, is perhaps, the most evident example of the successful link between nanotechnology and medicine. The evolution in the design and specificity of conceived nanoconjugates turned possible to overcome the main drawbacks related to drug delivery therapy in cancer, namely drug solubility, toxicity, targeting, or drug resistance. Moreover, it has provided, and for sure will continue to lead, new and powerful tools to improve both cancer diagnosis and treatment.

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CHAPTER

16 Nanophytomedicines: a novel approach for improving therapeutics via delivery of herbal medicine

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16.1 Introduction

Herbal medicine is a discipline that deals with the use of drugs traditionally recognized to fight against certain pathologies that affect humans. Indeed, extracts and essential oils extracted from medicinal plants have demonstrated enormous biological properties such as antimicrobial, antiinflammatory, and anticancer, antiparasitic effects. The pharmacological effects of medicinal plants are essentially linked to the presence of secondary metabolites rich in bioactive compounds belonging to different chemical families such as phenolic acids, flavonoids, terpenoids, and alkaloids. Although these molecules exert enormous pharmacological effects with various mechanisms of action, their application in a mixture is limited by the problem of penetration and bioavailability.

Indeed, these molecules are not able to cross the lipid membrane and thus they show poor aqueous solubility and slow down their biological efficacy and

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pharmacokinetics. Limitations of using natural products as medicines are absorption, stability, and therapeutic effects which can be overcome by using nanoformulations as a tool to improve drug topical delivery of herbal medicines (Mishra, Panda, Kumar, & Singh, 2017) as well as to enhance the activity and overcoming problems associated with herbal drugs (Gunasekaran, Haile, Nigusse, & Dhanaraju, 2014).

Nanocarriers as part of nanotechnology are one of the key novel drug delivery methods under investigation. These delivery systems have characteristics to deliver both lipophilic and hydrophilic molecules. Advances concerning fabrication, materials, techniques, and methods facilitate the development of new and superior reliability of nanocarriers. However, characterizations of nanocarriers are very critical to control their desired in vitro and in vivo behavior (Jain & Thareja, 2019). For that reason, future research must ensure the benefit and evaluate the risk ratio for many drugs included in nanocarriers (Bhaw-Luximon, Goonoo, & Jhurry, 2016).

The skin offers a convenient and accessible site for drug administration (Alkilani, McCrudden, & Donnelly, 2015) although its barrier function acts in such a way that it critically limits the absolute quantity of the drug to pass through a considerable area of it during the administration period, making it an important limiting factor in the delivery of therapeutic agents through the skin (Mishra, Pandey, Maheshwari, Ghode, & Tekade, 2019).

In this sense, to optimize transdermal drug delivery systems, studies are being performed to describe new vehicles or use sedate bearers to guarantee both the sufficient entrance of the medication and its restriction inside the skin (Alkilani et al., 2015). Nanocarrier systems are one of the significant novel drug delivery systems (Mishra et al., 2017) and are a revolution in the transdermal delivery of therapeutics. In this chapter, nanophytomedicines to deliver herbal medicine as well as their therapeutical applications were highlighted.

16.2 Human skin

The skin is the most visible and heaviest organ of the human body (Yasin, Ibrahim, Rashid, Razif, & Yusof, 2017), representing approximately 10% of the total body mass and covering about 1.7 m^2 of the body surface area (Pérez-Sánchez, Barrajón-Catalán, Herranz-López, & Micol, 2018). Human skin encloses the body and draws the limits of the self, it establishes the border between the inside and the outside in a living way because it is also open to the world, thus allowing vital bodily functions to occur within a controlled physiological environment (Madison, Swartzendruber, Wertz, & Downing, 1987). It is synergistic with internal orang systems and has a complex architecture that forms a protective barrier against microbes, chemicals, ultraviolet (UV) radiation, and the external environment (Yasin et al., 2017) and its sensory components perceive heat, cold, pain, pruritus, touch, and pressure. Also, the skin provides an ideal route for the

administration of therapeutic compounds for local and systemic effects, it presents an important barrier to the permeation of most compounds (Benson, 2011). However, the human skin may suffer alterations such as overexposure to ultraviolet radiation (photo-aging), gravity, poor nutrition, smoking, immune dysfunction, and inflammation which may significantly affect human health (Helfrich, Sachs, & Voorhees, 2008; Pérez-Sánchez et al., 2018; Yasin et al., 2017). Human skin is a multifunctional organ defined as a stratified epithelium, it is composed of three distinct tissue layers, each tissue layer consisting of different cell types that allow various functions. It can be broadly split into the overlying epidermis, dermis, and underlying hypodermis.

16.2.1 Epidermis

The human epidermis is on average 0.4 mm thick, ranging from 0.03 mm thick on the eyelids to 1.5 mm thick on the palms and soles (Barbieri, Wanat, & Seykora, 2014). The epidermis is the most superficial layer of the skin. It is a highly cellular, yet avascular structure that allows the main barrier against environmental aggressions (D'Errico, Lemma, Calcagnile, Santis, & Dogliotti, 2007). The main epidermal cell type that forms the superficial layers of the epidermis is sheets of keratinocytes from stem cell transdifferentiation but also contains non-epithelial cells, antigen-presenting dendritic Langerhans cells as well as melanocytes, and Merkel cells (Graham, Eckersley, Ozols, Mellody, & Sherratt, 2019). The epidermis is nourished by the diffusion of intercellular fluids, from the dermal vasculature (Abdo, Sopko, & Milner, 2020). It is also rich in vimentin, desmin, a-internexin and nestin. The epidermis itself can further be segmented, from the outside to the inside, into four micro-layers: the stratum corneum (horny layer), stratum granulosum (granular layer), stratum spinosum (prickle cell layer) and stratum basale (stratum germinativum). An additional layer can be formed on its surface by dead cells, known as the stratum lucidum (clear layer) or scales, which corresponds to a transition zone between the stratum granulosum and the stratum corneum of the epidermis which are continually renewed (Graham et al., 2019).

The stratum corneum is the outermost layer of the skin. It consists of a tightly bound sheet of 10–15 layers of corneocytes (dead, flattened keratinocytes which are continually renewed by type II nuclear receptor signaling). This receptor may be responsible for maintaining epidermis permeability (Hyter & Indra, 2013) and it is typically 10–20 μm thick and acts as the primary barrier against injury, and infection and prevents dehydration (Agache, 2004; Brandner & Jensen, 2008). The barrier property of this layer is assigned to both the terminally differentiated corneocytes and also to the surrounding lipid matrix (composed of ceramides, cholesterol esters, cholesterol sulfate, and free fatty acids with the notable absence of phospholipids) which collectively act as "bricks and mortar" model, where the corneocytes are likened to bricks and the extracellular matrix analogous to the mortar in a brick wall (Benson, 2011; Madison et al., 1987) that providing water content maintenance and acting as a biosensor and first protection against damage (Elias, 1983; Harding, 2004). Furthermore,

several studies have shown that the structural proprieties of this layer make it the major pathway of skin permeation and application of drugs in formulations (Bouwstra & Honeywell-Nguyen, 2002; Bouwstra, Gooris, Dubbelaar, & Ponec, 2002; Labouta, El-Khordagui, Kraus, & Schneider, 2011).

The stratum granulosum is a granular layer typically similar in thickness to that of the stratum corneum. Keratinocytes in the stratum granulosum are flatter and more irregular in shape than those in the stratum spinosum and have deep basophilic keratohyalin granules (Barbieri et al., 2014).

The stratum spinosum or spinous layer is known as the Malpighian layer localized between the stratum granulosum and stratum basale. It is composed mainly of two to six rows of lipid-releasing keratinocytes which adhere to each other by desmosomes (Graham et al., 2019).

The stratum basale is the innermost layer of the epidermis formed by a single layer of progenitor keratinocytes resting on the basement membrane zone that separates the epidermis from the dermis. These cells act as a source of proliferation, continuously replacing cells in the more superficial layers of the epidermis but also as a physical barrier between the dermis and the epidermis (Scott & Miller, 2011). In addition to keratinocytes, the epidermis also contains melanocytes that produce melanin synthesized from the amino acid tyrosine in melanosomes and play a major role in protecting the skin from ultraviolet radiation damage. Melanocytes represent approximately one out of ten cells in the basal layer which have hemidesmosomes but lack tonofilaments and desmosomes (Barbieri et al., 2014). Langerhan's cells located in the suprabasal epidermis and the dermis are mononuclear, dendritic, antigen-presenting cells and contain membrane-associated adenosine triphosphatase, vimentin, CD45 (common leukocyte antigen), and S-100 protein (immunohistochemical markers) (Scott & Miller, 2011). They act as sentinels of the cutaneous immune system (Collin & Milne, 2016).

16.2.2 Dermis

The dermis is the deeper part of the skin and an integral part of the body's connective tissue system (Yasin et al., 2017). In body skin, the dermis ranges from 1.6 to 6.1 mm thick, and in the mane and tail region, 3.7−10.5 mm (Scott, 2004). It is composed of elastic fibers, collagen, and cells. It also contains sweat pores, hair follicles, the epidermal appendages, arrector pili muscles, blood and lymph vessels, and nerves. These compositions contribute to the dermis' great tensile strength, elasticity, and mechanical properties of the skin (Quan & Fisher, 2015). The dermis consists of two layers. The papillary dermis is the first layer below the epidermis but is not usually seen (Scott & Miller, 2011), consisting of areolar connective tissue with thin collagen fibers and fine elastic fibers (Yasin et al., 2017). The dermal-epidermis junction localized of the interface between the epidermis and dermis is a basement membrane characterized by a complex network of dermal papillae; composed of a highly specialized extracellular matrix (comprised of proteins such as laminins and collagen IV) (1,4). The dermis provides structural and nutritional support to the skin (Barbieri et al., 2014) and is

involved in the regulation of cell growth, proliferation, migration, adhesion, and differentiation. It also contains abundant immune cells and fibroblasts which are involved in the synthesis of many of the extracellular matrix components. Also, it helps regulate body temperature due to the presence of blood vessels (Pérez-Sánchez et al., 2018).

16.2.2.1 Hypodermis

The hypodermis is the deepest and thickest layer of the skin. However, its absence is notable in some lean skin, such as that on the eyelid (Benson, 2011; Madison et al., 1987). It is composed mainly of fatty acids which provide insulation, thermoregulation, store nutrition and protect deeper tissues from injuries (Kanitakis, 2002) but contains also cells known as fibroblasts, connective tissue, and macrophages, cells that are part of the immune system and help keep the body free of intruders. Embedded in this skin layer are larger nerves and blood vessels (Benson, 2011; Madison et al., 1987). Recent studies suggest that the hypodermis which is rich in adipocytes may influence dermal physiology and structure (Ezure & Amano, 2015).

16.2.2.2 Functions of healthy human skin

Human skin is high complex orang that performs the following functions (Igarashi, Nishino, & Nayar, 2007; Menon & Kligman, 2009; Scott & Miller, 2010, 2011). The most obvious function of the skin is to provide an anatomical barrier between the internal and external environment and helps our bodily defenses to protect us from penetration of pathogen micro-organisms and mechanical damage but also preventing the inward and outward passage of water, electrolytes, and macromolecules (Archer, 2010). Skin is considered an active immunological organ. It plays an essential role in both innate and adaptive immunity. Immune responses in the skin involve an arsenal of immune-competent cells and soluble biologic responses including cytokines. In the innate immune response, substances of stratum corneum, such as polar lipids, free fatty acids, and glycosphingolipids contained in the horny layer and the intracellular spaces, provide antimicrobial properties and function as the first line of defense. In adaptive immunity response, after an infection incurs, Langerhans cells, the macrophage-like antigen-presenting cells that reside in the epidermis capture, process, and present pathogens to T cells in local lymphoid tissues (Abdo et al., 2020; Salmon, Armstrong, & Ansel, 1994).

Thermoregulation. Skin plays a crucial role in the regulation of body temperature through its support of the hair coat, regulation of cutaneous blood supply, and sweat gland function;

- Sensory perception. The skin contains sensory and autonomic nerves and several types of sensory receptors, which detect the incoming stimuli of touch, pressure, pain, itch, heat, and cold;
- Storage. The skin acts as a storage center for lipids (fat) and water but also a reservoir of electrolytes, water, vitamins, carbohydrates, proteins, and other materials;

- Produces vitamin D. The skin is a ready source of vitamin D following sun exposure. Solar ultraviolet-B radiation (UVB) stimulates the production of vitamin D3 from 7-dehydrocholesterol (7-DHC) in the epidermis of the skin;
- Antimicrobial action. The skin surface has antifungal and antibacterial properties that allow maintaining of the natural skin microflora;
- Excretion. The skin excretes waste products from the body in the form of sweat which is at most a secondary function to temperature regulation.
- The skin is a unique and variable ecosystem that provides many niches in which large populations of microbes are subjected to variable ecological pressures including humidity, temperature, pH, and the composition of antimicrobial peptides and lipids. Also, skin structures such as hair follicles and sebaceous, eccrine, and apocrine glands constitute discrete niches that harbor unique microbiota (Grice et al., 2008; Schommer & Gallo, 2013).
- Absorption. Skin absorption is a way used to administer medicine into the body by the use of adhesive patches or ointments.
- Pigmentation. Processes in the skin due to melatonin production, help to determine the color of the coat and skin. Pigmentation of the skin helps prevent damage from solar radiation (Slominski, Tobin, Zmijewski, Wortsman, & Paus, 2008).

16.3 Drug penetration

The transdermal penetration of substances is subject to large variability depending on interindividual factors such as age, ethnicity, and gender but also intraindividual factors such as the body region (Kilo et al., 2020). In this way, the exact pathways of drug penetration through the skin are subjected to numerous investigations (Alkilani et al., 2015). Moreover, that is why transdermal drug penetration must meet specific eligibility criteria and conditions as it must have an optimum partition coefficient, with a log P value (octanol/water) between 2 and 3 (Lane, Santos, Watkinson, & Hadgraft, 2012), the half-life of the drug, must be short (less than 10 h), It must have a skin permeability coefficient greater (0.5×10^{-3} cm/h), It must not produce any local sensitization or irritation, the daily dose should be low (20 mg/day) and a low molecular weight (less than 500 Daltons). It should also have potential characteristics for both lipophilic and hydrophilic phases (Mali, 2015). Drug molecule penetration through the skin is an intriguing phenomenon (Neupane, Boddu, Renukuntla, Babu, & Tiwari, 2020).

As mentioned there are critically two possible routes of drug penetration through the skin, namely the transepidermal pathway subdivided into the transcellular route (diffusion across corneocytes) and the intercellular route (diffusion across the lipid matrix) and via appendageal pathway or shunt route (diffusion into the sweat gland, hair follicles, and sebaceous gland) (Lane et al., 2012). The overall observed flow through the skin is governed by the combined flow of these two pathways (Neupane et al., 2020).

16.3.1 Transepidermal pathway

The transepidermal pathway requires that the permeant traverses the extracellular and/or intracellular spaces, from the epidermis to the dermis and hypodermis. In the transcellular route, the permeant is routed through the alternating layers of cells and the extracellular matrix (Ng & Lau, 2015). Drugs entering the skin pass through low lipid regions in the cytoplasm of the corneocytes. Corneocytes containing highly hydrated keratin promote an aquatic environment from which hydrophilic drugs can pass. However, this route involves a sequence of partitioning and diffusion into alternating lipophilic and hydrophilic regions, making this pathway very resistant to drug permeation, but typically the interiors of cells are more hydrophilic than the extracellular matrix (Ng & Lau, 2015; Rastogi & Yadav, 2014).

The intercellular route involves drug diffusion through a tortuous pathway along with the lipid matrix, without traversing the cells. This route presents a significant limitation for the following two causes: (1) The intercellular domain is a region of alternating structured bilayers. As a result, a drug must sequentially partition into and diffuse through repeated hydrophilic and lipophilic regions. (2) the "bricks and mortar" model of stratum corneum allows the permeant navigates the tortuous pathway yields by interdigitating the nature of the corneocytes. Small uncharged molecules penetrating the skin generally preferred this route (Fang, Aljuffali, Li, & Fang, 2014; Neupane et al., 2020; Prausnitz & Langer, 2008; Rastogi & Yadav, 2014).

16.3.2 Appendageal pathway

The appendageal route also known as the shunt pathway includes permeation through the sweat glands which are filled with aqueous sweat and the follicular glands with lipoidal sebum. The appendages occupy approximately 0.1%−1% of the total surface area of the human skin and are considered low-resistance shunts (Lane et al., 2012; Rastogi & Yadav, 2014).

16.4 Nanocarriers

The skin offers a convenient and accessible site for drug administration (Alkilani et al., 2015) although its barrier function acts in such a way that it critically limits the absolute quantity of the drug to pass through a considerable area of it during the administration period, making it an important limiting factor in the delivery of therapeutic agents through the skin (Mishra et al., 2019). In this sense, to optimize transdermal drug delivery systems, studies are being performed to describe new vehicles or use sedate bearers to guarantee both the sufficient entrance of the medication and its restriction inside the skin (Alkilani et al., 2015). Nanocarrier systems are one of the significant novel drug delivery systems (Mishra et al., 2017) and are a revolution in the transdermal delivery of therapeutics. These tiny systems are a powerful weapon against many diseases since they can deliver the drug to the target organ and they are

so small to be revealed by the immune system. Consequently, drug doses using nanocarriers and side effects are greatly reduced (Escobar-Chávez et al., 2012).

Nanocarriers have shown several properties include increase absorption of drugs in a given tissue, contributing to protection against the degradation of active drugs, inhibiting premature interaction of drugs with the biological environment, and allowing for higher and more efficient concentrations in the target tissue, as well as a decrease in the severity of undesirable toxic (Manju & Sreenivasan, 2010). They also improve cellular penetration, half-life, bioavailability, control pharmacokinetic and stability of drugs, in addition to their ability to improve drug efficacy via encapsulation of hydrophobic drugs within the core of the nanocarriers (Bhaw-Luximon et al., 2016; Escobar-Chávez et al., 2012). Furthermore, nanocarriers are mostly used as transport agents due to their small size and the potential to modify physical characteristics such as the charge and shape to carry molecule drugs to tissue (Manju & Sreenivasan, 2010) but also they can be linked to specific ligands so that their specificity to target tissues can be enhanced (Esfahani et al., 2018).

On the other hand, they are many factors that affect nano-based delivery systems including zeta potential which is essential for determining the stability of colloidal suspensions of nanoparticles, nanoparticle size by which stability, release, and cellular uptake of a drug are affected, and surface properties by which the surface charge of the nanoparticles affect their attachment to cell membranes (Khogta, Patel, Barve, & Londhe, 2020).

Herbal medicines have been serving as a crucial source of drugs around the globe for the treatment of several diseases since medicine began. They contain phytoconstituents which are hydrophilic but are big molecules and hence not able to cross the lipid membrane thus they show poor aqueous solubility and slow down their biological efficacy and pharmacokinetics. Limitations of using natural products as medicines are absorption, stability, and therapeutic effects which can be overcome by using nanoformulations as a tool to improve drug topical delivery of herbal medicines (Mishra et al., 2017) as well as to enhance the activity and overcome problems associated with herbal drugs (Gunasekaran et al., 2014).

Nanocarriers as part of nanotechnology are one of the key novel drug delivery methods under investigation. These delivery systems have characteristics to deliver both lipophilic and hydrophilic molecules. Advances concerning fabrication, materials, techniques, and methods facilitate the development of new and superior reliability of nanocarriers. However, characterizations of nanocarriers are very critical to control their desired in vitro and in vivo behavior (Jain & Thareja, 2019). For that reason, future research must ensure the benefit and evaluate the risk ratio for many drugs included in nanocarriers (Bhaw-Luximon et al., 2016).

16.5 Methods used in the formulation of nanophytomedicines

A range of different methods can be used in the formulation of nanophytomedicine including the solvent emulsification-diffusion method and the

solvent emulsification-evaporation method, nanoprecipitation, co-precipitation, high-pressure homogenization method, salting out method, self-assembly method and supercritical fluid method, and complex coacervation method (Harwansh, Deshmukh, & Rahman, 2019; Khogta et al., 2020; Mahapatro & Singh, 2011; Sahni, Baboota, & Ali, 2011). It identifies the more specifically used types of nanoformulation approaches for the dermatological and transdermal applications that have been involved as a tool to improve drug topical delivery of herbal medicines like nanoliposomes, nanoemulsions, transferosomes, Niosomes, ethosomes, dendrimers, polymeric nanoparticles, phytosomes, and cyclodextrins can be prepared with the help of the methods mentioned above (Diab, Jaafar-Maalej, Fessi, & Maincent, 2012; Sahni et al., 2011).

16.5.1 Nanoliposomes

Liposomal vesicles are promising drug delivery systems for topical administration (Sinico & Fadda, 2009). Liposomes are classified into various types based on their size including, unilamellar vesicles (further segmented into small unilamellar vesicles with size 20−100 nm and Large unilamellar vesicles with size >100 nm), oligolamellar vesicles with the size 100−500 nm, multilamellar vesicles with size > 500 nm, and giant unilamellar vesicles with size >1000 nm (Akbarzadeh et al., 2013; Anilkumar, Shalumon, & Chen, 2019; Kalepu, Sunilkumar, Betha, & Mohanvarma, 2013). The thickness of the membrane measures approximately 5−6 nm. These sizes and shapes depend on the preparation technique, the lipids utilized, and process variables (Sezer, 2012). The major compositions of liposomes are single phospholipids or a mixture of two or more phospholipids with additionally added cholesterol to enhance the bilayer characteristics of the liposomes (Anilkumar et al., 2019).

Liposomes are small spherical drug delivery systems that can carry both hydrophilic and lipophilic drugs. They can transport hydrophobic drugs between the bilayer and hydrophilic drugs inside the core due to their hollow lipid bilayer structures (Sezer, 2012). Liposomes present distinct advantages due to their structural versatility and their potential to encapsulate various materials. They can encapsulate drugs with large varying lipophilicity or solubility, either entrapped in the aqueous core of the phospholipid bilayer or at the bilayer interface (Aqil, Munagala, Jeyabalan, & Vadhanam, 2013). A liposome is a biodegradable, nontoxic, highly biocompatible vesicle (Sezer, 2012), and can improve solubility and intracellular uptake of the drug or herbal medicine they carry and enhance therapeutic activity and safety in addition to their ability to maintain therapeutic drug levels inside the bloodstream for a long time (Khogta et al., 2020). It protects the incorporated agent from the outer environment or side reaction, its charge, size, and surface properties can be easily changed simply by adding new ingredients, targeting the drug into the cell compartment and it has a lower nephron, cardio, and neurotoxicity (Bandopadhyay, Manchanda, Chandra, Ali, & Deb, 2020; Torchilin, 2005). The in vivo properties of liposomes, as well as their pharmacokinetics, have been investigated in many models. Drugs incorporated into

liposomes do not provoke undesirable immune responses and are not inactivated by biological surroundings (Torchilin, 2005). Various approaches have been developed to load liposomes with various biologically active substances including enzymes, peptides, and deoxyribonucleic acid (DNA). The method of preparation of liposomes depends on the physicochemical characteristics (Bandopadhyay et al., 2020) and follow generally three basic steps: (1) Lipid must be hydrated, (2) Liposomes have to be sized and (3) Nonencapsulated drug has to be removed. The level of transdermal drug penetration is influenced by lipid composition, lamellarity, mode of application, the charge on the liposomal surface, and total lipid concentrations (Sezer, 2012).

16.5.2 Nanoemulsions

Nanoemulsions are pioneering for encapsulating herbal bioactives and have been recognized as a tool for delivering and targeting herbal constituents (Harwansh et al., 2019). They are submicron-sized colloidal frameworks consisting of lipids and an aqueous core, balanced out by a blend of cosurfactants and surfactants (Lucia et al., 2020). Nanoemulsions are homogeneous, thermodynamically stable, and isotropic dispersed systems of two immiscible liquids aqueous and oil phase, which act as carriers for drug delivery (Harwansh et al., 2019). They have a size range from ranges from 5 to 100 nm (Mishra et al., 2017), colloidal frameworks consisting of lipids and aqueous core, balanced out by a blend of surfactants and cosurfactants. There are three kinds of nanoemulsions, which can be classified as water-in-oil nanoemulsion (water droplets are scattered in the continuous oil phase), oil-in-water nanoemulsion (oil is dispersed in the liquid phase), and bicontinuous nanoemulsions (Bandopadhyay et al., 2020). The advantages offered by nanoemulsion are as follow (Bandopadhyay et al., 2020; Harwansh et al., 2019; Jaiswal, Dudhe, & Sharma, 2015; Mishra et al., 2017):

- Nanoemulsions are promising to improve stability, solubility, bioavailability, and permeability of the herbal bioactives, sustained release of drugs, simplicity of manufacture;
- The nanoemulsions can channel the herbal constituents to the particular target site and maintain blood-plasma concentration for a long time;
- Nanoemulsions are non-irritant and non-toxic systems and they can be used for skin, parenteral and non-parenteral administration in general and they have been utilized in the cosmetic field;
- The herbal bioactives can be encapsulated into the nanoemulsion matrix so it enhances their physical stability;
- The oil or lipids are non-mutagenic and a reduced dose of this nanocarrier minimizes associated toxicities and provides better therapeutic effects so nanoemulsions are safe for human health.

Among the disadvantages the following ones are transdermal delivery using nanoemulsions has been reduced due to the stability problem for the long duration

of time due to their small droplet size and the inability of nanoemulsions to solubilize substances that have a high melting point (Bandopadhyay et al., 2020; Escobar-Chávez et al., 2012).

16.5.3 Transferosomes

Transfersomes have been described as exceptionally flexible vesicular drug delivery systems, comprising not less than one internal aqueous compartment encompassed by a phospholipid bilayer for transdermal delivery, by enhancing the skin permeation. Their mode of action uses the hydration and osmotic properties of the skin (Hardenia et al., 2019; Mishra et al., 2017).

Transferosomes have been employed as a promising system for the transdermal delivery of herbal drugs given their ability to pass through the Stratum corneum with an extensive variety of dissolvability as well as to penetrate via intracellular pores of the skin (Escobar-Chávez et al., 2012; Khogta et al., 2020; Pandey, Goyani, Devmurari, & Fakir, 2009). These Transferosomes called also transformable liposomes or deformable vesicles are certified to pass across a narrow constriction smaller than their size and use this approach to achieve the objective of drug delivery via the transdermal pathway. These types of nanoformulations seem to be most effective than liposomes (Escobar-Chávez et al., 2012; Hardenia et al., 2019). The characteristics of this tiny system allow the possibility to enhance in vitro skin delivery of a range of therapeutic agents and in vivo infiltration to accomplish therapeutic effective quantity while also using them as transdermal vaccine vectors (Gandhi, Chaskar, Jadhav, & Salunkhe, 2011). Tranferosomes are easy to prepare but they present limitations concerning the purity of natural phospholipids (Escobar-Chávez et al., 2012), and are chemically unstable due to their oxidative degradation (Bandopadhyay et al., 2020).

16.5.4 Niosomes

Niosomes are an emerging drug delivery system, in which the drug is encapsulated in a vesicle (Alyami, Abdelaziz, Dahmash, & Iyire, 2020). They are very small, and microscopic lamellar structures measuring between 10 and 1000 nm (Khogta et al., 2020). Niosomes are made on the admixture of nonionic surfactant and lipids such as cholesterol with subsequent hydration in aqueous media (Keservani, Sharma, & Ramteke, 2010). They are structurally similar to liposomes but more flexible and maintain drug-targeting advantages over them. The presence of nonionic surfactants in their structure, which are biodegradable and less toxic, allows them to be more chemically stable and overcome the problems of low drug encapsulation usually associated with liposomes (Alyami et al., 2020). Depending on the technique used, niosomes may be unilamellar or

multilamellar. These nanocarrier systems can enhance the skin permeation of drugs, accommodate the drug molecules with a wide range of solubility, and the surfactants used are biocompatible and non-immunogenic (Kazi et al., 2010; Makeshwar & Wasankar, 2013). Also, the fabrication materials for niosomes are less costly than those for fabricating liposomes (Debnath & Kumar, 2015).

16.5.5 Ethosomes

Ethosomes are noninvasive delivery carriers and innovative forms of flexible liposomes comprehending a generally extraordinary concentration of ethanol incorporated into the lipid bilayer that gives this nanosystem its flexibility (Bandopadhyay et al., 2020). Drugs can be incorporated into ethosomes according to their different physicochemical state, that is, hydrophilic, lipophilic, or amphiphilic which give them the ability to reach the systemic circulation and/or the profound skin layers (Bandopadhyay et al., 2020; Nafisi & Maibach, 2017). The size scope of ethosomes vesicles can be modulated from many nanometers to microns (Mishra et al., 2017).

The advantages of ethosomes include the following (Bandopadhyay et al., 2020; Chourasia, Kang, & Chan, 2011; Escobar-Chávez et al., 2012; Mishra et al., 2017):

- Ethosomes are efficient for the enhancement of skin transdermal and dermal permeability;
- Ethosomes are characterized by softness and malleability;
- Ethosomes can deliver a lot of groups of drugs such as peptides and protein molecules;
- Ethosomes can also deliver drugs in semisolid form and fluorescent probe to the skin;

Ethosomes are easy to prepare and no sophisticated technology is used, and they are considered safe and efficient. As a result, they could offer the future a huge opportunity to make better therapies, especially in transdermal delivery.

16.5.6 Dendrimers

Dendrimers are nonpeptidic fractal 3D structures made of highly branched macromolecules with a controlled three-dimensional structure around a simple core unit (Wani, Tarawadi, & Kaul-Ghanekar, 2015). They allow conjugation with numerous functional groups like targeting molecules, imaging agents, and drugs due to the nature of their branching structure (Escobar-Chávez et al., 2012). They improve the solubility, stability, and bioavailability of therapeutic drugs. They are easily prepared and functionalized with superb monodispersity, solvency, and a high potential for surface manipulation. They are successfully used to encapsulate

hydrophobic and labile molecules which act as increasing permeation of lipophilic drugs through biological membranes. The permeability of dendrimers through transdermal and dermal skin depends on physicochemical characteristics such as surface charge, generation size, molecular weight, composition, and concentration (Escobar-Chávez et al., 2012; Svenson, 2009). Finally, these nanocarriers have been used to transport photosensitizers and explored for drug delivery, gene therapy, and delivery of contrast agents (Uchegbu, Dufès, Kan, & Schätzlein, 2008) but have also been investigated to assess toxicity and biocompatibility (Duncan & Izzo, 2005).

16.5.7 Nanoparticles

Colloidal particles ranging in size from 10 to 1000 nm are known as nanoparticles. They can be synthesized from proteins, lipids, and carbohydrates, as well as several natural and synthetic polymers. They are constructed from materials designed to resist pH, temperature, enzymatic attack, or other problems (Escobar-Chávez et al., 2012). For delivery, a drug is dissolved, entrapped, encapsulated, or attached to a nanoparticle matrix (Aqil et al., 2013). Nanoparticles have been used successfully for vaccine development and the treatment of diseases because they are chemically stable and reproducible. They can be made of a lot of biodegradable materials and they also can include antibodies on their surface to reach target organs. Furthermore, both hydrophilic and hydrophobic drugs can be incorporated into nanoparticles, in addition to their ability to avoid the immune system due to their size (Escobar-Chávez et al., 2012).

Polymeric nanoparticles are used to deliver therapeutic agents for various types of cancers, bone healing, and vaccination. They are biocompatible, non-toxic, and biodegradable and they can be equipped with smart components to allow their delivery beyond certain biological barriers like the skin (Elsabahy & Wooley, 2012). Various polymeric nanocarriers consisting of polyethylène glycol (PEG), polylactide-*co*-glycolide (PLGA), poly ε-caprolactone (PCL), pluronic have been used for efficient delivery and uptake of herbal bioactive compounds (Wani et al., 2015).

Solid-lipid nanoparticles are submicron colloidal carriers, they offer interesting characteristics, for example, huge surface area, small size, high drug loading, and the interaction of phases at the interfaces, and are appealing for their potential to improve the execution of pharmaceuticals Hanumanaik (Lingayat, Zarekar, & Shendge, 2017; Mehnert & Mäder, 2012). They have also long-term stability and better control over the release kinetics of encapsulated compounds (Mishra et al., 2017).

16.5.8 Phytosomes

Phytosomes are a drug delivery system for polyphenolic phytoconstituents (Singh, Saharan, Singh, & Bhandari, 2011). They are little cell-like structures that result

from complexing polyphenolic phytoconstituents (like flavonoids, terpenoids, tannins, xanthones) with phospholipid (like phosphatidylcholine, phosphatidylserine, etc.) mainly phosphatidylcholine which binds components to each other on a molecular level (Jain et al., 2010). These phytosomes have the following advantages (Bombardelli, Della Loggia, Sosa, Spelta, & Tubaro, 1991; Kidd & Head, 2005; Singh et al., 2011):

- Bioavailability is enhanced due to their potential to cross the lipid-rich biomembranes and to protect.
- plant extracts (drugs) from destruction by digestive secretions and gut bacteria.
- Phytosomes can deliver standardized plant extracts and phytoconstituents through several routes of drug administration.
- Phytosomes improve the absorption of lipid-insoluble polar phytoconstituents through topical pathways showing better bioavailability, hence significantly greater therapeutic benefit.
- Phoshatidylcholine used in the preparation of phytosomes, as well as acting as a carrier also provides several therapeutic properties.
- Phytosomes show high stability profile since phytoconstituents molecules and phosphatidylcholine are chemically bonded.

16.5.9 Cyclodextrins

Cyclodextrins (CDs) are Chemically a group of cyclic oligosaccharides, formed by α-1,4-linked glucose units, with a lipophilic central cavity and hydrophilic outer surface (Bombardelli et al., 1991). CDs are non-toxic compounds and, of the three natural products, α-Cyclodextrin (αCD), β-cyclodextrin (βCD), and γ-cyclodextrin (γCD). The widely used in pharmaceutical applications is β-CD (Challa, Ahuja, Ali, & Khar, 2005). CDs conjugated nanoparticles have shown wide proprieties such as improved aqueous solubility of poorly water-soluble, targeted drug delivery, and increased bioavailability, safety, and stability (Loftsson, Jarho, Másson, & Järvinen, 2005). In addition to their well-known effects on encapsulation efficiency, drug solubility, and dissolution, and used in the preparation of various delivery carriers, such as liposomes, microcapsules, and nanoparticles. CDs offer distinctive advantages due to their unique ability to form inclusion complexes with a variety of organic and inorganic lipophilic molecules (Challa et al., 2005; Shelley & Babu, 2018). CDs can change biologically-active compounds that lack drug-like physiochemical properties into therapeutically-effective drugs. However, the type of CD and the amount and composition of pharmaceutical excipients involved in pharmaceutical preparations must be carefully selected (Saokham, Muankaew, Jansook, & Loftsson, 2018).

16.6 Applications of nanophytomedicines

16.6.1 Nanophytomedicines as anticancer drugs

Many drugs currently in use are either derived directly from natural sources or are chemical analogs of natural molecules. The main handicap in harnessing the previously unrealized potential of phytomedicines has often been the poor response in vivo due to their low solubility and stability, low lipid solubility, non-uniform particle size, poor absorption and bioavailability, destruction of some juice extracts (during gastric emptying), rapid clearance and biotransformation (Bilia et al., 2018). The treatment of cancerous tumors is complicated by their microenvironment and the abnormality of the blood vessels that feed them. The search for methods to improve the delivery of drugs to treat tumors and enhance the efficacy of cancer treatment remains a major challenge for current researchers (Ansari, Islam, & Sameem, 2012). Scientists have discovered nanoparticles that can be used as targeted drug delivery for cancer therapy where only the cancer cells will destroy without affecting healthy normal cells. It is also used to reduce the side effects as well as to achieve high localized concentration (Min et al., 2008). Paeonol (Pae; 2′-hydroxy-4′-methoxyacetophenone) has attracted intense attention as a potential therapeutic agent against various cancers, but its use is limited due to its hydrophobicity. Chen et al., prepared Pae-charged nanoparticles (Pae-NPs) with amphiphilic block copolymers using nanoprecipitation. The physicochemical characteristics and antitumor effects of nanoparticles were evaluated in various cancer cells. In vitro results show that even lower doses of Pae-NPs inhibited cell growth more effectively than equivalent doses of free Pae, also in vivo demonstrated that Pae-NPs could exert much stronger antitumor effects than free Pae. Therefore, Pae-NPs represent a promising delivery system to overcome the low solubility of Pae and enable its use in cancer treatment (Cong, Feng, Zhibo, Shu, & Qibin, 2017). In another study, Camptothecin (CPT), a natural plant alkaloid extracted from *Camptotheca acuminate*, an insoluble anticancer drug, was encapsulated in nanoparticles of chitosan glycol (HGC) by a dialysis method. the results show significant inhibition of tumor growth after injection iv of nanoparticles of CPT-HGC at doses of 10 and 30 mg/kg, compared to free CPT at the 30 mg/kg dose. Significant antitumor efficacy of CPT-HGC nanoparticles (Min et al., 2008). Tanshinone IIA (TA), is one of the fat-soluble active components extracted from the root of *Salvia miltiorrhiza* bunge, a traditional Chinese medicine. Chang et al. have adapted an optimal method for preparing TA nanoemulsions (TA-NE) to solve the problems of the low solubility of TA in water and insufficient dissolution rate. TA-NEs displayed more potent cytotoxicity than TA alone in human bladder cancer cells (Chang et al., 2011). Another plant *Gelidiella* sp. was used to synthesize silver nanoparticles and tested against human cancer cell lines Hep-2. The result showed that Hep-2 cell proliferation was significantly inhibited by silver nanoparticles (AgNPs) with an IC50 value of 31.25 μg/mL of the concentration (Saraniya, Bhimba, & Ratnam, 2012). In another study, AgNPs were synthesized using *ulvalactuca* and their cytotoxicity was

assessed against Hep-2, HT-29, MCF-7, and Vero cell lines. The result showed that Hep-2 cells proliferation was significantly inhibited by AgNPs with an IC50 value of 12.5 μg/mL of the concentration, MCF-7 cells with an IC50 value of 37 μg/mL of the concentration, and HT-29 cells with an IC50 value of 49 μg/mL of the concentration. It can be concluded that the synthesized AgNPs induce a cell-dependent inhibition concentration (Devi & Bhimba, 2012). Silver nanoparticles synthesized with *Citrullus colosynthis* root extract were tested against four human cancer cell lines, MCF-7 (breast carcinoma), HepG2 (hepatocellular carcinoma), and Caco-2 and HCT-116 (breast carcinoma). The cell viability test of these silver nanoparticles on human cancer cells showed that the Hep-G2 cell line and the HCT-116 cell line were the most sensitive cell line with IC50 21.2, 22.4 μg/mL, respectively. Where, as cytotoxic activity on MCF-7 and Caco-2 cell lines was not significant (IC50 > 30 μg) (Shawkey, Rabeh, Abeer, & Ashraf, 2013). It has also been reported that ZnONPs is capable of inducing significant selective toxicity against tumor T cells while no harm is being experienced by the normal body cell 130, some scientists investigated the anticancer activity of ZnONPs photosynthesized using *Mangifera indica* leaf extract against the growth of lung cancer (A549) and proved biosynthesized ZnONPs as a good anticancer agent because cytotoxic effects comparable to that of cyclophosphamide at low doses were recorded (Akintelu & Folorunso, 2020). In another study, silver nanoparticles extracted from *Malus domestica* and *Origanim vulgare* were used. AgNPs extracted from *M. domestica* have been shown to have a dramatic effect on MCF-7 which are breast cancer cells (Mariadoss et al., 2019). While the silver nanoparticles extracted from *O. vulgare* show a dose-dependent response against human lung cancer (A549 cancer cells) (Sankar et al., 2013). Recently, silver nanoparticles produced from the leaf extract of *Vitex negundo* and *Sesbania grandiflora* were tested against human colon cancer cells (HCT15 and MCF-7). *V. negundo* shows an antiproliferative effect on cancer cells, reducing DNA synthesis and inducing apoptosis (Prabhu, Arulvasu, Babu, Manikandan, & Srinivasan, 2013). Similarly, the silver nanoparticles obtained from *S. grandiflora* also cause cytotoxicity, oxidative stress, as well as apoptosis of tumor cells (Jeyaraj et al., 2013).

16.6.2 Nanophytomedicines as antidiabetic drugs

Diabetes is one of the most challenging diseases increasing in its prevalence worldwide. The overriding need today is to develop nanodrugs (carbohydrate hydrolyzing enzyme inhibitors) that can facilitate the production and application of materials to interact with the human body at a molecular level with a high degree of specificity, designed to achieve maximal therapeutic efficacy with minimal to no side effects (Saratale et al., 2017). The study and use of plants with antidiabetic qualities are of increasing interest due to their low toxicity and economic viability. Nanoparticles green synthesis is not time-consuming compared to

other biological processes (Shawkey et al., 2013). Syntheses of AgNPs from plant extracts also possess substantial antidiabetic activities have been reported (Balan et al., 2016; Kumar, Singh, Srivastava, Bhadouria, & Singh, 2019; Rajaram, Aiswarya, & Sureshkumar, 2015; Saratale et al., 2017; Vishnu & Murugesan, 2014; Yousefi et al., 2015).

The in vitro antidiabetic activity of biosynthesized silver nanoparticles from *Colpomenia sinuosa* was based on the evaluation of the inhibitory activity of the two enzymes α-glucosidase and α-amylase which are involved in human digestion. the results show that the inhibitory effect of α-glucosidase was somewhat remarkable than the effect of α-amylase, with an IC50 of 385 ± 0.02 and 490 ± 0.02 mg/mL, respectively, and significantly more potent than the acarbose molecule ($P < .05$) with an IC50 of 695 ± 0.01 mg/mL and 630 ± 0.01 mg/mL respectively for the two enzymes (Vishnu & Murugesan, 2014). Another study was designed and performed, where "green" chemistry was employed for the synthesis of AgNPs using an extract from the leaves of *Holoptelea integrifolia* (HI), the antidiabetic properties of the synthesized green AgNPs were tested using the α-amylase test. The antidiabetic property of synthesized green AgNPs has been shown to increase in a dose-dependent manner. At the highest concentration (100 μL), the observed % inhibition of extract AgNPs and acarbose was $71.28\% \pm 4.33\%$, $86.66\% \pm 5.03\%$, and $95.01\% \pm 5.41\%$ respectively (Kumar et al., 2019). A study deals with the synthesis and characterization of AgNPs using the aqueous leaf extract of *Lonicera japonica* and their antidiabetic activity. Antidiabetic targets such as α-amylase, and α-glucosidase. The antidiabetic ability of AgNPs was shown by the effective inhibition against carbohydrate digestive enzymes such as α-amylase and α-glucosidase with IC50 values of 54.56 and 37.86 mg/mL, respectively (Balan et al., 2016). Similar to results obtained in a previous report (Rajaram et al., 2015; Saratale et al., 2018). Other researchers suggest that novel synthesis of silver nanoparticles (AgNPs) using *Punica granatum* leaf extract (PGE) has shown effective inhibition against α-amylase and α-glucosidase (IC50; 65.2 and 53.8 μg/mL, respectively) (Saratale et al., 2018). Comparable results were observed in the literature (Balan et al., 2016; Rajaram et al., 2015).

Another plant *Chamaecostus cuspidatus* was used to synthesize gold nanoparticles, their hypoglycemic effect in a normal diabetic model and induced by streptozotocin was evaluated. Results show decreased glucose levels in diabetic rats, suggesting that the ability of synthesized gold nanoparticles exerts an insulin-like outcome on marginal tissues either by promoting glucose uptake or by inhibiting hepatic gluconeogenesis (Ponnanikajamideen, Rajeshkumar, Vanaja, & Annadurai, 2019). The biogenic AgNPs synthesized by *Halymenia poryphyroides* showed antidiabetic efficacy in vitro with an increase in the percentage (%) of inhibitory activity of $91.30\% \pm 0.02\%$ against the α-amylase enzyme and of $89.10\% \pm 0\%$, 01% against the α-glucosidase enzyme (Vishnu & Murugesan, 2013). Likewise, silver nanoparticles (AgNPs) extracted from *Argyreia nervosa* leaves extract (ANE) showed significant inhibition of α-amylase and α-glucosidase with EC50 of 55.5 and 51.7 μg/mL,

respectively, indicating its antidiabetic potential (Saratale et al., 2017). These outcomes are consistent with previous findings (Yousefi et al., 2015). We can therefore conclude after all these data that nanomedicines, therefore, improve the benefit/risk ratio of drugs by increasing their efficacy and bioavailability in the target tissue or organ while reducing the doses to be administered and the risk of toxicity.

16.6.3 Anti-schistosomal activity

Schistosomiasis is an acute and chronic parasitic disease caused by trematodes of the genus *Schistosoma*. It affects around 200 million people (Steinmann, Keiser, Bos, Tanner, & Utzinger, 2006). schistosomiasis control programmers are based mainly on chemotherapy (Gray et al., 2010). This treatment presents criticisms and some limits, such as the risk of re-infection and resistance.

Plants offer a wide range of bioactive compounds with schistosomiasis activity. Several studies show that nanoemulsions containing volatile oils are an important alternative for the control of schistosomiasis, including anti-*Schistosoma* and molluscicide activities (Aly, Husseein, Emam, & Rashed, 2017; Araújo et al., 2019; Mokbel, Baiuomy, Sabry, Mohammed, & El-Dardiry, 2020; Passos, dos Santos, Rocha, & Albuquerque, 2020). Araújo et al. (2019) investigated the effect of nanoemulsion from leaves of a *Xylopiaochrantha* on mollusks of the genus *Biomphalaria* (snail hosts of *Schistosoma mansoni*). This nanoemulsion caused mortality in *Biomphalaria tenagophila, Biomphalaria straminea, Biomphalaria glabarata* of different sizes at levels ranging from 50% to 100% in 48 h. Additionally, the formulation inhibited the development of deposited eggs (Araújo et al., 2019). In 2017, anti-helminthic effects against *S. mansoni* cercariae, schistosomules, and adults were also demonstrated by the work of (Aly et al., 2017), hey produced nanoemulsions based on an ethanol extract of crude powder of *Curcuma longa* rhizome. The nanoemulsion presented the schistosomicidal effects on cercariae and 24 h-old schistosomules of *S. mansoni*. In addition, it exhibited optimal activity against the adult stage with the decreased motor activity of the worms (Aly et al., 2017). More recently, Mokbel et al. (2020), evaluated the anti-schistosomal activity of curcumin and curcumin-loaded gold-nanoparticles (Cur-GNPs) with or without praziquantel (PZQ) against worms and eggs. According to this study, the result showed that the Curcumin caused a significant reduction in the worms and egg count (45.45%) in the third week. Cur-GNPs combined with PZQ 97.4% reduction of worm burden in the third week and the highest reduction in the intestinal and hepatic egg content. this combination alters the hematological, biochemical, and immunological changes induced (Mokbel et al., 2020).

Passos et al. (2020) published a work about the activity of *Ocotea pulchella* leaves EO nanoformulation, in the control of the schistosomiasis cycle, against adult *Biomphalaria glabrata*. The result showed that the nanoemulsion caused mortality of adult *B. glabrata*, its egg embryos, and *S. mansoni* (Passos et al., 2020).

16.6.4 Leishmanicidal activity

Leishmaniasis is one of the most important vector-transmitted diseases. The current treatments are limited due to high toxicity, high cost, and parasitic resistance (Macêdo et al., 2020). The research of new leishmanicidal drugs without these limitations is necessary. Hence, nanoemulsions are promising strategies for these Leishmanicidal activities.

In 2016, antileishmanial activity against *Leishmania amazonensis* promastigotes and amastigotes was demonstrated by the work of da Silva Santos et al. (2016). They developed the nanoemulsions based on the *Pterodonpubescens* fruit extracts. The nanoemulsions showed a better selectivity index and significant activity against parasites (IC50: 2.7 μg/mL for nanoemulsion of hexane extract; IC50: 1.9 μg/mL for nanoemulsion of supercritical extract) compared to the Miltefosine standard (0.7 μg/mL) (da Silva Santos et al., 2016).

Shokri et al. (2017) demonstrated the antileishmanial activity of *Lavandula angustifolia* and *Rosmarinus officinalis* medicinal plants' essential oils and nanoemulsions on *Leishmania major*. The result showed that both essential oil and the nanoemulsion of Lavander and Rosemary showed antileishmanial activity on promastigote with IC50 = 0.11 μL/mL, IC50 = 0.26 μL/mL, and IC50 = 0.08 μL/mL respectively. The nanoemulsions of both plants were more effective than essential oil (Shokri et al., 2017). In addition, de Moraes, Tavares, Soares Rocha, de Paula, and Giorgio (2018), evaluated the activity of nanoemulsions prepared with essential oils of copaiba- and andiroba against *Leishmania infantum* and *L. amazonensis* in vivo and in vitro. the result showed that the Copaiba- and andiroba-based nanoemulsions exhibit activities against *L. infantum* and *L. amazonensis* and reducing parasite burden in the spleen and liver and improving histopathological features (de Moraes et al., 2018).

16.6.5 Larvicidal and insecticidal activity

Mosquitoes serve as vectors of several diseases, some of them such as dengue, malaria, and West Nile virus (WNV), causing serious health problems for humans (Michaelakis et al., 2009). Mosquito control has relied largely on the use of chemical larvicidals.

The insecticidal activities of nanoemulsified essential oils against *Culex pipiens* were determined in several studies. Azmy et al. (2019) demonstrated the activity of the nanoemulsionated EO from *Cirtus sinensis* (prepared by the ultrasonic method) against larvae of *C. pipiens*, The Larvicidal activity of the formulated nanoemulsion was more toxic and with LC50 was 27.4 ppm (Azmy et al., 2019). In 2020, Mohafrash, Fallatah, Farag, and Mossa (2020) showed the application of *Mentha spicata* essential oil (EO) nanoemulsion against *C. pipiens*, and housefly, *M. domestica*. presented a high larvicidal activity against *C. pipiens*

(LC50, 43.57 μg/mL) and *M. domestica* (LC50, 65.13 μg/mL) compared with normal oil and lambda-cyhalothrin insecticide (Mohafrash et al., 2020). In the same year, Jesser et al. (2020) demonstrated that the nanoemulsion obtained based on essential oils of *Geranium maculatum* enhance the toxicity of geranium EO against larvae of *C. pipiens* (EO LC50 0Oiensnsionv NEs LC50 0siensnsionv) and *Plodia interpunctella* (EO + β-cypermethrin LD50 0O + β μg larvae − 1, NEs + β-cypermethrin LD50 0s + β-cypermethri) (Jesser et al., 2020). More recently, El Gohary, Farag, El-Sayed, Khattab, and Mahmoud (2021), evalueted the larvicid activity of both the two oil forms (*Syzygium aromaticum* bulk EO and encapsulated EO), against third instar larvae of *C. pipiens*. The result showed that the Encapsulated nanoformulation EO presents higher toxicity (LC50 = 20 ppm) than the bulk EO (LC50 = 39 ppm) (El Gohary et al., 2021).

Other scientific groups also have conducted studies on *Aedes aegypti*, one of the main dengue-transmitting mosquitoes. de Oliveira et al. (2020) exhibit that the nanoemulsion loaded with volatile Oil from *Piper alatipetiolatum* was more effective than the volatile oil, and interrupt the development of immature forms of *A. aegypti*, the nanoemulsion presented higher ovicidal activity (47.7%−100%), larvicidal activity (LC50 6.37 ppm), and pupicidal activity (LC50 9.33 ppm) against *A. Aegypti* de Oliveira et al. (2020). In addition, Faustino et al. (2020) described a similar effect on *A. aegypti* larvae, in this work, after 72 h of application the nanoemulsion from the *Protium heptaphyllum* Resin to *A. aegypti* larvae, the nanoemulsion showed larvicidal action with LC50 = 2.91 μg/mL (Faustino et al., 2020). Similarly, Lucia et al. (2020) showed that the Nanoemulsions based on thymol-eugenol mixtures presented larvicidal activity against *A. aegypti* (Lucia et al., 2020). More recently, in 2020, larvicidal activity against *A. aegypti* was also demonstrated by the work of Suresh et al. (2020). They investigated the insecticidal effectiveness of the seed essential oil of *Crithmum maritimum* L. and its encapsulated forms (nanoemulsion) against larvae of the mosquito *A. aegypti* and cotton leaf worm *Spodoptera litura*. The results noticed that the nanoemulsion showed significant toxicity against larvae and pupae of *A. aegypti* and *S. litura*. In addition, reducing the longevity and fecundity in both targets (Suresh et al., 2020).

Many researchers explore essential oils-based nanoemulsions against various species of insects. Heydari, Amirjani, Bagheri, Sharifian, and Sabahi (2020), elucidate the effects of nanoemulsion formulations of *Mentha piperita* on the *cotton aphid*. The result showed that the nanoemulsion formulations presented high contact toxicity (the average value of LC50 was about 3879.5 ± 16.2 μla.i./L) against the *cotton aphid* (Heydari et al., 2020). In 2020, Benelli et al. (2020) also evaluated their toxicity against first instar larvae of *Lobesia botrana*, a major vineyard pest. According to this study, the result of toxicity assays, both the *Carlina acaulis* EO and the *C. acaulis*-based NE were highly toxic to *L. botrana* larvae, with LC50 values of 7.299 and 9.044 μL/mL for *C. acaulis* EO and NE, respectively (Benelli et al., 2020). The impact of sub-lethal concentrations of *Pimpinella anisum* L. EO and nanoemulsion was evaluated on enzymatic and macromolecular parameters of the red flour beetle *Tribolium castaneum*, by Hashem et al. (2020).

The result provided an insight into the structure and interactions of ALT and AST protein with essential oils as Nano/Bio-insecticides and demonstrates that the aniseed EO-based nanoemulsion effect is stronger than that of EO (Hashem et al., 2020). In another study, the abamectin-loaded nanoemulsion (2% abamectin and 5% castor oil polyoxyethylene (EL-40)), had more effectiveness against to third-instar larva of *Plutella xylostella* and presented the lowest LC50 (0.0686 mg/L), causing the larval body to blacken and shrivel (Feng et al., 2020). Also, Venunathan (2019), demonstrated the larvicidal activity of Nanoemulsion of *Acorus calamus* L. against *Pulse Beetle* at a lower concentration (Venunathan, 2019).

The insecticidal activity of nanoemulsions containing essential oil of lemongrass or eucalyptus was evaluated by the Topical Application Method and Exposure Impregnated Paper Exposure, against adult flies (*M. domestica* and *Lucilia cuprina*). The results demonstrate a potential insecticidal effect of nanoemulsions of lemongrass oil in concentrations of 10, 30, and 50 μL/mL against *M. domestica* and *L. cuprina* (Velho et al., 2020). Recently, in 2020, the insecticidal activity of nanoemulsion of *Piper aduncum* extract was evaluated against cabbage head caterpillar *Crocidolomia pavonana* F. (Lepidoptera: Crambidae). The results showed that nanoemulsions caused mortality of *C. pavonana* larvae, and also interfered growth and development of *C. pavonana* larvae (Erlina, Lina, Reflinaldon, Djamaan, & Arneti, 2020). Also, Nascimento et al. (2020) demonstrated the insecticide activity of the essential oil from leaves of *Ocotea elegans* Mez and its nanoemulsion against *D. peruvianus* (Nascimento et al., 2020).

The nanoemulsions containing volatile oils are an important alternative for the control of vectors that are important in the cycle of disease transmission and relevant activity against pathogens. However, these nanoemulsions act by the production of (Reactive oxygen species) (ROS) which disrupt bodily functions and stimulate the immune system and alter gene expression leading to altered protein. in addition, NPs affect the pigmentation and integrity of the cuticle. The smaller the NP size, the greater the toxicity and penetration into the insect's body, and the mechanisms of NP−insect interactions (Shahzad & Manzoor, 2021).

16.6.6 Schizophrenia and psychic disorders

This work attempts to spotlight recent trends and highlight novel approaches to the administration of herbal medicine relating to the development of nanophytomedicines and their applications in the treatment of various diseases, notably schizophrenia and other mental disorders.

Mental health is an essential parameter that reflects the emotional, psychological, and social well-being of an individual. At present, the general population is under daily pressure in their professional and personal life resulting in continuous stress in their daily life, thus leading to an imbalance of the conscious being resulting in mental disorders, accompanied by pessimistic thoughts, a feeling of

hopelessness, and helplessness, and in some cases suicide. Likewise, schizophrenia is considered a pernicious mental disorder that affects approximately 1% of the world's population. Antipsychotic medication is the first line of treatment for symptom management in patients with schizophrenia. Unfortunately, some people are on treatment for schizophrenia who may develop resistance to the monotherapy, in the case of drug-resistant or refractory schizophrenia.

To this purpose, combined pharmacotherapy is increasingly used and demanded nowadays for the treatment of various diseases such as schizophrenia, depression, multi-drug resistance, etc.

Currently, clozapine is well known to be the only drug approved by the USFDA for the treatment of refractory schizophrenia. However, some patients may still not respond to clozapine, so another alternative has also been considered, involving the combination of antipsychotics and herbal medicines to improve the prognostic factor.

In this sense, it should be noted that the conventional combined administration of antipsychotics poses certain limitations, such as drug-related toxicity, adverse drug effects, difficulty in crossing the blood-brain barrier, etc. Hence, to overcome the drawbacks associated with conventional therapies, the interest needs to explore new approaches to optimize the administration of antipsychotics and herbal drugs *via* the development of systems based on nanotransporters such as polymer nanoparticles, lipid carriers nanostructures, solid lipid nanoparticles, liposomes, and polymeric micelles to deliver them to their target site by overcoming biological, biophysical, and biomedical barriers.

This work summarizes the combination of antipsychotics with other antipsychotics and herbal drugs and their delivery to the brain using nanotransporters for the management of schizophrenia and related disorders.

A very recent research review was conducted (Baboota & Ali, 2020) based on a detailed study of a hundred articles on the study of the combination of antipsychotics with other herbal drugs, as well as their potential for improving therapeutic effect and patient compliance. In addition, other research studies have been conducted involving the use of nanotransporters, to optimize the delivery of combinatorial antipsychotics and thereby increase their therapeutic efficacy in schizophrenia and related disorders.

16.7 Combination of antipsychotics with herbal drugs

In recent years, the use of herbal additives has become increasingly important in the treatment of psychiatric illnesses. Herbal medication used in combination with antipsychotics may reduce its side effects. This work will be devoted to the discussion of the various clinical trials carried out involving the use of herbal medicinal products associated with antipsychotics, notably clozapine (Kane & Correll, 2010; Wang et al., 2015).

In this context, a preclinical study conducted by Tian, Wang, Wang, Sze, and Zhang (2016) to assess (in vivo) the effects of the concomitant use of herbal

medicines such as *Fructus Schisandrae*, *Radix Rehmanniae*, *Radix Bupleuri*, and *Fructus Gardeniae* in combination with clozapine on the pharmacokinetics of clozapine. To do this, rats were injected intraperitoneally with clozapine at a rate of 10 mg/kg alone and in combination with the aforementioned plants orally. The results of this study showed that the formation of norclozapine and the active N-oxide metabolite of clozapine is found to be reduced in acute treatment, without having any effect on the pharmacokinetics of clozapine. Whereas, under chronic treatment, no significant changes in the pharmacokinetics of clozapine and its metabolites have been observed. Overall, the herbs used in this study were shown to have limited interaction effects on the pharmacokinetics of clozapine whether during acute or chronic treatment, and may maintain the therapeutic effect of clozapine and help reduce its side effects. Moreover, to explore the pharmacodynamic parameters relating to possible drug interaction, another study carried out by Lei et al. (2017) showed the potential of the use of the substitute of *Calculus bovis* (*C. bovis sativus*) in combination with haloperidol to reduce the dose of haloperidol while maintaining its therapeutic efficacy compared to the use of haloperidol alone.

This study was conducted in schizophrenic rats induced by MK-801, during which the researchers observed the pharmacodynamic effects of haloperidol in combination with the substitute *C. bovis*. During this study, a determination of the plasma concentrations of haloperidol by the intragastric and intravenous route was carried out in these rats after the oral administration of a single dose of the *C. bovis* substitute (CBS) or after one week of pretreatment with the substitute of *C. bovis* (50 mg/kg). Therefore, the combination of haloperidol with CBS may lead to a significant reduction in locomotor activity and an increase in the percentage of center distance compared to the use of haloperidol alone.

Besides, in the co-administered oral groups, the results showed that the area under the curve and the peak drug concentration were significantly higher compared to treatment with haloperidol alone. This led to the conclusion that CBS led to an improvement in bioavailability and has a synergistic effect on haloperidol (Baboota & Ali, 2020). Other studies have shown the effects of the P-glycoprotein (P-gp) substrate on the pharmacokinetics and pharmacodynamics of drugs.

Another study conducted on rats by Lee et al. (2013) evaluated the effects of silymarinon the pharmacokinetics of the P-glycoprotein (P-gp) substrate, risperidone, and metabolites of risperidone. Consequently, after the concomitant administration of risperidone (6 mg/kg) and silymarin (40 mg/kg), the maximum concentration of the metabolite of risperidone was increased to 1 and 3 times, nevertheless, for other pharmacokinetic parameters there was no significant difference.

However, repeated administration of silymarin for 5 days before risperidone, showed a 2.4-fold and 1.7-fold increase in risperidone and 9-hydroxyrisperidone Cmax, respectively, 1.7-fold and 2.1-fold increase in AUC0-t. Thus, these findings suggest that silymarin repeated exposure increased the oral bioavailability of risperidone and 9-hydroxyrisperidone by inhibiting Pgp.

Another randomized, triple-blind controlled study was conducted by Parvizi et al. (2019) to evaluate the effect of the Persian herbal medicine *Cuscuta*

epithymum in combination with risperidone on cognitive impairment and clinical symptoms in patients treated for schizophrenia. During this study, groups of patients received an appropriate dose of risperidone with 500 mg of the Persian herbal medicine *C. epithymum* in capsule form twice a day. The results demonstrated a significant improvement in cognitive, negative, and positive symptoms after 8 weeks of treatment compared to the placebo formulations of the Persian herbal medicine *C. epithymum*.

Thus, the data from different studies have made it possible to conclude that herbal medicine can have promising results for its use in combination with antipsychotics allowing an improvement of their therapeutic efficacy, the reduction of the side effects of antipsychotics, and the improvement of the adherence in schizophrenic patients resistant to antipsychotic therapy.

16.8 Nanotechnology for combinational delivery of antipsychotics

Over the past decades, many studies have been carried out (Patel, Mundada, & Sawant, 2019; Theochari, Xenakis, & Papadimitriou, 2020) on nanotransporters to show the potential of these nanoscales (10−200 nm) materials containing safe and biodegradable materials to alleviate problems associated with antipsychotic therapy such as low therapeutic efficacy, low oral bioavailability, peripheral drug toxicity, and patient compliance. Furthermore, a study showed recent advances in nanotechnology thus promoting the delivery of drugs to specific human tissues or to cellular targets beyond biological barriers whatever the mode of administration, either by oral, parenteral, transdermal, topical, ocular, or nasal route (Theochari et al., 2020). Another research conducted by Mishra, Patel, and Tiwar (2010) showed that nanotransporters might be a better vector for drug delivery, by protecting the drug from degradation in the gastrointestinal tract, the release of the drug in a controlled manner, and the modification of the internalization pathways. Moreover, MH Patel and co-workers showed the important role of various types of nanotransporter systems such as polymer nanoparticles, solid lipid nanoparticles, nanostructured lipid transporters, liposomes, polymeric micelles, and nanoemulsions, etc. in the combined administration of antipsychotics in the treatment of schizophrenia and associated disorders due to their security and biocompatibility (Patel et al., 2019). Unfortunately, due to the lack of scalable industrial techniques and stability issues over long-term storage, pharmaceutical industrial development of nanotransporters is still limited.

16.9 Perspectives and conclusions

Nanophytomedicines are particles that can deliver drugs including phytocompounds and can therefore resolve the problem of penetration and availability of

herbal medicine for several therapeutical applications. In this chapter, it has been shown that these nanocarriers positively showed remarkable results for improving therapeutics via the delivery of herbal medicine. However, these applications should be investigated for several applications in vivo and in vivo. Moreover, further studies concerning clinical trials should also be carried out to validate the clinical applications of these nanophytomecines.

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CHAPTER

Multifunctional nanocomposites for theranostics

17

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17.1 Introduction

Multifunctional nanosystems that associate diagnostic and therapeutic modalities are a novel trend in nanotechnology. Progress in nanotechnology expressively influenced the therapy and detection of illnesses (Ding et al., 2015; Li, Deng, Peng, & Wang, 2014; Liu et al., 2014; Muthu, Leong, Mei, & Feng, 2014). It is known that, before treatment, clinical diagnosis is a critical step that significantly affects therapy outcome and overall survival. Recently, clinicians are targeting to accomplish diagnosis and therapy simultaneously, thus, multifunctional nanosystems including nanocomposites offer a platform for theranostics to concurrently achieve diagnosis and treatment (Sumer & Gao, 2008), making possible the monitoring of drug localization and image the biological outcome of a therapeutical approach (Fig. 17.1).

The theranostic strategies can be produced by using several different approaches including solid lipid nanoparticles, micelles, dendrimers, silica nanoparticles, magnetic nanoparticles, quantum dots, and polymeric carries that can achieve improved synergistic effects and diminished side effects when compared to drugs without a drug delivery system (Jing et al., 2014; Li et al., 2013; Peng et al., 2015). The main aim of theranostics is to enable the diagnosis and treatment at the earliest stage of diseases. In this chapter, various nanocomposites designed for imaging as well as for the controlled release of drugs are described.

17.2 Nanocomposites

A nanocomposite is a two-phase structure, where, at least one constituent must present a nanosized dimension up to 100 nm (Zhang, Le, Wang, & Chen, 2011). A key property of a nanocomposite is a large surface area which results in higher interaction

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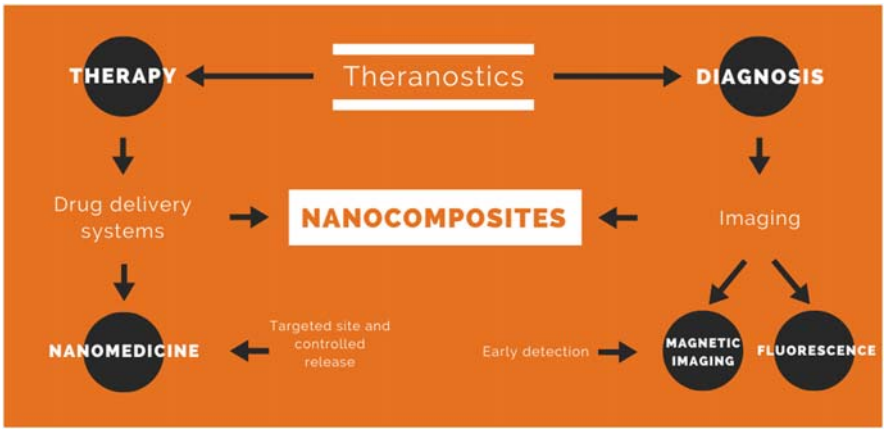

FIGURE 17.1

Nanocomposites as multifunctional drug delivery systems for theranostic approaches.

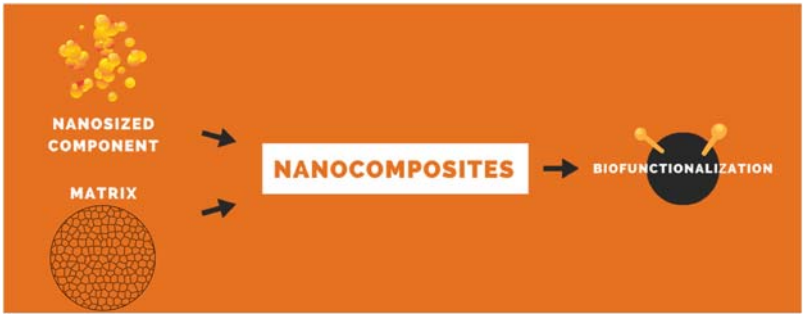

FIGURE 17.2

Nanocomposites are two-phase structures composed of a matrix and a nanosized component. The generated nanocomposite may be further biofunctionalized to increase therapeutic and diagnostic efficacy.

between its nanocomponents with the matrix (Payghan, 2014). The nanocomposite approach can avoid nanoparticle agglomeration by using a matrix where the nanoparticle can be dispersed (Chivrac, Pollet, Schmutz, & Avérous, 2008), moreover, the nanocomposite biodegradability increases after producing a composite with nanosized systems (Sothornvit, Hong, An, & Rhim, 2010) (Fig. 17.2).

Similarly, a nanocomposite drug delivery system hypothetically can accomplish important requirements aiming to provide effective therapy since these systems:

1. Improve drug pharmacodynamics and pharmacokinetics profiles.
2. Can eradicate unhealthy cells without affecting healthy cells.
3. Prolong and control the release of compounds.

4. Enhance cellular uptake of delivered drugs.
5. Can reduce side effects by targeting therapy and by decreasing the dose of drugs (Feldman, 2019; Kaurav, Manchanda, Dua, & Kapoor, 2018).

Owing to the fact that the nanocomposites field is a novel and rapidly expanding area, it is consistently generating new materials with different properties. Nanocomposite science produces a flexible platform for manufacturing new nanomaterials, which have diverse properties and functionalities. Three different morphologic types of nanocomposites are obtained including phase-separated systems, exfoliated systems, and intercalated systems gave the different preparation methodologies and nature of the components (Rahman, Chang Hui, & Hamdan, 2018).

The types of framework systems for nanocomposites can be one, two, and three-dimensional combinations of organic and inorganic materials. According to the matrix of the systems, the nanocomposites can be classified as ceramic-matrix, metal-matrix, or polymer-matrix nanocomposites. Specifically for biomedical applications, the bionanocomposites are usually hybrid systems derived from synthetic or natural biodegradable polymers and organic/inorganic fillers (Hule, 2007). Eminent areas of interest in nanocomposites for biomedical sciences are the production of nanofibers by the electrospinning method, which can be used as tissue scaffolds (Vasita & Katti, 2006), the use of metallic nanoparticles incorporated into polymeric matrices, which present antimicrobial activity and can be used as a contrast agent for magnetic resonance imaging (MRI) (Yaqoob, Adnan, Rameez Khan, & Rashid, 2020), and finally, the hydroxyapatite-based systems for bone repair and implantation (Szurkowska, Laskus, & Kolmas, 2018).

Nanocomposites exhibit numerous advantages to manufacture drug delivery systems given their capacity to preserve the system's stability and improve its physical and mechanical properties; nevertheless, since these systems are heterogeneous, the application of the nanocomposite is subjected to the composition of the system. Therefore, the production of multifunctional composites that can bypass drawbacks related to specific characteristics of individual components may enhance the quality of drug delivery systems and improve the treatment outcome of several challenging diseases.

17.3 The dual approach of theranostics

Advanced theranostic nanosystems retaining multifunctional properties can diagnose and deliver therapeutics to unhealthy tissue with the assistance of targeting ligands and biomarkers (Jia et al., 2013; Yu, Park, & Jon, 2012) (Table 17.1). The entrapment, conjugation, or encapsulation of imaging agents and drugs in nanosystems can result in combined loading and, it can eventually accomplish the purpose of theranostics at the cellular level (Muthu & Feng, 2013; Muthu & Singh, 2009). The therapeutic agents used in theranostics comprise small chemical molecules, proteins, peptides, and genetic material.

Table 17.1 Properties of multifunctional theranostic systems and combination therapy options.

Combination treatment	Imaging probe type	Characteristics
Chemotherapy Radiotherapy Photothermal Photodynamic	Small molecule dyes	Easy metabolize High quantum yield
Radiotherapy Photothermal Photodynamic Gene therapy Immunotherapy	Quantum dots Carbon dots Metal nanoclusters Upconverting nanoparticles	Antiphoto bleaching Long cycle time
Radiotherapy Photothermal Photodynamic Gene therapy Immunotherapy	Fluorescent nanoparticles	Targeting and safety

Aiming to deliver the combination of imaging agents and drugs concurrently, drug delivery systems including nanocomposites have been designed and developed to achieve a controlled and sustainable release of the therapeutic agents and to treat and image the affected cells in specific diseases. For precise circumstances such as pH, hypoxia, and temperature, specific markers have been carried out to accurately regulate the drugs' pharmacokinetics (Caldorera-Moore & Peppas, 2009).

Nanocomposites produced for cancer theranostics are intelligent platforms, which usually comprehend a synergic treatment with chemotherapy and stimuli-responsive nanocarriers for photothermal therapy (PTT) and/or photodynamic therapy (PDT) (Fig. 17.3). These combinations are capable of significantly enhancing the therapeutic efficiency (Wang & Cheng, 2019). PTT is a local therapy modality, which is minimally invasive and nontoxic. This method is based on activating a photosensitizer agent by electromagnetic radiation, including near-infrared (NIR), microwaves, and visible light aiming to transform the energy into heat. The heat generates hyperthermia, which triggers several cellular phenomena, such as cell membrane lysis and protein denaturation resulting in cell apoptosis (Lapotko, 2009). Different from PTT, PDT induces an antitumor response by triggering the generation of radical oxygen species (ROS), thus, the presence of oxygen is mandatory (Eskiizmir, Ermertcan, & Yapici, 2017). Even though PTT presents suitable efficacy, it is not an easy task to perform optimized NIR radiation on each tumor and, while heating the tumor tissue to approximately 50°C or higher, this method can damage the adjacent tissue, thus causing unwanted side effects (Nomura et al., 2020). Thus, a targeted therapy seems to be an important option to overcome possible side effects on healthy cells.

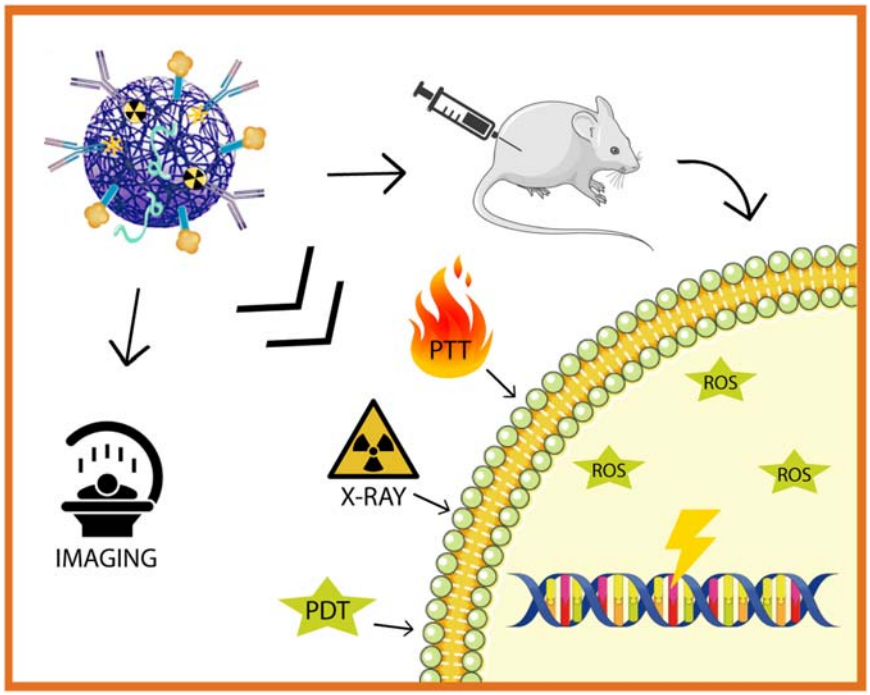

FIGURE 17.3

Nanocomposites produced for cancer therapy offer various treatment combinations including PTT, PDT, chemotherapy and radiotherapy combined with diagnosis through different imaging approaches.

Imaging agents in drug delivery systems can be used to observe nanosystems accumulation and affinity in tissues and to detect interactions between the microenvironment and nanocarriers in a noninvasive way. The theranostic probes are a type of nanoagents that can deliver an improved therapeutic response through imaging guidance and positioning of the treatment (Xing et al., 2020).

The traditional diagnostic methods are MRI, X-ray, positron emission tomography (PET), photoacoustic imaging, and computed tomography (CT), however, fluorescence imaging offers benefits when compared to those, including low toxicity, radiation, and invasiveness and real-time rapid response. Based on these advantages, several fluorescent probes have been described predominantly for tumor diagnosis. One interesting example is the development of targeted probes, which help to visualize the normal tissue boundary with the tumor aiming for surgical assistance; moreover, the use of NIR fluorescent probes seems to be a promising approach for tumor vascular imaging. Amid the developed theranostic probes, glutathione (GSH)-responsive prodrugs, proteinase-responsive prodrugs, hypoxia-activated prodrugs, H_2O_2-activated prodrugs, photon-activated prodrugs,

quantum dots, carbon dots, metal nanoclusters, and upconversion nanoparticles have been extensively studied. These probes present different features, but normally they exhibit suitable performance for diagnosis in biomedical research (Reviewed by Xing et al., 2020).

17.3.1 Nanocomposites for theranostics

In this section, recent developments in multifunctional nanocomposites theranostic platforms for the therapy of cancer (Table 17.2) and other disorders including brain disorders such as cerebral ischemia, cardiovascular diseases, osteoporosis, infections, and bone regeneration will be summarized, including magnetic nanoparticles, drug-polymer conjugates, micelles, polymeric/magnetic nanoparticles and polymeric/silica nanoparticles.

17.3.1.1 Multifunctional nanocomposites for cancer therapy

Cancer is one of the most lethal diseases in the world, and the number of new cases rises each year (Ferlay et al., 2015). In 2018, it was estimated over 18 million new cases, resulted in more than 9.5 million deaths worldwide (Ferlay et al., 2019). The most common cancer types are lung, breast, and colorectal cancer, which comprehend for more than 30% of all cancer cases (Ferlay et al., 2019).

The conventional approaches for cancer handling comprise surgery, chemotherapy, immunotherapy, and radiotherapy. There is a rising number of preclinical and clinical studies showing satisfactory outcomes for patients with different cancer types and stages (Amaria et al., 2019; Das, Ciombor, Haraldsdottir, & Goldberg, 2018; Tray, Taff, & Adams, 2019; Valentino, Borra, Allione, & Rossi, 2018) where overall cancer morbidity and mortality are decreasing. Even though there are novel improvements in diagnostic and treatment approaches, the patient's overall survival rate has not significantly improved over the past decades (Jemal, Siegel, Xu, & Ward, 2010; Siegel, Miller, & Jemal, 2020). Therefore, there is a necessity for the development of novel approaches to specifically detect early-stage cancers and to target the therapies established on specific markers of different types of tumors, which could lead to personalized treatment.

Currently, innovative treatments including targeted therapies are being extensively studied (Mun, Babiker, Weinberg, Kirson, & Von Hoff, 2018), due to the fact that chemotherapy targets rapidly dividing cancerous cells and normal cells (Baudino, 2015) resulting in severe side effects (Oun, Moussa, & Wheate, 2018). Novel strategies for cancer therapy are extremely necessary to not only reduce side effects but also to improve patient diagnosis and prognosis. Recent advances in nanocomposite materials focused on passive and active targeting approach for improving drug concentrations at the tumor site whereas preventing the undesirable toxicity to healthy tissue (de Castro Sant' Anna et al., 2018). Nanotechnology combined with molecular biology appears to be an important path for new therapy strategies guaranteeing personalized oncological care linked to low-cost and noninvasive treatments (de Castro Sant' Anna et al., 2018).

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics.

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
Bimetallic zeolitic imidazolate framework nanocomposites (Mn-ZIF-8)	Chemotherapy: 5-Fu	T_1-weighted MRI (Mn-based NPs)	Glioma (U87-MG tumor-bearing mice)	After injecting the system into the mice, a high accumulation of Mn2 + in tumors was observed. Targeted delivery significantly improved the therapeutic efficacy of Mn-ZIF-8/5-Fu, resulting in an 80% survival rate over 40 days of treatment.	Pan et al. (2019)
PLGA-based hybrid nanocomposites (LDM-PLGA/PPF/VEGF shRNA)	Chemotherapy: DOX and VEGF shRNA (targeted therapy)	T_2-weighted MRI and fluorescence imaging (quantum dots, superparamagnetic Fe_3O_4)	Breast cancer (EMT-6 tumor-bearing mice)	The codelivery of DOX and VEGF shRNA into tumor cells effectively suppressed VEGF expression, exhibiting synergistic antitumor effects both in vitro and in vivo.	Shen et al. (2017)
Nickel ferrite/carbon nanocomposite ($NiFe_2O_4$/C)	SDT	T_2-weighted MRI ($NiFe_2O_4$/C)	Melanoma (B16/F10 tumor-bearing mice)	The results established the applicability of the nanocomposite as a theranostic agent for concurrent SDT and MRI.	Gorgizadeh et al. (2019)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
Nanosized graphene oxide (GO)-PEG-folate nanocomposite	PDT PTT	Multicolor fluorescence imaging (GO)	Melanoma (B16/F10 tumor-bearing mice)	The average half-life span of the mice treated with the nanocomposite GO-PEG-folate and PDT was ~1.8 times longer than the mice treated with DOX. The study accomplished an effective system of using GO-PEG-folate nanocomposite as a theranostic nanomedicine for simultaneous in vivo fluorescent imaging and combined PDT and PTT for antitumor treatment.	Kalluru, Vankayala, Chiang, and Hwang (2016)
Zinc(II) phthalocyanine mono-α-substituted with 4-sulfonatophenoxyl (PcS) nanovesicle structure nanocomposite (NanoPcS)	PDT (zinc(II))	Fluorescence imaging	Hepatocellular carcinoma and adenocarcinoma (HepG2 and HeLa tumor-bearing mice)	The in vivo specific binding between albumin and PcS, of injected NanoPcS, was confirmed using a transgenic mouse system. Fluorescence imaging and antitumor tests suggested that NanoPcS has superior tumor-targeting ability and the potential for PDT.	Li et al. (2019)
Iron platinum-dimercaptosuccinic acid/PEGylated graphene oxide-folic acid (FePt-DMSA/GO-PEG-FA) composite nanoassemblies (FePt/GO CNs)	FePt (Fe catalyzes H_2O_2 decomposition into ROS and induces cell apoptosis)	T_2-weighted MRI (Fe) and CT imaging	Breast cancer (4T1 tumor-bearing mice)	The results indicated that FePt-based NPs displayed suitable biocompatibility and favorable MRI/CT imaging ability in vivo and in vitro. The decomposition of FePt decreased the T_2-weighted MRI signal and increase the ROS signal. This enabled real-time and in situ monitoring of Fe release in tumor cells.	Yue et al. (2017)

Iron oxide (Fe_3O_4) NPs (G23-DOX/alg-Fe_3O_4) nanocomposite	Chemotherapy: DOX	T_2-weighted MRI	Glioma (U87-MG-luciferase tumor-bearing mice)	The nanocomposite was able to cross the BBB by targeting gangliosides. In the mice treated with DOX, algFe_3O_4 NPs, and G23-alg-Fe_3O_4 NPs, the tumor sizes showed no obvious variation until 7 days postinjection, however, the tumors shrank significantly in the mice treated with the system.	Su, Tsai, Tomanek, Chen, and Cheng (2016)
Polypyrrole@MIL-53 nanocomposite	Chemotherapy: DOX PTT	T_2-weighted MRI (MIL-53)	Breast cancer (4T1 tumor-bearing mice)	The nanocomposite displayed in vitro and in vivo synergism of chemotherapy and PTT, MRI-guided.	Huang, Li, Zhang, and Meng (2018)
PFH/DOX@PLGA/Fe_3O_4-FA nanocomposite	Chemotherapy: DOX HIFU	T_2-weighted MRI (superparamagnetic iron oxide NPs)	Hepatocellular carcinoma (Bel-7402 tumor-bearing mice)	The system suppressed tumor growth based on the enhanced and synergistic chemotherapy and HIFU ablation, providing an efficient theranostic nanoplatform for cancer treatment.	Tang et al. (2018)
PEGylated hollow gold NPs (mPEG@HGNPs)	Radiotherapy PTT	CT imaging	Breast cancer (4T1 tumor-bearing mice)	mPEG@HGNPs exhibited a favorable tumor-targeting effect and good CT contrast enhancement in breast tumor models.	Wang, Deng, et al. (2019)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
Carbon nanoparticles (CPs), silver nanoparticles (AgNPs) and MnO_2 (CPs@MnO_2-AgNP) nanocomposite	PTT	Fluorescence imaging	Hepatocellular carcinoma (SMMC-7721 cell line)	This redox-responsive nanocomposite was constructed as a multifunctional nanosensor for glutathione and improved the MnO_2 nanomaterial-based applications in GSH sensing.	Wang, Wang, et al. (2019)
Transferrin receptor antibody (TfR Ab) and DOX—loaded Fe3O4@ZnO nanocomposites	Chemotherapy: DOX Radiotherapy	MRI	Hepatocellular carcinoma (SMMC-7721 tumor-bearing mice)	After the treatment, a noninvasive visualization monitoring exhibited a suppression in tumor growth by the targeted chemoradiotherapy.	Zhang, Patel, Ding, Xiong, and Wu (2016)
Doped titanium dioxide NPs (TiO_2(Gd) NPs)	Radiotherapy with nanosensitizer (TPP)	Fluorescence imaging (IR806)	Breast cancer (MCF-7 tumor-bearing mice)	TPP in combination with a single X-ray radiation exposure achieved complete tumor ablation without side effects during treatment. Moreover, the mitochondria-targeted nanosensitizer could significantly reduce treatment doses and greatly amplify antitumor efficiency.	Chen, Li et al. (2019)

Double-mesoporous core-shell nanosystems based on Pt NPs functionalized with lanthanide complexes (mPt@mSiO_2-GdDTPA)	PTT (Pt NPs)	MRI (Gd)	Adenocarcinoma (HeLa tumor-bearing mice)	The nanosystems displayed a higher r_1 value than the medical contrast agent Magnevist and were successfully applied to in vivo MRI.	Zhao et al. (2017)
Gd-doped silicon NPs, zeolitic imidazolate framework-8 (ZIF-8), HOOC-PDMAEMA-SH, and FA-PEG into one single nanoplatform (FZIF-8/DOX-PD-FA) nanocomposite	Chemotherapy: DOX PTT: Ce6	MRI (Si-Gd NPs) and fluorescence imaging (Ce6)	Breast cancer (MCF-7 tumor-bearing mice)	The pH-responsive ability of HOOC-PDMAEMA-SH was able to prevent drug leakage. Moreover, The tumor volume of the FZIF-8/DOX-PD-FA + PTT-treated mice significantly decreased when compared to the other treatment groups.	Qin, Peng, He, Li, and Zhang (2019)
Mesoporous silica-coated gold cube-in-cubes core/shell (RGD-CCmMC/DOX)	Chemotherapy: DOX, RGD (targeted therapy) PDT (singlet oxygen) PTT (gold cube-in-cube core)	MRI and fluorescence imaging	Breast cancer (4T1 tumor-bearing mice)	The nanocomposite was found to be biocompatible and effectively obliterates the tumors in vivo.	Zhang et al. (2019)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
Au-BSA core/shell NPs (Au-BSA-DOX-FA) nanocomposites	Chemotherapy: DOX	CT imaging (Au)	Gastric cancer (MGC-803 tumor-bearing mice)	In vivo, antitumor experiments demonstrated that Au-BSA-DOX-FA nanocomposites have selective antitumor activity effects and no adverse effects on normal tissues and organs. Additionally, the system exhibited selective targeting activity, X-ray attenuation activity, and pH-sensitive drug release activity.	Huang, Yang, et al. (2017)
Mn-porphyrin&Fe_3O_4@SiO_2@PAA-c(RGDyK) nanocomposite	Chemotherapy: DOX	T_1- and T_2-weighted MRI and fluorescent imaging	Lung cancer (A549 tumor-bearing mice)	The nanocomposites exhibited highly sensitive MRI contrast function by synergistically enhancing positive and negative MRI signals. The nanocomposites showed great potential for integrating imaging diagnosis and drug-controlled release providing real-time imaging with greatly enhanced diagnostic accuracy during targeted therapy.	Huang, Yuan, et al. (2018)

Mesoporous organosilica NPs (HMONs) loaded with ICG and PFP	Chemotherapy: PTX PTT: ICG	Ultrasound (PFP) and photoacoustic imaging	Breast cancer (MDA-MB-231-tumor-bearing mice)	The platform was found to have suitable properties for both ultrasound and photoacoustic imaging. In addition, both in vitro and in vivo results show that the NPs provide potent synergistic chemo-PT therapy.	Wu, Williams et al. (2019)
AuNPs-PEI nanocomposites	PTT	Photoacoustic imaging	Colon carcinoma (CT26 tumor-bearing mice)	The nanocomposites containing PEI outperformed the other tumors as measured by tumor growth rate.	Mulens-Arias et al. (2019)
HMONs with PDA interlayer (DI@HMONs-PMOF)	Chemotherapy: DOX PTT: ICG	MRI and photoacoustic imaging	Breast cancer (4T1 tumor-bearing mice)	The results suggested that the existence of ICG can cooperatively enhance the MRI. In addition, the significantly improved synergistic therapeutic efficacy was confirmed both in vitro and in vivo.	Chen, Zhang, et al. (2019)
Bio-Metal-Organic Framework (Fe_3O_4@Bio-MOF) coated FA-chitosan conjugate	Chemotherapy: Curcumin and 5-Fu	T_2-weighted MRI	Breast cancer (MDA-MB-231 tumor-bearing mice)	The selective uptake of 5-Fu-loaded Fe_3O_4@Bio-MOF by folate receptor-positive cells was confirmed. The nanocarrier exhibited no significant toxicity, while the drug-loaded nanocarrier showed selective and higher toxicity against the cancerous cells than normal cells.	Nejadshafiee et al. (2019)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
Hybrid Au/Ag doped carbon quantum dot nanocomposite	PTT	Not tested in vivo	Adenocarcinoma (HeLa tumor-bearing mice)	PTT heating experiments were promising.	Liu, Wang, and Du (2020)
A single-light-triggered ICG-loaded PEGylation AgNPs core/polyaniline shell (Ag@PANI) nanocomposites (ICG-Ag@PANI)	PTT: ICG	Photoacoustic and NIRF imaging	Adenocarcinoma (HeLa tumor-bearing mice)	The dual-modal imaging confirms the accumulation and distribution of ICG-Ag@PANI in the tumor region via the EPR effect.	Tan et al. (2016)
Neodymium vanadate ($NdVO_4$)/Au heterojunction nanocrystals (NCs)	PTT PDT	PTT and photoacoustic imaging	Adenocarcinoma (HeLa tumor-bearing mice)	$NdVO_4$/Au was internalized efficiently via endocytosis and caused apparent phototoxicity on HeLa cells. In vivo experiments showed that the system could act as a high-efficiency NIR light-triggered anticancer agent with a suitable tumor inhibition effect.	Chang et al. (2019)
PEGylated Fe@Bi_2S_3 nanocomposites	Radiotherapy PTT	*T2*-weighted MRI (Fe core) and CT (Bi_2S_3)	Breast cancer (4T1 tumor-bearing mice)	The imaging effect provided by the system was better than commercial products. When the nanocomposites were used for synergistic therapy there was a significant reduction in tumor size and the nanocomposites exhibited no obvious toxicity to cells and mice at a therapeutic dose.	Li, Cheng et al. (2018)

HA-coated Fe_3O_4@polydopamine NPs	Chemotherapy: DOX PTT	MRI	Adenocarcinoma (HeLa tumor-bearing mice)	The nanocomposite presented a suitable antitumor effect by photothermal-chemo combination therapy. H&E and Ki67 staining tests showed obvious necrosis and weak cell proliferation in the region of the tumor.	Lin et al. (2020)
Magnetic HMONs loaded with Ce6 and DOX, assembled with alginate/chitosan PEM, and adsorbed with P-gp shRNA (M-MSN(Dox/Ce6)/ PEM/P-gp shRNA) nanocomposite	Chemotherapy: DOX PDT: Ce6	MRI and CT imaging	Breast cancer (EMT-6 tumor-bearing mice)	The system presented great potential as a multifunctional delivery platform, which is promising for imaging-guided cancer combination therapy with high efficacy.	Yang et al. (2017)
Au and ferroferric oxide NPs coating polypyrrole particles (PPy@Fe_3O_4/Au) nanocomposite	PTT	MRI and CT imaging	Adenocarcinoma (HeLa tumor-bearing mice)	The PPy@Fe_3O_4/Au nanocomposites exhibited suitable CT imaging and MRI performance, which provides more comprehensive and accurate diagnostic information. Moreover, PPy@Fe_3O_4/Au nanocomposites could efficiently kill cancer cells by hyperthermia and even completely ablate tumors.	Yan et al. (2018)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
SPIONs with PCLA-PEG-PCLA (NC-SPIOs-IR820-PTX) nanocomposite	Chemotherapy: PTX PDT: IR820 — photosensitizer)	MRI and NIR fluorescence imaging	Breast cancer (4T1 tumor-bearing mice)	The synergistic therapeutic effects of NC-SPIOs-IR820-PTX were able to induce tumor targeting and enhance the cotherapy results, resulting in significant tumor inhibition effects. Thus, NC-SPIOs-IR820-PTX theranostics could be applied for magnetic field-guided tumor targeting as well as multimodal imaging, and imaging-guided combined therapy.	Liao et al. (2017)
GO and AuNPs core with polyaniline shell (GO-Au@PANI) nanocomposite	Chemotherapy: DOX PTT: PANI	SERS imaging	Breast cancer (4T1 tumor-bearing mice)	The GO-Au@PANI system presented a high-performance chemo-photothermal therapeutic nanoagent. The theranostic applications of GO-Au@PANI provide it with great potential for personalized and precise cancer medicine.	Chen et al. (2016)

FA and PEG-modified octopod platinum-copper alloy nanoframes (OPCNs-PEG-FA) nanocomposite	Radiotherapy PTT: OPCNs	Infrared thermal imaging and photoacoustic imaging	Hepatocellular carcinoma (HepG2 tumor-bearing mice)	The multifunctional nanotheranostics of OPCNs-PEG-FA achieved simultaneous imaging and synergistic dual-modal radiotherapy/PTT tumor ablation. The nanotheranostics agent not only improved synergistic tumor suppression but also exhibited no obvious systemic toxicity in vivo.	Li, Zu et al. (2018)
HA-coated FeOOH@polypyrrole (FeOOH@PPy) nanorods (HA-FeOOH@PPy NRs) nanocomposite	PTT	Photoacoustic imaging	Breast cancer (MDA-MB-231 tumor-bearing mice)	The photothermal anticancer activity results of the designed nanocomposite evidenced its promising potential in cancer treatment. The tumor-bearing mice completely recovered after 17 days of PTT treatment without obvious side effects.	Phan et al. (2018)
Surface-superparamagnetic iron-oxide functionalised tantalum carbide (Ta_4C_3) MXene (Ta_4C_3-IONP-SPs composite MXenes)	PTT	T_2-weighted MRI and CT	Breast cancer (4T1 tumor-bearing mice)	The high photothermal-conversion efficiency of Ta_4C_3-IONP-SPs composite nanosheets achieved complete tumor eradication without reoccurrence, demonstrating the highly efficient breast-tumor hyperthermia performance.	Liu et al. (2018)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
MOF@POP-PEG (HUC-PEG) nanocomposite	PTT PDT	CT and photothermal imaging	Cervical cancer (U14 tumor-bearing mice)	The HUC-PEG exhibited suitable physiological stability and favorable biocompatibility. For the tumors treated with PTT, HUC-PEG had high efficiency in tumor inhibition.	Zheng et al. (2020)
Zeolitic imidazolate framework (ZIF-8) core-shell with MnO_2 on the surface of porphyrinic ZrMOF NPs (ZrMOF@MnO_2) nanocomposite	PDT	T_1-weighted MRI	Glioma (U87-MG- tumor-bearing mice)	ZrMOF@MnO_2 hybrid NPs have enhanced PDT efficiency owing to the intracellular balance of GSH and MnO_2.	Liu et al. (2019)
Fe_3O_4-black TiO_2 (Fe-Ti NCs) nanocomposite	PTT PDT	MRI	Breast cancer (MCF-7 tumor-bearing mice)	Fe−Ti NCs had superior photothermal properties compared to those of individual NPs. Moreover, their therapeutic applications were confirmed.	Saeed et al. (2019)
Polydopamine stabilized graphene quantum dots (GQD)-GCpD/CpG oligodeoxynucleotide (CpG ODN) NPs PC@GCpD(Gd)) nanocomposite	Immunotherapy PTT PDT	MRI and fluorescence imaging	Breast cancer (EMT6 tumor-bearing mice)	CpG ODN was delivered to the targeted endosomal Toll-like receptor 9 to stimulate the secretion of pro-inflammatory cytokines and the maturation of dendritic cells, thereby resulting in the activation and infiltration of T-lymphocytes. almost completely suppress the tumors under laser irradiation.	Wu, Guan et al. (2019)

GSH-platinum (IV) (Pt(IV)) prodrug-loaded phase-transitional NPs (Pt(IV) NP-cRGD) nanocomposite	Chemotherapy: Pt(IV)	Ultrasound imaging	Ovarian cancer (SKOV3 tumor-bearing mice)	Pt(IV) NP-cRGD combined with ultrasound imaging exhibited excellent echogenic signals, suitable therapeutic efficacy, and limited side effect, suggesting precise theranostics against ovarian cancer.	Huang et al. (2019)
Bismuth-based NPs coated with SiO2 and functionalized with S-nitrosothiol (Bi-SNO NPs)	Radiotherapy PTT	CT imaging and NIR thermal imaging	Cervical cancer (U14 tumor-bearing mice)	Synergistic tumor inhibition was found and no obvious toxicity of Bi-SNO NPs was observed in the treated mice within 14 days. Thus, the Bi-SNO was found to be an effective nanoagent for cancer theranostics with well-controlled morphology and uniform size.	Zhang et al. (2020)
Fe_3O_4 with ^{99m}Tc and IR-1061 (FIP-^{99m}Tc) nanocomposite	PTT: IR-1061	NIR fluorescence imaging, photoacoustic imaging (IR-1061), and CT imaging (^{99m}Tc)	Breast cancer (4T1-Luc tumor-bearing mice)	FIP-^{99m}Tc confirmed the fast accumulation and clear delineation of metastatic lymph nodes and it could effectively prevent further lung metastasis after resection of the primary tumor.	Cai et al. (2020)
GO/bismuth selenide/PVP NP (GO/Bi2Se3/PVP) nanocomposite	PTT	CT imaging and photoacoustic imaging	Adenocarcinoma (HeLa tumor-bearing mice)	After intratumoral or intravenous injection of the nanocomposites, irreversible photothermal ablation of tumors was achieved.	Zhang et al. (2017)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
Gadolinium porphyrin and Zinc porphyrin (GdTPP/ZnTPP) nanocomposites	PDT	MRI and fluorescence imaging	Adenocarcinoma (HeLa tumor-bearing mice)	The system achieved combined functions for visualized cancer theranostics.	Wang et al. (2020)
Electrospun hyaluronic acid-ceramide (HACE) and Soluplus (SP) nanocomposite	Chemotherapy: resveratrol	NIR fluorescence imaging	Breast cancer (MDA-MB-231 tumor-bearing mice)	The HACE/SP NC can be a promising theranostic nanosystem for CD44 receptor-expressed cancers.	Lee et al. (2016)
Maghemite (γ-Fe2O3) nanoflower-like multicore NPs and a spiky copper sulfide shell (IONF@CuS) (Iron Oxide Nanoflowers @ CuS Hybrids) nanocomposite	Magnetic hyperthermia PTT PDT	MRI and photoacoustic imaging	Prostate cancer (PC3 tumor-bearing mice)	Complete tumor regression was obtained for the PTT-treated animals. The integration of the dual heating capability (magnetic hyperthermia + PTT) with the PDT offered a unique asset to tackle tumors by multiple cytotoxic strategies to improve the therapeutic outcome in a broader spectrum of clinical conditions.	Curcio et al. (2019)
Lu-based upconversion nanophosphor (UCNP) and Bi-based nanomaterial loaded with iron phthalocyanine and coated with folate-conjugated amphiphilic PEG (UCNP@NBOF-FePc-PFA)	Radiotherapy PTT PDT	CT imaging and luminescence	Breast cancer (4T1.2 tumor-bearing mice)	A highly effective tumor ablation effect was verified, thus, the nanomaterial offered a novel method for the construction of a new theranostic platform.	Liu, Zhang, et al. (2020)

β-cyclodextrin-$(76)_{21}$ [β-CD-$(PLA\text{-}PDMAEMA\text{-}PEtOxMA)_{21}$] unimolecular micelles loaded with AuNPs and DOX nanocomposite	Chemotherapy: DOX	CT imaging	Hepatocellular carcinoma (HepG2 tumor-bearing mice)	The system achieved high CT imaging and antitumor efficacy under in vitro and in vivo acid tumor conditions.	Lin et al. (2017)
Gadolinium oxide (Gd_2O_3) NPs with PPy, modifying with HA and loaded aluminum phthalocyanine (AlPc) (Gd_2O_3@PPy/AlPc-HA) nanocomposite	PTT PDT	MRI, fluorescence, and photoacoustic imaging	Breast cancer (4T1 tumor-bearing mice)	HA and AlPc were adsorbed on PPy for HA-mediated tumor targeting and PDT respectively. It was observed enhanced tumor uptake effect after intravenous injection and antitumor efficiency was achieved under the combined therapy, which was significantly better than any other mono-therapy.	Cheng et al. (2018)
Tyrosine (Tyr)-HA-PEI, radiolabelled with $^{131/125}$ I and loaded with a p53 mutant restoring regent, Prima-1 (Prima-1@PEI−HA−Tyrs−131I) nanocomposite	Chemotherapy: Prima-1 (targeted therapy) Radiotherapy	SPECT and CT imaging	Thyroid cancer (8305C tumor-bearing mice)	The system displayed suitable tumor imaging and a long radiation treatment cycle. The ^{131} I-labeled NPs demonstrated antitumor effects in vitro and in vivo, due to radiosensitization of Prima-1 by reactivation of the p53 mutants.	Huang et al. (2020)

(*Continued*)

Table 17.2 Recent developments of multifunctional nanocomposites for cancer theranostics. *Continued*

System	Treatment	Diagnosis	Cancer type	Outcome	Reference
T-UCNPs@Ce6@mSiO_2 nanocomposite	PDT	MRI	Breast cancer (MDA-MB-435 tumor-bearing mice)	The designed nanocomposite could improve the uptake of HER2-positive cells and tumors by modifying the site-specific peptide, and the in vivo experiments showed suitable MRI and PDT via intravenous injection.	Zeng et al. (2016)

5-Fu, *5-Fluorouracil;* AgNPs, *silver nanoparticles;* Au, *gold;* BBB, *blood-brain barrier;* BSA, *bovine serum albumin;* Ce6, *chlorine e6;* CN, *composite nanoassemblies;* CP, *carbon nanoparticles;* CT, *computed tomography;* DOX, *doxorubicin;* EPR, *enhanced permeability and retention;* FA, *folic acid;* Fe, *iron;* Gd, *Lanthanide;* GO, *graphene oxide;* GSH, *glutathione;* H_2O_2, *oxygen peroxide;* HA, *hyaluronic acid;* HIFU, *high-intensity focused ultrasound;* HMONs, *mesoporous organosilica NPs;* HOOC-PDMAEMA, *succinic-poly(2-(diethylamino)ethyl methacrylate);* ICG, *indocyanine green;* Mn, *Manganese;* MOFs, *metal-organic frameworks;* MRI, *magnetic resonance imaging;* NPs, *nanoparticles;* NIR, *near-infrared;* NIRF, *near-infrared fluorescence;* PAA, *poly(acrylic acid);* PANI, *polyaniline;* PCLA, *poly(ε-caprolactone-co-lactide);* PcS, *zinc(II) phthalocyanine mono-α-substituted with 4-sulfonatophenoxyl; PDA: polydopamine; PDT: photodynamic therapy;* PEG, *poly(ethylene glycol);* PEI*: polyethyleneimines;* PEM, *polyelectrolyte multilayers;* PFH, *perfluorohexane;* PFP, *perfluoropentane;* PLGA, *poly(lactic-co-glycolic acid);* POPs, *porous organic polymers;* PPF, *PEI-PEG-FA;* PPy, *polypyrrole;* Pt, *platinum;* PTT, *photothermal therapy;* PTX, *paclitaxel;* PVP, *polyvinylpyrrolidone;* RGD, *Arg-Gly-Asp peptide;* ROS, *reactive oxygen species;* SDT, *sonodynamic therapy;* SERS, *surface-enhanced Raman scattering;* shRNA, *short hairpin RNA;* SPIONs, *superparamagnetic iron oxide NPs;* TfR Ab, *transferrin receptor antibody;* TPP, *4-carboxybutyl triphenylphosphonium bromide;* VEGF, *vascular endothelial growth factor*.

The targeted delivery of nanocomposites can overcome complications related to conventional chemotherapy, including rapid clearance, insolubility of compounds, lack of selectivity, and several side effects associated with the toxicity to healthy cells (Allen, 2002). A possible direction to resolve these problems is the use of theranostics formulations. Several theranostic systems have been studied for tumor therapy and imaging (Ashley et al., 2011) and particular attention has been found to the use of nanocomposites, as a consequence of their capacity to cross biological barriers and accumulate in tumor cells.

Nanocomposites theranostics can significantly advance therapeutic and diagnostic effectiveness (Allen, 2002; Yu et al., 2012). Thus, these multifaceted drug delivery systems could reach challenging regions of the body such as the brain. Along with that, some biomolecules or biofunctionalized nanocarriers could penetrate the brain-blood barrier (BBB) and the brain tumor barrier, contributing to a better outcome for brain cancers, including glioblastomas.

Recently, a bimetallic zeolitic imidazolate framework (Mn-ZIF-8) was synthesized and it presented high surface area and good dispensability, which could be used for possible high drug loading. This drug delivery system was produced as a pH-responsive nanocomposite for the delivery of 5-Fluorouracil (5-Fu) targeting gliomas. The results demonstrated a significantly improved therapeutic efficacy of 5-Fu and it was able to prolong the survival of a glioblastoma mice model. Moreover, given the pH responsiveness property, it enhanced the accumulation of Mn^{2+} at the tumor site, resulting in a suitable T_1-weighted MRI signal (Pan et al., 2019). Another nanocomposite based on ZIF-8 containing MnO_2 (ZrMOF@MnO_2) used PDT to treat gliomas and it provided a T_1-weighted MRI signal improving the PDT efficiency owing to the intracellular balance of GSH and MnO_2 (Liu et al., 2019). By using iron oxide (Fe_3O_4) nanoparticles, Liu et al. developed a nanocomposite loaded with doxorubicin (DOX) and alginate tagged with a peptide (G23; sequence: HLNILSTLWKYRC) on the surface, which is permeable to the BBB. This system nanocarrier can cross the BBB and enter the brain to treat gliomas while providing enhanced T_2-weighted images. The tumors of mice treated with G23-Dox/alg-Fe_3O_4 reduced significantly when compared to DOX only. Interestingly, the nanoparticles and alginate have their use authorized by the United States Food and Drug Administration, thus, this nanocomposite would be safe for human use since it does not show side effects (Su et al., 2016).

Was observed a recent increase in the number of systems targeting breast cancer (Cai et al., 2020; Chen et al., 2016; Chen, Li et al., 2019; Chen, Zhang, et al., 2019; Cheng et al., 2018; Huang, Li et al., 2018; Lee et al., 2016; Li, Cheng, et al., 2018; Liao et al., 2017; Liu et al., 2018; Liu, Zhang, et al., 2020; Nejadshafiee et al., 2019; Phan et al., 2018; Qin et al., 2019; Saeed et al., 2019; Wang, Deng, et al., 2019; Wu, Williams et al., 2019; Wu, Guan et al., 2019; Yang et al., 2017; Yue et al., 2017; Zeng et al., 2016; Zhang et al., 2019; Shen et al., 2017). These nanocomposites exhibited promising antitumor results and they were able to significantly achieve an efficient theranostic effect. Shen and collaborators proposed a strategy of encapsulating quantum dots, superparamagnetic Fe_3O_4 nanocrystals, and DOX into a

biodegradable poly(d,l-lactic-*co*-glycolic acid) (PLGA) polymeric nanocomposite (Shen et al., 2017). This system was modified with polyethylene glycol and folic acid (FA) and loaded with a vascular endothelial growth factor (VEGF)-small hairpin RNA (shRNA). The nanocomposite enhanced the T_2-weighted MRI signal and the codelivery of VEGF shRNA and DOX effectively suppressed VEGF expression resulting in a synergic antitumor effect (Shen et al., 2017).

An interesting study produced a hyaluronic acid-ceramide-based nanocomposite with Soluplus by using the electrospraying method for the release of resveratrol (Lee et al., 2016). This drug is a known anticancer agent that can induce oxidative stress resulting in cell death, however, it has poor aqueous solubility and is unstable in solution, thus, the authors improved the drug solubility by trapping it into a micellar composite structure. The results confirmed a selective tumor targetability with an increased uptake for the CD44 + cells in a breast cancer mouse model using NIRF imaging, therefore, this system could be a promising theranostic option for CD44receptor-positive tumors (Lee et al., 2016).

Impressive research was developed by Liu, Zhang, et al. (2020), in which, the authors proposed a unique strategy combining PTT, PDT, and radiotherapy to treat breast cancer. The developed nanocomposite contained a Lu-based upconversion nanophosphor (UCNP) and an iron phthalocyanine-loaded Bi-based nanomaterial (UCNP@NBOF-FePc-PFA). The system provided a tri-modal tumor treatment and the in vivo results demonstrated a greatly efficient tumor ablation effect, moreover, this nanocomposite exhibited upconversion luminescence capacity, X-ray attenuation, PTT effect, and X-ray and NIR that triggered ROS generation (Liu, Zhang, et al., 2020).

Other elegant nanocomposite approaches for cancer theranostic targeted hepatocellular carcinomas by using different tactics (Li et al., 2019; Tang et al., 2018). It is known that the overexpression of albumin receptors and albumin-binding protein SPARC (secreted protein, acidic and rich in cysteine) on tumor cells increases albumin accumulation and degradation. Consequently, albumin is considered an interesting candidate as a cancer diagnosis biomarker and possibly a target for specific drug delivery. Because of that, a research group designed a theranostic nanocomposite system to target these cells that overexpress albumin (Li et al., 2019). The design strategy of this system comprises a nanostructured self-assembly nanocomposite based on the zinc(II) phthalocyanine mono-α-substituted with 4-sulfonatophenoxyl (PcS) molecule, displaying an albumin-dependent disassembly (NanoPcS), resulting in an effective theranostic agent for tumor-targeted fluorescence imaging and time-modulated PDT (Li et al., 2019). Finally, Tang et al. conjugated folate onto the surface of a nanocomposite for targeting hepatocellular carcinoma cells by receptor-ligand interaction, which facilitates the uptake of these systems into the tumor cells (Tang et al., 2018). Perfluorohexane (PFH) was used to generate PFH/DOX@PLGA/Fe_3O_4-FA nanocomposite, which significantly improved the high-intensity focused ultrasound ablation efficacy and when combined with DOX, it caused a higher percentage of tumor necrosis when compared to the other treated groups; moreover, it achieved the contrast-enhanced ultrasound

imaging (Tang et al., 2018). Thus, this system successfully suppressed tumor growth providing an efficient theranostic nanocomposite for cancer treatment.

17.3.1.2 Multifunctional nanocomposites for other diseases

Multipurpose nanodevices are a fascinating prospect for more personalized and efficient medicine. From a future perspective, it is challenging since each disease and individual has unique characteristics that have to be taken into account to meet its needs when building a nanotheranostics system (Wong, Sena-Torralba, Álvarez-Diduk, Muthoosamy, & Merkoçi, 2020). Nevertheless, its application reduces prognosis mistakes and therapeutically failure due to empirical treatment, since it can provide an early and more accurate diagnosis, targeted release, and real-time imaging. Commonly, the nanotheranostics agent is a nanocomposite system, especially for its multiple functionalization opportunities, targeting possibilities, polymer coating/stabilization ability, and carrier function and not to mention its varied bioimaging approaches (either through fluorescence or MRI/electronic transmission). Most of the nanocomposites have been assessed for anticancer therapy, however, other applications are arising reported in the literature. Herein we outline the utmost research on different disease treatments with nanocomposite theranostics (Fig. 17.4).

Recently, Kumar and collaborators (Kumar et al., 2020) highlighted the potential of nanotheranostics to address current issues in the neurodegenerative diseases field, such as Alzheimer's and Parkinson's diseases. Up-to-date studies call attention to nanocomposites, metal nanoparticles, biosensors, quantum dots, and biomarkers that can be further explored on a combined strategy such as nanotheranostics.

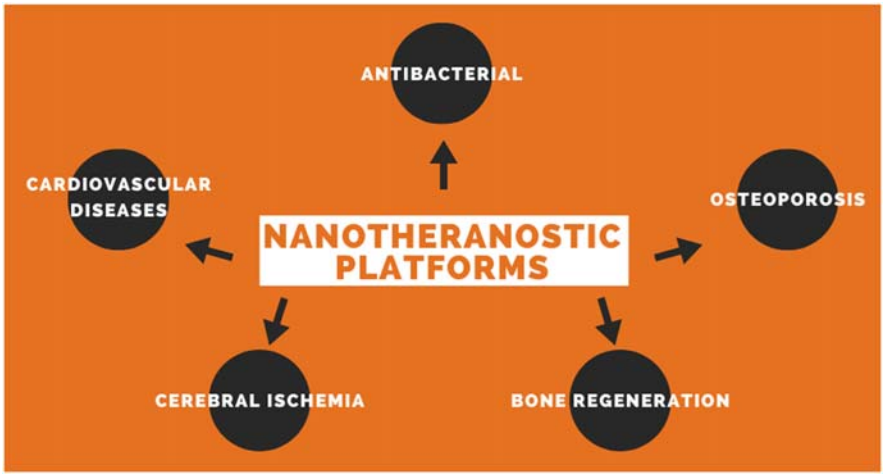

FIGURE 17.4

Scheme illustrating diseases currently addressed with nanotheranostic composites based on published research.

A limitation of the treatment of neurodegenerative diseases is the individuality of each patient, in line with other diseases. Every patient has a different genome coding and neural functioning characteristics, thus, it is difficult to assess all of them on a standard-treatment basis. Another issue found is the proper targeting of proposed treatments, due to absorption by the systemic circulation and the challenge of the BBB. Also relevant, monitoring of the treatment is currently inefficient and the therapeutic alternatives are expensive. All of these characteristics urge attention to the development of a complex nanocomposite combined with a theranostic approach that could potentially solve most of the issues related to neurodegenerative disease treatment. In 2018, Chen and collaborators (Chen et al., 2018) highlighted that the current approach to treating Alzheimer's disease by inhibiting amyloid-β aggregation might be uncertain, since most treatments lead to failure, and this mechanism has been weakly correlated with cognition loss in the literature. Thus, researchers have proposed a nanocomposite focused on diminishing tau hyperphosphorylation and oxidative stress, to reduce cognition decrease in vivo. The proposed nanocomposite had methylene blue as a carried drug, which inhibits tau aggregation, in combination with iron oxide nanocrystals (for bioimaging) onto the surface of mesoporous silica nanoparticles for a synergistic effect. The nanoplatforms had also self-assembled ultra-small ceria nanocrystals, which are tau hyperphosphorylation inhibitors, and coated with Amino-T807 (selective tau binder). The Amino-T807 was grafted via the macrocyclic chelator NOTA, which could be further conjugated to $_{68}$Ga for PET imaging. With this approach, the authors achieved a simultaneous inhibition of tau hyperphosphorylation and aggregation, accompanied by the inhibition of neuronal apoptosis and consequent therapeutical success. However, the study did not assess BBB barrier crossing, which might be a topic for further improvement and analyses on this promising nanocomposite (Chen et al., 2018).

Beyond Alzheimer's disease, brain stroke (or infarction) is also an important disease that leads to many deaths every year. Almalki and collaborators (Almalki, Alghamdi, Alzahrani, & Zhang, 2020) recently highlighted the importance of nanotheranostics to address common issues regarding stroke treatment, like specificity, targeting, and bioimaging, and enumerated in their review some outstanding studies with nanoparticles and nanocomposites for this purpose. For example, a study using core-shell nanopolymerosomes with magnetic nanoparticles and targeting siRNA revealed a theranostic potential to targeted deliver, provide imaging contrast and affect the neural stem cells (Almalki et al., 2020).

In a similar situation, osteoporosis is a disease in which its current hormonal treatment lacks specificity and targeting. Serious side effects occur in patients undertaking hormonal therapy to treat osteoporosis, due to the accumulation of the hormone in nonselective sites (such as the breast, heart, and uterus). Therefore, the nanotheranostics approach is a good choice to mitigate this problem. Chen et al. (2020) have reported an upconversion nanocomposite of 17β-estradiol (E2)-laden, mesoporous silica surface-modified with EDTA, for targeted osteoporosis hormonal treatment. The upconversion core (nanoscale crystals

doped with rare-earth ions) coated with mesoporous silica provided imaging capacity to the complex, together with a slow-release profile (drug release within 50 h). Moreover, EDTA functionalization provided bone-targeting ability. The proposed nanocomposite proved to be efficient in bone targeting and local delivery of the hormone (good cellular uptake rates and osteogenic activity), decreasing side effects and increasing the efficiency of the therapy (Chen et al., 2020).

Tissue engineering and bone regeneration can also be assessed by nanotheranostics composites, to monitor the treatment in situ and increase its efficiency. Li and collaborators (Li, Guo, et al., 2018) indicated the application of nanotheranostics to bone regeneration through a composite nanogel. The authors developed a simple poly(citrate-siloxane) elastomer to provide desired mechanical properties, osteogenic capacity, and photoluminescent property (bioimaging) to conjugate with BGNs (silica-based bioactive glasses). The research group used sol-gel-derived BGNs nanoparticles monodispersed in the elastomer environment to potentiate biomineralization and osteoblastic cell response. It could be seen that the biomineralization activity, osteogenic differentiation ability, low inflammatory response, and osteoblast's biocompatibility were achieved within the proposed nanocomposite. Besides, bioimaging capacity was demonstrated with its detection for more than 2 months in vivo (Li, Guo, et al., 2018).

In a recent review, Agrawal and collaborators (Agrawal, Nooti, Singh, & Rai, 2021) shed light on nanomaterials-based theranostics to approach vascular diseases, such as atherosclerosis. Mostly using nanoparticles, research is going towards nanocomposite applications due to its multiple mechanisms possibilities and addressing of current disadvantages and limitations listed by them, to note, nanomaterial-related toxicity, limited targeted ability, and difficulty to assess the blood distribution (Agrawal et al., 2021).

Considering all previously mentioned studies, it is ultimate that the second most addressed field for nanotheranostics composites is bacterial infections. In a recent review, Mosselhy and collaborators (Mosselhy, Assad, Sironen, & Elbahri, 2021) claimed attention to the application of nanotheranostics to assess drug-resistant *Staphylococcus aureus* (MRSA) infection and biofilm formation. They highlighted different nanosystems like nanofibers and nanoparticles as potential platforms for treatment, diagnosis, and real-time accompanying of the pathogen infection. According to the review, different mechanisms can be approached by nanotheranostics, such as PTT and PDT (similar to cancer treatment). Moreover, it can provide rapid and accurate bacterial identification, together with treatment monitoring. In agreement, some recent studies have approached this technology to fight different bacteria with varied nanocomposite systems (Mosselhy et al., 2021).

In 2017, Huang, Chen, Zhu, Xu, and Liu (2017) developed a polypeptidic ruthenium/selenium nanotheranostics composite for antibacterial activity. The targeting function was achieved by the peptide UBI29−41, which also helped the stabilization of the selenium nanoparticle core. The selenium nanoparticle core, beyond its antibacterial activity, is also efficient in wound healing function. Selective imaging function was assessed by coating with fluorescent ruthenium

complexes, also possessing antibacterial activity. The signalization of the system with ruthenium and the peptide provided specificity to the nanocomposite, being able to distinguish between cancer-induced infection, bacterial infection, and inflammation sites. Moreover, the selenium and ruthenium combination proved to have a synergistic inhibitory effect, especially against gram-positive bacteria. Mechanisms involved in antibacterial activity were adsorption via electrostatic interactions with the phosphoric acid on a bacteria cell wall, disruption of cell membrane followed by ROS generation inside the bacteria, and DNA destruction. Also, biocompatibility assays were performed (cytotoxicity and hemolysis) and its toxicity was considered negligible, however, there is still a place for optimization of biocompatibility in further assessed nanoplatforms in this fashion (Huang, Chen, et al., 2017). It is worth mentioning that Enshaei and collaborators (Enshaei et al., 2001) used a different nanotheranostics approach to treat bacterial infections. They used the electro-responsive polymer (poly(3,4-ehtylendioxythiphene)) loaded with chloramphenicol, forming nanoparticles with activity against *Escherichia coli* and *Streptococcus sanguinis*. This smart nanotheranostics electro-responsive platform provided antibiotic controlled release by cyclic voltammetry and diagnostic capability through electrochemical detection of β-nicotinamide (microbial metabolism product) (Enshaei et al., 2001).

More recently, Liu and collaborators (Liu et al., 2021) proposed a self-assembled nanogel of thiolated silver nanoclusters impregnated in chitosan for both gram-positive and gram-negative infections, in vitro. Ultra-small silver nanoclusters are known for their antibacterial activity, however, their potential for toxicity and aggregation capacity complicates their usage. Thus, the group used chitosan to enhance biocompatibility, and antibacterial activity, diminish aggregation by fine dispersion, and promote the slow release of silver ions. Moreover, these nanoclusters are also photoluminescent in the UV-vis range and had their photoluminescence increased by 1.8-fold with the chitosan conjugation. The nanocomposite was demonstrated to have almost the same activity after 6-month storage at 4°C, indicating stability. It was tested against *S. aureus, E. coli, Bacillus subtillis*, and *Pseudomona aeruginosa*, bacteria representative of the main pathogenic ones, and functioned as both bacteriostatic and bactericidal with all of them, having a proposed mechanism of action by ROS production (slight increase). The complex had reduced cytotoxicity in comparison to nonconjugated nanoclusters, but it still needs improvements for biocompatibility in further animal testing (Liu et al., 2021).

Taking into account the utmost research on nanocomposites for theranostics, it can be stated that this technology has the potential to be widely used for many diseases. The nanocomposite complexity can range from multicomposed platforms of more than four components, with complicated synthesis and individual roles; or it can comprise a conjugation of up to three components in affordable and easy synthesis, with multiskilled components. Even promising, the technology is still at its basic steps towards development out of the cancer field, and it is of great contribution to the science of the future.

17.4 Perspectives

One of the most attractive options for theranostic applications is the nanocomposites that associate biofunctionalized nanoparticles and a suitable photosensitizer. Nevertheless, the limited available data related to unknown effects that these approaches could trigger on biological systems turn the applicability of multifunctional nanocomposites challenging. For complex drug delivery systems as nanocomposites to be recognized as safe for human treatment, more studies including the use of other in vivo models, besides immunocompromised mice models, are indispensable.

From a cancer therapy point of view, achieving an efficient antitumor effect without harming the healthy adjacent tissue is still a demanding goal of novel therapeutic approaches. Thus, multifunctional nanocomposites could bypass several treatment drawbacks including systemic toxicity, drug degradation, and bloodstream circulation time. The data assessed in this chapter exhibited promising theranostic systems; however, it was also observed the lack of standardization of evaluation methods between research laboratories. Importantly, the complex mixtures could be a risk if the nanocomposite release dynamic is not assessed. For commercially applicable nanocomposites, there is a necessity for the prediction of the metabolization of secondary products aiming to develop a risk assessment framework for the delivery systems.

With that said, the perspective of theranostics' applications is optimistic. With key attention to personalized medicine, theranostics offers an evolution from conventional therapy to a precision medicine approach. The theranostics systems can achieve the target tissue by avoiding host defenses and delivering treatment drugs and diagnostic agents to treat and diagnose at cellular and molecular levels. It is also important to state that theranostic systems could move directly in clinical trials for established markers and drugs.

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CHAPTER

Nanofibers for diagnosis, drug delivery, and therapy 18

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18.1 Introduction

Nanotechnology has expanded as a life-requiring interdisciplinary approach for many fields including agriculture, environmental remediation, medicine, and energy (Arachchige, Rienzie, & Adassooriya, 2019; Madanayake, Adassooriya, & Salim, 2021; Perera, Nadeesh Madusanka, Attalage, & Perera, 2015; Rienzie, Ramanayaka, & Adassooriya, 2019), etc. Therefore, the unique properties of nanomaterials (NMs) with respect to their high surface-area-to-volume ratio and quantum effects enable them to utilize in a range of fields. Also, the capability to fabricate into different shapes and sizes with functional candidates and their improved optical, electronic, magnetic and catalytic properties can master them as promising motifs (Madanayake, Rienzie, & Adassooriya, 2019) for these applications. Recently, the utilization of nanotechnology-based platforms has gained significant attention due to their improved bioavailability, stability, and targeted control (Balan, Indrakumar, Murali, & Korrapati, 2020). Therefore, nanotechnology-based medical applications have been exploited a lot for the detection, diagnosis, therapeutics, and treatment of diseases. More importantly, NMs can mitigate the negative impacts of traditional medical approaches including lower bio half-life, poor absorption due to enzymes, and nonselectivity against healthy cells (Ashwanikumar et al., 2016). Also, the smaller size of NMs and their properties can ensure controlled drug delivery and release, enhancing the passive accessibility and targeted drug delivery (Visweswara Rao & Hua Gan, 2015).

In recent years, the focus on the use of 1D nanostructures in different applications has attracted a lot. Ease of surface modification and unique nanoscale properties allow them to be used more intensively. Nanostructure including nanorods, nanowires, nanofibers (NFs), and nanotubes are grouped under this category. The high aspect ratio of these structures has made them more significant in many applications (Jeevanandam et al., 2020). The NFs are one-dimensional (1D) structures having at least one of their dimensions to lie within 1 and 100 nm scale. Extension in one specific direction distinguishes the 1D nanostructures clearly from nanoparticles (NPs) with zero dimension (0D) and from two-dimensional

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(2D) structures (Einarsrud & Grande, 2014). Potential of NFs to imitate biological systems including human tissues triggered to fabricate of them with different functional units. In addition, the high surface area to volume ratio alongside their penetrable nature allows them to be used in biomedical applications (Vasita & Katti, 2006).

The arena of NFs is a promising strategy in biomedical applications for many reasons. Firstly, the high surface area of NFs accedes to improve the adhesion into biological systems alongside medically important chemicals. Also, the capability to engineer them into modernized macro- structures permits them to induce their use in living systems with greater biocompatibility (Balan et al., 2020). Hence, this chapter will give an overview of the use of different NFs in biomedical applications as advanced formulations currently being used.

18.2 Classification of nanofibers

Currently, diverse numbers of NFs are being used in a range of fields. Generally, these can be grouped as carbon-based NFs, polymeric-based NFs using synthetic and natural molecules, and virus-based NFs using harmless bacteriophages (Cai et al., 2018; Kadavil, Zagho, Elzatahry, & Altahtamouni, 2019; Ma, Yang, Zhai, Yang, & Mao, 2020; Tran, Zhang, & Webster, 2009).

Most widely used materials to synthesize NFs and numerous publications are reported in the literature for their implications (Zahmatkeshan et al., 2018). Synthetic biopolymers including poly(ethylene glycol)-poly(DL-lactide) (PELA), poly(ε-caprolactone), poly(vinyl alcohol) (PVA), poly(D,L-lactide-coglycolide) (PLGA), poly-L-lactic acid (PLLA), poly(vinyl pyrrolidone) (PVP), poly(ethylene oxide) (PEO), poly(ethylene glycol) (PEG) have attracted for NF building blocks (Cui, Zhu, Yang, Li, & Jin, 2009; Li et al., 2012; Meng et al., 2011; Pinto, Rodrigues, Saraiva, & Lopes-da-Silva, 2015; Qiu et al., 2013; Son, Ryu, Lee, & Nam, 2013; Szilágyi et al., 2011; Uyar, Hacaloglu, & Besenbacher, 2011; Wang, Liang, et al., 2019; Zandi, Lotfi, Tamjid, Shokrgozar, & Simchi, 2020; Zhao et al., 2017). Therefore, these precursors are used as the main platforms for the synthesis of polymeric NFs.

Self-assembled peptide NFs have more recently emerged as another platform for NF synthesis. These have attracted a lot for numerous biomedical fields including tissue engineering, and drug delivery systems (DDS). The capability of introducing multifaceted functional groups via branched amino acid chains creates novel possibilities for the development of NFs with enhanced properties for biomedical applications. Furthermore, molecular structure and charged functional groups can help to assemble peptides to form NF. Therefore, binding affinities of peptide NFs with different structures including aromatic compounds would preferably work as a perfect release system (Ashwanikumar et al., 2016). For instance, certain dipeptides are capable of assembling as unidirectional NFs via hydrogen

bonding between adjacent peptide backbones to form desirable nanostructures (Debnath, Roy, & Ulijn, 2013).

Recently, cellulose NFs including their derivatives have attracted a lot for DDS and therapeutic applications as a new generation NM possesses a wide range of practical applications. Carboxymethyl cellulose (CMC), hydroxypropyl cellulose, bacterial cellulose, and cellulose acetate NFs are a few such examples of cellulose NFs motifs (Allafchian, Hosseini, & Ghoreishi, 2020; Aytac, Sen, Durgun, & Uyar, 2015; Konwarh, Karak, & Misra, 2013; Meneguin et al., 2020) experimented. Mechanical capability, stability, and biocompatibility alongside their degradability make them ideal candidates for DDS. Therefore, biopolymers have impressed a lot with nanomedical implications with their less riskiness to human health. Moreover, biopolymers consist of better biocompatibility, clinical functionality, and nonimmunogenicity than respect to synthetic polymers (Zandi et al., 2020).

Recently, human-friendly bacteriophages are used in biomedical applications. These bacteriophages for biomedical applications do not show any harm to human health because of the absence of promoters to express severe human diseases (Mohan & Weiss, 2014; Raja et al., 2019). Also, the phage's capability to self-assemble to form filaments and crystalline structures triggered use in these applications. Conjugating alien chemical groups (peptides) into the N-termini of phage proteins with no harm to their composition has made it easier to apply in biomedical applications. Also, these NFs can scale up as a low-cost approach for effective vaccination (Huai et al., 2016). Therefore, virus-based NMs have attracted a lot in different medical applications including chemical and biological sensing, disease diagnosis, therapy, tissue regeneration, screening peptides or antibodies, and proteomics analysis (Huai et al., 2016; Mao, Liu, & Cao, 2009; Raja et al., 2019; Xu, Cao, Li, & Mao, 2020), etc.

The bonding nature of carbon NMs allows for the development of structures having unique properties. The sp^2 hybridization constructs with van der Waals forces and strong in-plane bonds allow the making of carbon nanostructures (Eatemadi et al., 2014). Being small in size, inherent physicochemical properties and cost-effectiveness make them fruitful in numerous applications including multifunctional composites, electronics, energy, and biomedical applications (Aoki, Haniu, Kim, & Saito, 2020; Huang, Hsu, Kuo, Hsieh, & Teng, 2011; Wang et al., 2018). Also, versatility to alter the chemical properties of carbon NFs can be engineered to improve their solubility and biocompatibility properties for biological applications (Tran et al., 2009). Carbon NFs are cylindrical 1D NMs having diameters varying from hundreds of nanometers and lengths ranging from a few micrometers to millimeters. These are quite similar to graphite as conductive materials with respect to their electronic structure. Also, these are having high thermal stability opening novel possibilities for numerous applications (Naseri, Samadi, Ebrahimi, Kheirabadi, & Moshfegh, 2020).

Synthesis of carbon NFs mainly utilize hydrocarbons such as gaseous alkanes and oxides of carbon for their production (Vijayakumar, Samal, Mohanty, & Nayak, 2019).

Other than that, inorganic material-based NF complexes are also used for different applications reported in the literature (Ludwig et al., 2018; Wang, Qiu, et al., 2019).

18.3 Nanofiber fabricating techniques

There are some widely discussed techniques to synthesize NFs for different applications and these include Thermally Induced Phase Separation (TIPS), template synthesis, drawing, Chemical Vapor Deposition (CVD) and electrospinning, etc.

18.3.1 Thermally induced phase separation

Thermodynamic variations of polymers separate into multiphasic structures at a given temperature is the principle of this technique. The TIPS includes five main steps viz., polymer dissolution, phase separation, gel formation, gel extraction, and vacuum freezing of the solution which will divide a polymer into low and polymer − rich phases (Qin et al., 2017; Soltani, Khanian, Choong, & Rashid, 2020). This is a promising technique to obtain well − organized and unified polymer matrices. TIPS is an easy, quick, and flexible procedure to manufacture NFs (Soltani et al., 2020).

18.3.2 Template synthesis

Fabricating NFs using templating can generate bio—interfaces with adjustable cellular responses at the nano and micron scales for numerous applications. This is a promising technique to fabricate NFs having conductive polymers, semiconductors, and metals. More importantly, templating can engineer easily to control the pore sizes of NFs. However, the diameter of NFs is controlled by the diameter of the pores of the membrane (Soltani et al., 2020).

18.3.3 Drawing

Drawing is a novel technique allowing the users to construct NFs using polymer precursors. Principally, this is happened by drawing out polymer droplets at a specified speed to synthesize NFs and the rate is dependent on the polymer type. Drawing synthesizes suspended NFs by conjugating previously deposited polymer droplets using a sharp probe tip. Then these conjugated liquid droplets solidify to form NFs by rapid evaporation of the solvent (Nain, Wong, Amon, & Sitti, 2006). This technique allows us to understand the properties of individual NFs because the synthesis depends on the deposition of polymer solution dropwise forming suspended NFs. This method is useful in nano-optics, nanoelectronics, and tissue engineering (Bajakova, Chaloupek, Lukáš, & Lacarin, 2011) applications.

18.3.4 Chemical vapor deposition

This is a commercially available technique to synthesize NFs, especially for carbon-based NMs. Here, a carbon source is decomposed to form NFs under a suitable substrate catalyzed by a metal. For instance, Hulicova-Jurcakova, Li, Zhu, De Marco, and Lu (2008) synthesized graphitic carbon NFs using methane and acetylene on the alumina substrate supported by Ni catalyst. Also, carbon NFs were produced using CVD using the same support and catalyst for the synthesis are some of the examples for carbon NFs synthesized (Zheng et al., 2004). The NFs expand around the catalyst and the size of metallic NPs affect its diameter (Saifuddin, Raziah, & Junizah, 2013; Soltani et al., 2020). However, operation at higher temperatures is the main limitation where it requires considerably a larger amount of energy for the synthesis. In addition, certain precursor molecules and by-products cause toxic effects on users (Soltani et al., 2020).

18.3.5 Electrospinning

Electrospinning is the first choice for preparing NFs in medical applications because of their ability to scale up industrially. More importantly, the ability to use a range of polymeric units from synthetic to natural chemicals allows them to be more suitable candidates in a range of applications. Therefore, this method of synthesis makes it a cost-effective motif for mass-scale production (Leung & Ko, 2011). Effective self-assembly of NFs into nano scaffolds is affected by bonding via hydrogen bonds, van der Waals, electrostatic effect, hydrophobic effect, and $\pi - \pi$ stacking interactions (Guo et al., 2019). Implying a high voltage to a polymeric sample or into a precursor molecule leads to generating NFs (Kajdič, Planinšek, Gašperlin, & Kocbek, 2019). It is a superior technique compared to other methods of NF preparation such as phase separation, self-assembly, template synthesis, and mechanical drawing. Because electrospinning is a simple, economical, and easily customizable technique to manufacture superfine NFs. Furthermore, electrospinning has great flexibility to stipulate materials for drug delivery, diagnosis, and therapeutics. It is a cost-effective approach to producing drug loading scaffolds with high loading and encapsulation efficiency with controllable release rates (Zandi et al., 2020). In addition, electrospinning is the preferred method to synthesize NFs with different precursors ranging from natural to synthetics chemicals including biodegradable and nonbiodegradable molecules (Pillay et al., 2013).

18.4 Nanofibers in drug delivery

Tailoring DDS with controlled rates with appropriate dosages is an utter requirement for medical applications. In addition, enhanced biocompatibility with minimum side effects (Cinar et al., 2017) has focused a lot on nanomedicine. Based

on the specific binding abilities of NFs, they can be engineered as appropriate candidates for DDS (Ashwanikumar et al., 2016).

Zandi et al. (2020) fabricated a core-sheath NFs system for dual drug delivery for medical applications using gelatin/PVA solution. It was observed that the analytes were released in three phases. Initially, a burst release (first eight hours) having a non-Fickian diffusion mechanism followed by a decreased release rate lasting for 33 h as the second stage and finally with a steady state release. Authors have speculated that the developed core-sheath NF platform has a dual delivery capacity with the potential to be used in wound healing applications (Zandi et al., 2020). Meng et al. (2011) prepared PLGA and PLGA/gelatin NFs via electrospinning to deliver fenbufen (FBF). It was speculated that higher gelatin content improves the hydrophilicity of NFs enhancing the release rates of FBF. Moreover, NFs arrangements are influenced by the release pattern of the test drug. Furthermore, this DDS has great stability under different pH values in contrast to solvent-cast films. A multiple DDS was introduced by Balan et al. (2020) for carcinoma treatments using chitosan-loaded polycaprolactone NFs to supply resveratrol and ferulic acid. Anticancer efficacy of NFs was displayed 30% and 50% reduction in the cell viability on epidermoid carcinoma cells.

Self-assembled peptide NFs have attracted immensely for controlled drug delivery for biomedical applications application because of ease in drug loading and high drug loading capacity, biocompatibility, reversible character, and biodegradability make them ideal candidates (Ashwanikumar et al., 2016; Cinar et al., 2017). For instance, Ashwanikumar et al. (2016) engineered a pH-sensitive RADA-F6 peptide NFs to deliver the anticancer drug 5-fluorouracil (5-FU) under alkaline conditions for the treatment of colon cancer was reported. Intracellular restructured reduced glutathione-responsive peptide NFs DDS for doxorubicin (DOX) for tumor cell treatment also reported synergistic chemotherapy effects (Guo et al., 2019). Peptide-based NFs can be successfully tailored for targeted hydrophobic DDS. For instance, Liu et al. (2014) speculated that Nap-GFFYG-RGD peptide NFs are potent agents for curcumin (CUR) delivery for cancer treatment. Peptide NF-based strategies are capable of stamping out the requirement of cell transplantation or viral gene transfection therapeutics for effective regenerative medicine in tissue engineering (Kumar et al., 2016).

The CMC has been recognized as a promising candidate for DDS due to its hydrophilicity, biocompatibility, and biodegradability. Hence, CMC/PVA NFs loaded with flufenamic acid drug delivery were prepared by Allafchian et al. (2020) for therapeutic applications. Dialysis tube testing had shown that this NF composite is having a time-based behavior for drug loading and releasing with time. Cellulose NF-TiO_2 nano complex implanted with diclofenac sodium, penicillamine-D, and phosphomycin was designed as a long-term release for dermal applications (Galkina, Ivanov, Agafonov, Seisenbaeva, & Kessler, 2015). Interactions between titania and the grafted drugs control their release kinetic of them for extended durations of release. Hence, this can be regarded as a potential therapeutic agent for anesthetics, analgesics, and antibiotics. Fakhri, Tahami, and Nejad (2017)

exploited Fe_3O_4-Ag_2O quantum dots cellulose NFs conjugated with etoposide and methotrexate another DDS. It was reported that the drugs were released at a constant rate achieving their equilibrium at 24 and 30 h as a promising DDS. Also, certain studies have shown that bacterial cellulose NFs and hydroxypropyl cellulose NFs can be used as potential candidates to deliver lipophilic and hydrophobic drugs (Aytac et al., 2015; Meneguin, et al., 2020). For instance, Meneguin, et al. (2020) used spray dried bacterial cellulose NFs as a recent application for lipophilic pharmaceutical drugs for novel enteric DDS based on natural polymers. These are some of the promising applications of different NFs in drug delivery and diagnosis is another arena that is promised with these advanced formulations.

18.5 Nanofibers in disease diagnosis

NMs-based procedures are recognized as promising tools for detecting illnesses. Because they are imperatively tiny, easy to handle, and more cost-effective than spectroscopic techniques (Regius & Jain, 2014). Early detection is crucial for the efficient treatment of diseases to increase the life expectancy of patients with critical conditions (Wongkaew, 2019). Identification of cancer biomarkers in body fluids has impressed for diagnosis compared to surgical biopsies. Markers from blood, urine, and saliva possess little invasiveness, simplicity, and suitability for high throughput assays. Detection of DNA point mutations using NFs scaffolds was reported in cancer diagnostic applications. For instance, Tripathy et al. (2018) demonstrated the use of a label-free electrochemical biosensor for BRCA1 gene-specific point mutation detection using electrospun graphene doped manganese III oxide(GMnO) NFs. These NFs enhance the resolution of the electrode surface through improved reaction kinetics. Therefore, this platform is highly sensitive to alterations in conductivity due to charge transfer resistance from GMnO leads to detecting the mutations more accurately.

Virus NFs are another biosensor-mediated approach for diagnosis. Certain anti-antibodies in blood serum such as anti-p53 antibodies can act as markers of cancers. Pan et al. (2015) employed a modified immunological approach by combining phage NF as a capture probe for the enzyme-linked immunosorbent assay with conventional methods. It was reported that combined application had the greatest accuracy and efficiency for the anti-p53 antibody detection with various malignant cell types. Szot-Karpińska et al. (2020) demonstrated a bacteriophage-based diagnostic sensor for C-reactive protein (CRP). The biosensor was developed by depositing negatively charged bacteriophages on positively charged carbon NFs. The level of CRP content in the blood of a given patient is an indication of tissue damage, infections, and inflammations. Moreover, CRP plays an important role in detecting different disease conditions including cardiovascular, tumors, and autoimmune diseases (Gabay & Kushner, 1999; Szot-Karpińska et al., 2020). Authors have justified this approach as a novel technique over conventional immunological methods

such as artificial antibodies to diagnose viral and bacterial infections to mitigate antibacterial resistance.

At present, analyzing the volatile organic components in exhaled air is pointed as a trending strategy for pinpoint detection of noncontagious diseases (Asal & Nasirian, 2019; Regius & Jain, 2014). For instance, measuring the concentration of acetone in exhaled air can differentiate diabetes patients from healthier personnel (Asal & Nasirian, 2019). Hence, semiconductor metals-based gas sensors show promising results in detecting these gas components. Principally it involves the variation in resistance stimulated by chemisorption of oxygen anions (O^-, O^{2-}) that interact with reducing gases. This alters the depletion area along with its resistance optimizing for disease diagnosis. Choi et al. (2014) showed that diagnosis of diabetes and halitosis can be achieved using SnO_2 NFs by detecting acetone and hydrogen sulfide, respectively. It was speculated that the threshold level was 1 ppm for hydrogen sulfide and 100 ppb for acetone which are significantly below the dosages concentrations in exhaled air of healthy. The sensitivity of lateral flow assay can be enhanced by incorporating carbon NFs into a nitrocellulose membrane was shown by Tang, Liu, Zhang, Li, and Li (2019) for *Staphylococcus aureus* testing is another example of utilizing NFs in disease diagnosis.

18.6 Nanofibers in therapeutic applications

Nanotherapeutics in cancer treatments have attracted immensely to minimize the systemic toxicity and mitigate multidrug resistance of conventional strategies (Guo et al., 2019). Yu, Zhang, Liu, and Liu (2019) synthesized supramolecular NFs containing Au nanorods, peptide fabricated iron oxide NPs having functionalized β-cyclodextrin for cancer therapeutics. It was reported that these NFs cause severe mitochondrial destruction to human adenocarcinoma cells while suppressing the metastasis upon irradiating with near-infrared light to model rats bearing these cells. Therefore, authors have stated this as a promising approach to mitigate tumor evasion and metastasis. Zhang, Liu, Yang, and Zhu (2015) synthesized a silk fibroin-sodium alginate NF composite for tissue engineering applications using TIPS. Culture experiments proved that NF matrices can affix humans allowing them to regenerate well on the surface. Gelatin and silica hybrid NFs were produced by Lei et al. (2012) for bone tissue engineering applications. NFs thus synthesized had shown an enhanced mechanical property with higher biodegradation stability and apatite forming ability. Also, biocompatible delivery of DOX and CUR, for the treatment of glioblastoma are reported as chemotherapeutic strategies (Karavasili, Panteris, Vizirianakis, Koutsopoulos, & Fatouros, 2018). Norouzi, Abdali, Liu, and Miller (2018) developed PLGA NFs grafted salinomycin antibiotics for cancer therapeutics. It was shown that NFs were stable for 30 days with a continuous release of the therapeutic agents for two weeks. Also,

authors have attributed that more than 50% of the human glioblastoma cells experienced apoptosis within 48 h with treated concentrations. It was highlighted that reactive oxygen species generated intracellularly led to malignant cell apoptosis. In addition, these NF agents were capable of upregulating certain tumor suppressor genes too. Ding et al. (2016) fabricated docetaxel-loaded poly-D, L-lactide NFs to prevent against reoccurrence of localized breast cancers. It was speculated using mice experiments NF complex has brilliant biocompatibility with a minimum density of inflammatory cells in tested sites. Conjugated therapeutics applications were also reported in the literature. For instance, Zhang, Liu, Xiong, et al. (2015) formulated a photo and chemotherapeutic agent by grafting multi-walled carbon nanotubes (MWCNTs) and DOX with PLLA NFs. Here MWCNTs are effective heat producers in the presence of near-infrared light (NIR). The NIR radiation induces a blowup release of DOX from NFs. Also, enhanced temperature around the tumor area promotes the cytotoxicity of malignant cells via phototherapy and chemotherapy.

Recently, virus NFs have attracted therapeutic applications as well. For instance, Huai et al. (2016) utilized genetically modified phage NFs for the treatment of *Candida albicans* infections. Where this was achieved by immunization of patients with secreted aspartyl proteinases 2 by expressing such an epitope on the wall of virus NFs. Here bacteriophages used for biomedical applications do not show any harm to human health because of their absence of promoters to express severe human diseases (Mohan & Weiss, 2014; Raja et al., 2019). Also, the phage's capability to self-assemble to form filaments and crystalline structures triggered is a plus point. In addition, the ability of viruses themselves to arrange into hierarchically ordered structures, such as rope-like bundles and liquid crystals allows them to master these applications. Conjugating the alien chemical groups (peptides) into the N-termini of phage proteins with no harm to their composition has made it easier to apply in biomedical applications. Also, these NFs can be scaled up as a low-cost approach for effective vaccination (Huai et al., 2016). Therefore, virus-based NMs have attracted a lot in different medical applications including chemical and biological sensing, disease diagnosis, therapy, tissue regeneration, screening peptides or antibodies, and proteomics analysis (Huai, et al., 2016; Mao et al., 2009; Raja et al., 2019; Xu et al., 2020), etc. In addition, reverse reconstruction of the damaged intervertebral disks using bacterial cellulose NFs as a promising tactic to design highly sophisticated artificial tissues by mimicking native tissues was speculated by Yang et al. (2018).

18.7 Conclusion and future perspectives

Nanomedicine is a promising approach for most of the drawbacks of traditional methods used in medicine. Therefore, nanotechnology has advanced day by day to overcome these limitations in drug delivery, diagnosis, and therapeutics. The

focus on utilizing advanced nanoformulations such as NFs has accelerated their applications in those fields. NFs has a tremendous flexibility capacity to conjugate different functional motifs for these applications. Polymeric-based, carbon-based, peptide-based, as well as phage-based NFs, have been the crucial players currently used in medical applications. Different techniques have been introduced to synthesize NFs having diverse pros and cons unique to them. However, electrospinning approaches have become the first choice of selection for NF fabrication mostly. Controlled drug delivery with appropriate dosages is the main feature for the higher attraction of NMs in nanomedicine. Therefore, enhanced biocompatibility with minimum side effects of NFs created more opportunities among researchers to advent novel NF-based formulations. Also, the specific binding capabilities of NFs can master them as appropriate candidates for DDS. Detection and diagnosing the disease is the area of focus to apply NFs because of their easiness of handling and cost-effectiveness compared to conventional strategies. Moreover, early detection of diseases can be achieved using NFs mediated systems. However, NFs are promising for biomedical applications still it is important to study whether these formulations may have a toxic effect on nontargeted biosystems is imperative. Furthermore, it is noteworthy to mention that natural precursor molecules to can be used as more promising candidates over synthetics may mitigate the limits. Therefore, NFs can be augmented as a promising candidate for medical applications.

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CHAPTER

Nanovectors for theranostic applications 19

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19.1 Introduction

Nanomedicine, an offspring of nanotechnology, is the latest term whose description is evolving. Nanotechnology has an important role in early-and late-stage interventions to manage various pathological conditions such as asthma, cancer, cardiovascular diseases, diabetes, infectious diseases, rheumatoid arthritis, genetic disorders, multiple allergic conditions, neurodegenerative and immune diseases (Fig. 19.1) (Murthy, 2007). There have been significant advances in biological and physical technologies over the decade to make nanomedicine a reality. For instance, new tools are being developed that allow imaging of biological structures at the nanoscale, quantification of forces, and rapid assessment of dynamic behavior of assemblies at the molecular level. The major aim of nanomedicines is to provide better control on intracellular machinery, thus leading to improved tools in diagnosis and personalized disease treatments with minimal side effects. The first generation of nanosystems developed basically for passive targeting of diseases and due to lack of precise binding and toxicity, the second generation of nanovectors was synthesized for active targeting. However, the second-generation vectors failed to provide effective targeting and high therapeutic efficacy. This led to the conceptualization of the third generation of multifunctional and multipurpose NPs under a single platform to elevate imaging, targeting, and treatment capacity. Yet, research is ongoing towards synthesizing fourth-generation vectors to reduce toxicity and improve compatibility in more than one approach, and deliver synergistic effects (Fig. 19.1) (Murthy, 2007).

The basic unit of nanotechnology-based therapeutic regimen is a nanoparticle, and their unique function to deliver drugs to the intracellular compartments makes them a nanocarrier commonly defined as "nanovectors". Not long ago, nanotechnology-derived nanovectors evolved to overthrow the drawbacks of a few nonviral and viral vectors. The magnitude of nanovectors allows flexibility, ease of use, cost-effective production, and superior biological safety with a higher transfection rate. Nanovectors, which are nanocarriers (1−100 mm diameter in size), qualified to transport and deliver more than one bioactive agent, is

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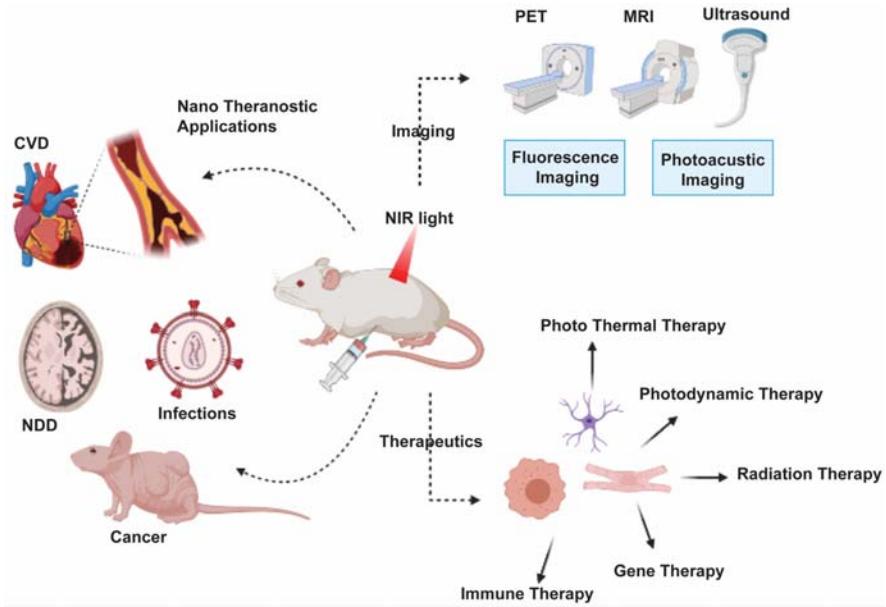

FIGURE 19.1

Nanotheranostic application of nanovectors in various diseases role of nanovectors as contrast agents in functional imaging and image-guided theranostics of different diseases.

emerging as a novel class of delivery platform for imaging enhancers and therapeutic agents. With their high surface area, nanovectors allow high drug load and cross blood barriers, and deliver therapeutic agents to target cells (Fig. 19.2). The primary aim is to achieve precise drug action at the delivery site and with less systemic off-target effects.

Nanovectors might also act as promising delivery tools for therapeutic DNA, RNA, pDNA, and proteins. These therapeutic carriers will be more active and efficient in reaching the active site due to less immunogenic attack. It is anticipated that nanovectors functionalized within may have their intrinsic therapeutic ability that will work synergistically with the drug being loaded in the long term. Various organic, inorganic, and polymeric nanomaterials provide a basic unit of nanovector to stack particular ligands on the surface, which provides them ideal molecular sensing, efficient contrast for functional imaging, cargoes of drugs, delivery agents (gene or drugs), and therapeutic agents in thermal therapies of tumor ablation. Though a significant number of nanovector-based therapeutic and diagnostic agents such as theranostic systems have been engineered for the treatment of several highly morbid and mortality-yielding pathological conditions (Fig. 19.1), the safety of nanovectors became indispensable for human applications. A novel class of nanovectors known as biomimetic NPs gained attention

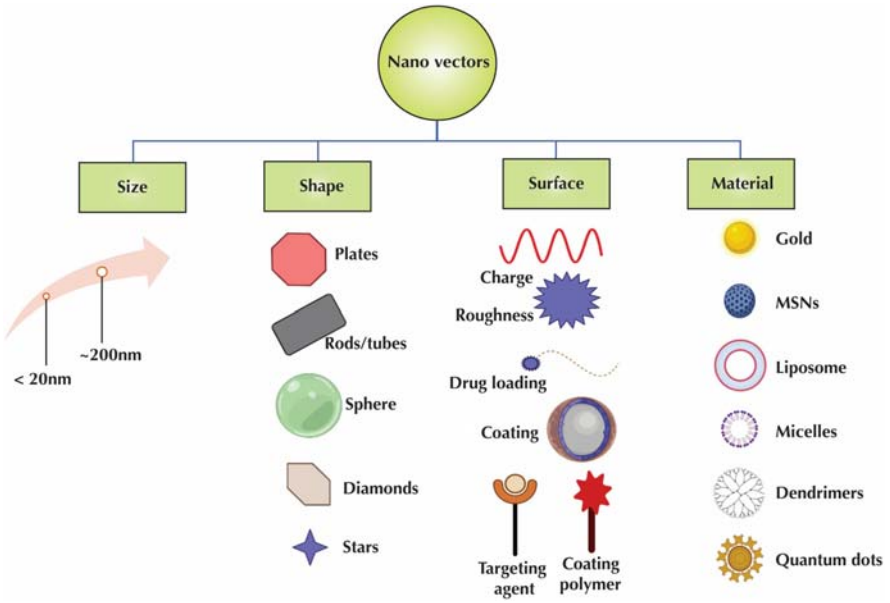

FIGURE 19.2

Schematic representation of intrinsic properties of nanovectors size, shape, surface charge, biomaterial, and presence of ligand affect nanovector therapeutic purposes.

due to their biocompatibility, biodegradability, nontoxicity, and no off-target effects. Coating of NPs core with cell-derived membranes has been used to overcome toxic profile and escape from immune attacks. The nanotheranostic approach is a well-established strategy in molecular targeting of both diagnosis and therapy. The visualization of active sites can help predict the therapeutic regimen, the prognosis of diseases, and posttherapy response or change of ongoing therapy. The future focus of nanomedicines is to develop hybrid nanovectors for improved accuracy and sensitivity since recent cancer therapeutics with these hybrid nanovectors proved successful.

19.2 Features of nanovectors

The healthcare and pharmaceutical industry benefit most from nanotheranostics-a new and booming sector of nanotechnology. It also plays a vital role in molecular biology by adding molecular sensors, and imaging contrast agents (CAs) for the diagnosis and delivery of a therapeutic drug. These innovative vectors and carriers can make a difference in the treatment of various pathological conditions via nanotheranostic diagnostic platforms (quantitative assays and imaging) and

therapeutic strategies through gene therapy or by identification of biomarkers and targeting methods. An essential element of nanotheranostic is forming a nanovector or nanocarrier that can accommodate all critical requirements of a functional theranostic agent in one platform (Kang et al., 2008; Miao, Oldinski, Liu, & Chen, 2019). Usually, the physical and chemical characteristics (like size, shape, charge, and surface modification) of nanovectors decide their outcome (Penet, Krishnamachary, Chen, Jin, & Bhujwalla, 2014). For example, tiny NPs, $<$20 nm, have active biodistribution, but their renal clearance is also rapid. On the other hand, NPs with $>$200 nm tend to accumulate in organs like the liver and spleen and undergo mononuclear phagocytic clearance (Zhang, Li, Lykotrafitis, Bao, & Suresh, 2009). In healthy blood vessels, the pore size of the endothelial tight junction is $<$10 nm, whereas, in the tumor microenvironment (TME), the size of the tight junction is $>$200$-$1200 nm. Furthermore, the lack of lymphatic drainage in TME facilitates retention of nanovectors, thus enhancing the permeability and retention (EPR) effect. The nanovectors allow visualization and monitoring of drug entry, distribution, the kinetics of drug delivery, and drug efficacy. With the use of a single nanovector, it is likely to tune treatment while simultaneously catering to real-time monitoring of disease progression and drug response (Sneider, Vandyke, Paliwal, & Rai, 2017).

The size and shape of nanovectors used in theranostics are directly associated with their effectiveness (4). For example, globular vectors are regularly utilized in theranostics, since rod-shaped, filament-like, worm-like, well-shaped, or disk-shaped, have distinctive traits like drug storing and absorption ability, circulation half time, target intake, absorption at the target site, etc. (Fig. 19.2). Also, a wide range of vector size is necessary for the transport and absorption of nanovector by the target site. According to *Banerjee* et al., nanovector size can range from 50 to 200 nm (Fig. 19.2). The study found that rod-shaped vectors had higher cellular uptake than sphere-shaped vectors irrespective of active targeting agents (Banerjee, Qi, Gogoi, Wong, & Mitragotri, 2016). Coming to transportation properties, nanorods had higher intestinal transport than spheres (Banerjee et al., 2016). As per another study, worm-like vectors with poly(ethylene glycol)-polybutadiene (PEG-PBD) probe had practical anticancer benefits (Loverde, Klein, & Discher, 2012). The distinct small size sponsors their blood circulation time beyond conventional chemotherapeutics and doubles the absorption rate by tumor vasculature (Penet et al., 2014). In addition, by modifying of shape from a sphere to rods one can achieve change in resonance and optical properties, thus, giving a therapeutically useful range from 800 to 1200 for deep penetration via photoimaging and photothermal methods (Singh et al., 2018).

Another feature is that nanovectors own surface-area-to-volume ratio that is adequate to provide loading space to imaging CAs and targeting ligands in therapy is added benefit for them to match nanotheranostics (Loverde et al., 2012). The unique inherent properties of nanocarriers enormously benefit the field of customized medicine in diagnosis and therapy (Mura & Couvreur, 2012). These

extended qualities of nanovectors make them preferable in treating multiple diseases that conventional treatments fail to address (Fig. 19.2). Other essential elements while operating with nanovectors in nanotheranostics are knowledge of the type of target tissue or cells and their associated biomarkers. Also, the course of administration or path nanovectors will pursue to travel their target position can be taken into consideration (Natfji, Ravishankar, Osborn, & Greco, 2017) (Fig. 19.2).

Stability and protection of nanovectors while in the body system, that is, attack by the external bodily stimuli (pH or body fluids or oxygen levels) or resist the immune attack to reach their particular target and get consumed. The physiological cellular microenvironment such as pH, hypoxia, and interstitial fluid pressure provides receptor-mediated targeting of nanovectors. For example, hypoxia in healthy cells can induce neoplastic properties in the cells, and low pH promotes the TME around the neoplastic cells (Penet et al., 2014). When the interstitial fluid pressure changes, the pathway for a foreign material is exposed to enable free influx or efflux due to a change in cellular permeability. The foreign body can be a disease carrier. It may contain several other undesired markers that may create shifts in the cellular functions with the decrease or increase in the permeability of cells (Wang et al., 2017). For instance, prolonged permeability may cause wobbly membranes, making it challenging to target the vasculature in the TME. Moreover, cancerous stromal cells surrounding the tumor will undergo uncontrolled proliferation and initiate cancer progression (Natfji et al., 2017). Extracellular matrix (ECM) can also be a target that can modify physicochemical properties of cells and induce tumor development (Wang et al., 2017). Hence, the molecular receptors expressed by various cancer cells can be used to design nanovectors. For example, in many cancer types, matrix metalloproteinases (MMPs) induce tumor growth, angiogenesis, metastasis, and invasion; and are regarded as targets for tumor discovery since cancer cells specifically overexpress these markers (John & Tuszynski, 2001). It is important to mention that the cell-surface markers are highly dynamic in their expression patterns and highly specific regarding their tissues of origin.

The tissue microenvironment in disease is not the same as healthy tissues, such as during infections and neoplasms, the blood flow and pH change. As a result, many other biomarkers (receptors, antibodies, cytokines) are specifically expressed by these diseased tissues and give a foundation for personalized medicine. In the last decade, nanovectors have proved their role in early diagnosis and targeted drug delivery of therapeutic agents in cancerous and noncancerous disease conditions. Our human body is not a single section but a collection of multifaceted and multifunctional organs and systems, and all individual is different in their genetic and phenotypic makeup. These variations also affect how an individual differs in response to a particular therapy. Thus, developing nanotheranostics that can generate biological responses to accomplish personal demands necessitates a broad perception of several pathophysiological variations of several disease types.

19.3 History of nanovectors

Nanovectors are progressing in multiple ways, with significant advancements in their synthesis, functional elements, and applications in nanomedicine. Precise targeting (active or passive), photo-induced therapy, and regulated drug release form some of the basic features known to produce a clinical purpose. Based on their predetermined function and application purposes, nanovectors are classified into three "generations" (Leon et al., 2020; Murthy, 2007).

19.3.1 First generation nanovectors

The first groups of vectors are made of simple and basic formulations such as liposomes and Au carrying chemotherapeutic drugs or gene targets (siRNA or DNA) and got approval for medical applications in the past (Godin et al., 2010; Martinez et al., 2012; Williams, Pijpers, Ridolfo, & van Hest, 2017). The first-generation nanovectors can easily penetrate tumor environments due to large intra- and inter-endothelium channels favoring extravasation of nanocarriers (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017). Additional modifications with stealth or PEG prevented their intake by the reticuloendothelial system (RES), thus extensively increasing the time of vectors in circulation (Milton Harris & Chess, 2003) and expanding the probability of tumor homing (Martinez et al., 2012; Williams et al., 2017). Among the many first-generation NPs, liposomes conjugated with or without PEG have gained popularity. Myocet and Doxil nanovectors got approval for use, and DaunoXome, SPI-77, Onco-TC, and OSI-211 have been evaluated in clinical settings (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017). Liposome-based vectors and metal nanovectors for diagnosis, albumin conjugated with paclitaxel for therapeutics, and polymers loaded with drugs have been engineered in the past (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017).

19.3.2 Second generation nanovectors

Second-generation nanovectors differ from earlier classes with additional functions preventing degradation of loaded drugs or delivering nanovectors designed with biomarkers capable of targeting tissues whose expression is specific (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017). Adding multiple biorecognition, triggered activation, and environmentally responsive tools in second-generation nanovectors provided better targeting of tissues or diseased sites, both in vitro and in vivo (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017). The most studied example of the second class of nanocarriers are antibody (Ab) conjugated NPs. Other than Abs, a group of targeting vectors such as aptamers, peptides, and ligands targeting biomarkers specifically present on target tissues have been studied at large (Godin et al., 2010; Martinez et al., 2012;

Williams et al., 2017). Though the targeting efficiency enhanced with beneficial results, this class of vectors did not penetrate the market and was unable to fulfill clinical purposes, which led to the next generation of vectors' design (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017).

19.3.3 Third generation nanovectors

The third-generation nanovectors (TGN) provided a change in traditional thought and decoupled the diversity of tasks needed for NPs to travel to intravascular sites (Godin et al., 2010; Martinez et al., 2012). This group could address challenges regarding penetration of vectors to biological barriers and improved deposition at the target site (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017). TGN can produce a time course of functions, including aggregated NPs and nanoconstituents' united coordination. These new contemporaries of NPs are exemplified by manipulating multiple nanobased commodities that synergistically produce discrete functionalities (Godin et al., 2010; Martinez et al., 2012; Williams et al., 2017).

19.3.3.1 Nanovectors for theranostic applications

Theranostics represent current and emerging nanotechnology forms; however, the nanovectors should accommodate more than one agent such as stabilizers, CAs, therapeutics, and targeting ligands. The size, shape, and nature of the material and surface properties are key to developing theranostic nanosystems (Fig. 19.2). The majority of the ongoing focus is to find potential alternative agents with diverse architecture and properties to engineer a structure-function relationship of nanovectors. Their high surface-to-volume ratio and structural and functional properties change/depend on the nanoconstituents and preparation method. For biomedical applications, the nanoconstituents must be well characterized, stable, biocompatible, and able to present advantages compared to conventional therapies such as encapsulation of water-insoluble molecules, retention capacity, biodistribution, conjugation with either diagnostic or therapeutic ligands, enhanced subcellular penetration, controlled drug release, and target specificity. Numerous nanomaterials are under study for clinical purposes, varying from inorganic to organic, and many of them are under investigation. Fig. 19.3 provides a glimpse of the types of nanovectors used in a theranostic application through a systematic grouping of vectors based on the biomaterial composition.

19.3.4 Inorganic nanovectors

Nanovectors can be made using either single or hybrid nanomaterials (inorganic core & organic shell). However, in this section we will emphasize various inorganic constituents of nanovectors that can be found in various mixtures and shapes; that have been used in diagnosis and therapeutics (Fig. 19.3).

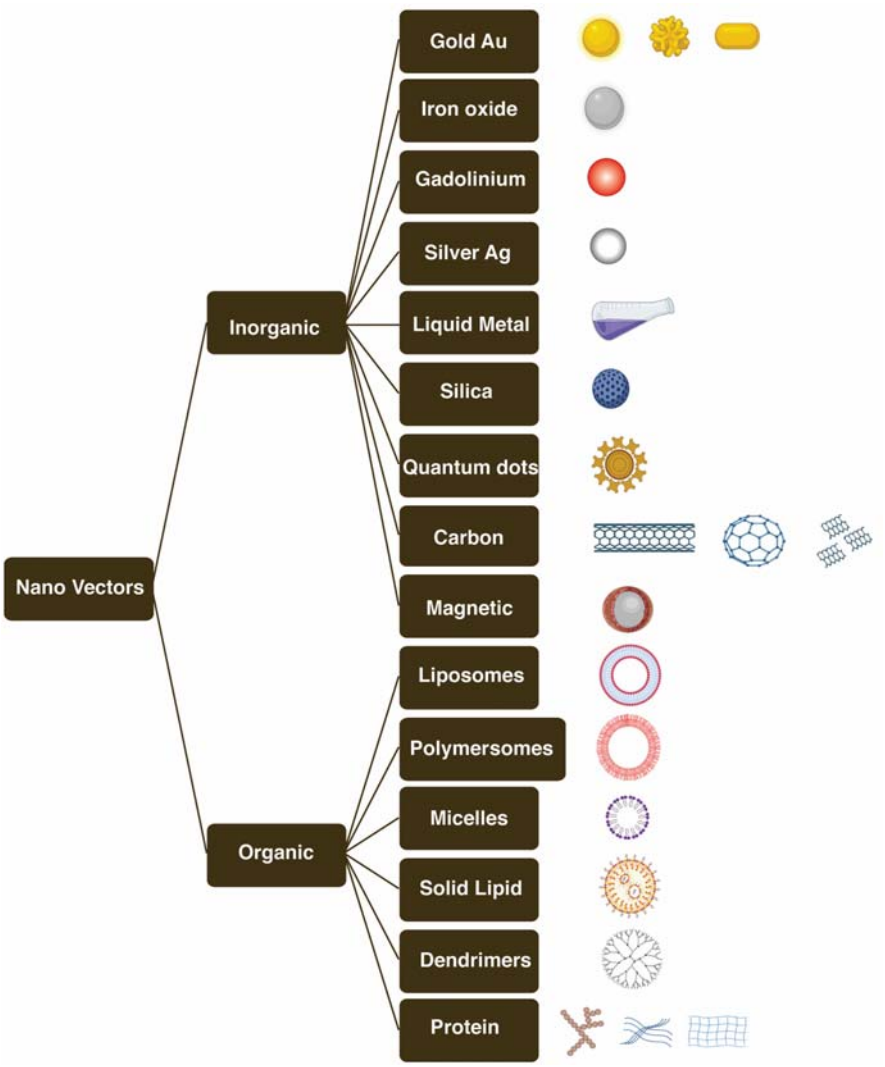

FIGURE 19.3

Nanovectors and their architecture examples and classification of nanovectors based on their chemical composition.

19.3.4.1 Au

Properties such as simple chemistry, electrical, optical, low toxicity, and biodegradability allow the synthesis of gold NPs through simple methods at large. Gold NPs serve as excellent delivery agents of therapeutic drugs or biological agents suited for photothermal therapy of various cancer types and biomarker-based

diagnosis of noncancerous diseases. Au-based nanovectors showed promising benefits in the diagnosis and treatment of parasitic diseases as well. Studies have reported that AuNPs hold great protective ability against siRNA degradation, implicating that AuNPs as excellent gene delivery carriers. In addition, the non-toxic profile and biocompatibility of AuNPs make them prolific drug carriers. In the past, AuNPs were conjugated with drugs like methotrexate and doxorubicin (DOX) conjugated, since these drugs alone failed to penetrate target sites. Upon conjugation with AuNPs, enhanced uptake and higher toxicity on affected cells have been reported (Chen et al., 2007; Wang et al., 2011). The low stability of peptide-drug-conjugates, low half-life, low specificity, rapid clearance, and reduced penetration capacity of phytochemicals were improved upon liking with AuNPs (Kalimuthu et al., 2018; Srinivas Raghavan, Kondath, Anantanarayanan, & Rajaram, 2015). The tuneable shape and size provide extended space for cross-linking various agents like the linkers, drugs, ligands, Ab, and aptamers for strategic delivery.

The intrinsic plasmonic properties of AuNPs can absorb infrared light and shift to an excited state; this property makes them suitable partners for targeted thermal therapies (photothermal and photodynamic therapy). The maximum absorption capacity of AuNPs at 795 nm has been used in photothermal therapies that can kill cancer cells. Also, 30 nm AuNPs conjugated with IgG in targeting cluster of differentiation 8 (CD8) expressing tumor cells demonstrated for the first time (Pitsillides, Joe, Wei, Anderson, & Lin, 2003). The only successful AuNPs approved by the Food and Drug Administration (FDA) and are in ongoing clinical investigations are PEGylated AuNPs because of enhanced absorption and accumulation at the tumor site in the NIR region (Dickerson et al., 2008). In delivery and therapeutics, AuNPs can also be used in multiple imaging methods by adding more than one CAs to improve the characterization of diseases and prevent multiple drug challenges to the immune system of subjects (Louie, 2010). AuNPs have been used in imaging cardiovascular diseases, viral infections, and diagnosis and therapy of Alzheimer's diseases (AD) (Draz & Shafiee, 2018; Sivanesan and Rajeshkumar, 2019; Varna, Xuan, & Fort, 2018).

19.3.4.2 Iron oxide

Ferromagnetic or superparamagnetic properties of iron oxide NPs play a key role in biomedical applications. However, the superparamagnetic property is the most desired one. Under the magnetic field, the superparamagnetic property will provide the alignment of domains with magnetism. When magnetism is removed, temperature increases and the generated heat under magnetic fields on/off is useful in hyperthermic tumor ablation. Off lately, iron oxide NPs in imaging and therapy of neurodegenerative disease due to assimilated targeting, ability to enter blood-brain barriers (BBB) in Parkinson's disease (PD) and AD rising hope for patients. Iron oxide NPs' major roles come through MRI-compatible CAs and drug carriers, bio-catalytic activity, or photo-responsive therapy. Iron oxide-based CAs are famous for imaging the liver, vascular, lymph

nodes, and inflammation of the damaged tissues. Iron oxide nanoparticles (Fe_3O_4 NPs) are commonly used in T_1 or T_2-weighted imaging for biosecurity. The superior thermal effect following the oscillating magnetic field, magnetic iron oxide NPs, is extensively used in magnetic hyperthermic ablation of tumors (Zhao, Yu, Qian, Chen, & Shen, 2020).

Fe3O4 NPs mimic peroxidase or oxidase enzyme The catalytic activity for cancer therapy through Fenton reactions. The Fe_3O_4 NPs produce hydroxyl radical (−OH) from endogenous hydrogen peroxide (H_2O_2). The release of hydroxyl radical (−OH) affects tumor progression and abnormal cell killing. Lastly, studies have shown that superparamagnetic iron oxide nanoparticles (SPION) are applied to deliver biological agents such as proteins, Ab, siRNAs, and nucleic acids as therapeutic and regenerative medicine tools. Therapeutic applications of iron oxide NPs include anemia, cancer, macrophage polarization, and magnetic fluid hyperthermia (Dadfar et al., 2019; Zhao et al., 2020). For regenerative medicine and immunotherapies-based applications, progenitor cells and immune cells such as dendritic or T cells were labeled with SPION to track in vivo delivery and mode of action (Dadfar et al., 2019; Zhao et al., 2020). Microbubbles loaded with iron oxide NPs are famous for crossing BBB and delivering therapeutic agents. The USPIO-loaded microbubbles allowed excellent monitoring and treatment in brain tumors, AD, and PD (Dadfar et al., 2019; Zhao et al., 2020) Noteworthy to mention that iron oxide-based NP formulation Nanotherm^{VR} was approved to treat glioblastoma.

19.3.4.3 Gadolinium

Gadolinium (Gd) NPs belong to metallic nanovectors and are known for their potential to act as CAs for functional imaging since most of the nanoformulations engineered have been used in MRI (T_1 weighted) so far. Hybrid nanovectors made with Gd oxide−Au nanoclusters (Gd_2O_3-AuNCs-ICG), where bovine serum albumin [acted as a stability agent to enhance indo-cyanine green delivery following MRI or computed topography (CT)/X-ray of tumor cells]. Au nanoclusters' presence triggered fluorescence and oxygen radicals under near-infra-red (NIR) irradiation, implying they as suitable diagnostic and therapeutic partners (Han et al., 2016). Furthermore, By tuning morphological and optical properties Au-Gd core-shell NPs, CAs have been developed for their application in MRI-guided diagnosis, targeting, and therapeutics of several diseases. Gd NPs have better permeability and retention capacity, high relaxivity, biological distribution, and passive uptake by most cancer cells. Apart from these imaging applications, recent studies show that Gd nanovectors can also be used as radiosensitizers in tumor ablation. Multifunctional silica GdNPs named AGuIX with 5 nm size exhibited rapid clearance by kidneys which was most useful in enhanced imaging through MRI and image-guided radiotherapy (Mignot et al., 2013). Noteworthy to mention that Gd chelates conjugated with lipid-based or polymer-based materials have proven crucial in the diagnosis and therapy of multiple cancers (Narmani et al., 2018).

19.3.4.4 Silver

The unique features of silver nanovectors have appeared in various industrial and medicinal applications (Adassooriya, Sandaruwan, Kottegoda, Karunaratne, & Park, 2014; Madusanka, Sandaruwan, Kottegoda, & Karunaratne, n.d.; Zhang, Hu, et al., 2016). Silver nanovectors represent biological and physicochemical properties linked to their size and shape. The inherent properties coupled with catalytic, electronic, photonic, and therapeutic partners enable their application in functional imaging, diagnostics, drug delivery, and therapeutics (Austin, MacKey, Dreaden, & El-Sayed, 2014; di Pietro, Strano, Zuccarello, & Satriano, 2016). As with other metallic nanovectors, AgNPs also display surface plasmon resonance, a suitable trait of a theranostic agent. Furthermore, for theranostic applications, Ag nanocubes, nanorods, and nanospheres are extensively used (Austin et al., 2014). Ag nanovectors hold stronger photoacoustic signals, are easily degraded in the body, antimicrobial properties add further value in their application in photoacoustic imaging, and provide a tuning-based change via surface functional modification (Wang, Chuang, & Ho, 2012). AgNP's The ability of AgNP to induce chromosomal aberrations and oxidative DNA damage has given cancer or gene therapy opportunities. Further, the capacity to stimulate reactive oxygen species (ROS) that invokes mitochondrial-dependent apoptosis has attracted its use in multiple therapeutics (Bhushan & Gopinath, 2015). The potential application of Ag vector is in image-guided therapy, where AgNPs provide optical detection range and got full attraction as a theranostic platform in personalized medicine. Notwithstanding the potential of AgNPs in theranostic importance, toxicity at specified quantities produced adverse results on human cells, thus restricting their usage (Bhushan & Gopinath, 2015). Hence, most Ag nanovectors are composed of stabilizing agents to leverage toxicity. Lately, it has been recorded that hybrid formulations having $Ag+$ graphene quantum dots (GQDs) tied with carboxymethyl inulin have alleviated their toxicity and prevented the expansion of pancreatic tumor cells (Nigam Joshi et al., 2017).

19.3.4.5 Liquid metal

Light-induced liquid metal transformation, a smart application toward theranostics, has drawn researchers' attraction, notably to molecular imaging. Liquid metals such as gallium, gallium-indium, and gallium-indium-tin alloys have lower melting points, investigated for functional imaging purposes because of their stability and static nature with water. Recently, liquid metal nanovectors have shown advantages in photoacoustic outcomes. Also, fluid metal vectors capable of inducing ROS and heat following irradiation with NIR light allowed their use for effective tumor ablation. However, its high toxicity is a primary drawback to broad theranostic applications (Chechetka et al., 2017).

19.3.4.6 Silica

The adaptive mesoporous structure of silica (2−50 nm) provides a high surface-to-volume ratio, which enables loading of high drug dosage and uniform distribution

of loaded moiety. Chemical binding, physical adsorption, and drug conjugation methods can be used to embed drugs and confer better biological safety than non-conjugated mesoporous analogs (Tang & Cheng, 2013). Furthermore, silica NPs can be easily excreted through the renal system to orthosilicic acid in the human body (Park et al., 2009; Xu et al., 2012). Silica has superior biocompatibility and safety profile when compared with other inorganic nanoconstituents. Silica is also resistant to external stress and deterioration due to the presence of Si-O-bonds (Tarn et al., 2013). Silica has been a significant game player in targeting, delivery, bioimaging, therapeutics, and bioadhesive applications. Silica NPs have compatibility with several molecular imaging tools to accommodate enzyme, Ab, protein, or short peptides as effective CAs. In therapeutic applications, silica NPs have been used in diabetes, HIV, arthritis, and various other cancer types since they could provide therapeutic benefits via gene or drug delivery. In 2011, ultrasmall nonporous silica NPs got persimmon to use in cancer imaging by FDA. Silica has been used in multiple theranostics modalities like imaging disease conditions including cancers, brain regeneration, cardiac detection, cardiac stem cell therapy, and several infectious illnesses.

19.3.4.7 Quantum dots

In the last decade, scientists have given great attention to multimodal drug-delivery systems, and quantum dots (QDs) are considered one of the most effective tools used in theranostic applications due to their unique physicochemical properties (Ho & Leong, 2010; Tan et al., 2011; Wagner, Knipe, Orive, & Peppas, 2019). QDs range in size from 2−50 nm and consist of electrical or optical properties in their semiconducting material. QDs can be synthesized with a core-shell concept to restrain the core size as per the desired radius (Torchilin, 2011). QDs display strong photoluminescence with a high molar quenching coefficient, making them the best competitors for cell labeling and cancer biomarkers detection. Along with photoluminescence, QDs display a range of emission wavelengths falling within visible and NIR with exceptional light stability (Valizadeh et al., 2012). The earlier progress of QDs was in medicine and biology of developing biosensors to surpass detection limits. The QDs surface-to-volume ratio facilitates the development of "smart" nanoplatforms for extending QDs usage in diagnosis and therapeutic tools (Ho & Leong, 2010; Tan et al., 2011).

In imaging applications, QDs used because of their high sensitivity and resistance to photobleaching. In a few scenarios, they can be used as nanovectors to carry drugs in therapeutic theranostic applications. Size and composition tuneable light emission, high fluorescent quantum yields, photochemically robust, fluorescence, and large stokes shift makes them the most suitable agents in gene delivery and photothermal therapeutics. QDs are biocompatible and excreted by the liver and kidney in the human body. Recently, semiconducting nanocrystals emerging as rapid tools for multiple biomedical applications because of their photophysical characteristics (Ho & Leong, 2010; Tan et al., 2011; Wagner et al., 2019). The biomedical applications of QDs include molecular imaging, drug or gene delivery,

and theranostics of cancer, HIV-associated encephalopathy, and amyloidosis. However, few fears surfaced about QDs toxicity through ROS production by Cd containing QDs (Walling, Novak, & Shepard, 2009). But, strategies to overcome the toxic nature of QDs, and increase biocompatibility, adding proteins, lipids, or polymers have been tested.

19.3.4.8 Carbon

Carbon-based NPs (CBNs) comprises various architectures and compositions, giving rise to individual NPs such as graphene oxide (GO) and its derivative GQDs, carbon nanotubes (CNT), nanodiamonds (NDs), and C_{60} (Patel, Singh, & Kim, 2019). In recent years CBNs have attracted biomedical engineers' attention because of their unique innate properties such as mechanical, thermal, optical, electrical, and structural diversities. Surface modifications with a broad range of functional agents make CBNs a potential nanovector in molecular imaging, fluorescence tracking, biosensing, gene or drug delivery, and photo-triggered thermal therapeutics (Maiti, Tong, Mou, & Yang, 2019). The unique optical properties (flexible emission spectrum, intrinsic fluorescence, superior photostability) of CBN make them useful in imaging and screening tissues or cells. Furthermore, surface area coupled with elector mechanical properties makes them the most desired theranostic agents. CBNs perform well in fluorescence, Raman, phosphorescence, photoacoustic, and two-photon imaging methods (Maiti et al., 2019; Zhang, Wu, Wu, Zhu, & Zhang, 2018). The intrinsic antiinflammatory function of CBNs exploited in the past to treat various inflammatory diseases like rheumatoid arthritis, atherosclerosis, autoimmune encephalomyelitis, and ulcerative colitis. It is encouraged to mention the use of carbon nanotubes (CNT) in drug delivery for cancer treatment, anticancer immune therapy, neurodegenerative, and AD because of their vital antioxidant capacity (Rym, Shi, Alberto, & Cécilia, 2020).

Among the many carbon analogs, CNTs have pulled increasing attention as an extremely qualified vehicle for carrying many drug particles into the living cells; since their general morphology promotes noninvasive diffusion across the cell membranes (Panczyk, Wolski, & Lajtar, 2016). CNT forms strong covalent bonds with loaded drugs, thus stabilizing the loaded drug in intra and extracellular environments. However, this covalent interaction does not sustain to release of the loaded drug, which is a disadvantage in CNT-based drug transport. Though the CNT mediated drug delivery through both covalent and noncovalent interactions shortcoming, using the inner hollow groove of CNT for loading drugs might give ideal conditions and add extra stimuli response elements like temperature, electricity, or pH might provide regulated drug release (Kang, Kim, & Shin, 2017). Fullerenes (Huang et al., 2018), fluorescent nanodiamonds (Su et al., 2017), and GO and GO nanosheets (Goenka, Sant, & Sant, 2014) have been studied as effective theranostic nanovectors recently. CBNs can serve as multifunctional, uniting various imaging tools and curative drugs for theranostic applications. Notwithstanding the inevitable challenges and unsolved barriers, CBNs have possible clinical benefits as imaging and treatment partners.

19.3.4.9 Magnetic

Magnetic NPs belong to a significant class of nanovectors and hold biocompatibility, and are cheap to manufacture (Jinhao, Hongwei, & Bing, 2009; Shabatina, Vernaya, Shabatin, & Melnikov, 2020; Wang et al., 2012). Most importantly, the magnetic behavior under triggered magnetic fields gives a new path for diagnosis and therapeutics using magnetic NPs (Veiseh, Gunn, & Zhang, 2010). Magnetic NPs have their tissue engineering applications, gene targeting, and biosensors in the medical sector (Gao et al., 2007). The magnetic vectors' surface charge is crucial for biological applications since charged vectors can nonspecifically interact with cells. In contrast, neutrally charged vectors have enhanced half-life in circulation (Belyanina et al., 2017), thus majorly neutrally charged vectors are designed using magnetic biomaterial to prevent nonspecific binding. Polymer coating of magnetic vectors to favor their selective interaction and enhance their biosensing properties has been practiced and increasingly utilized (Shubayev, Pisanic, & Jin, 2009). The nanoscale size, higher EPR, and surface volume for attaching molecular therapeutics provide them a huge advantage in several theranostics (Greish, 2007). Magnetic vectors coupled with Gd, iron oxides, nickel, and manganese formulation have gained considerable attention in theranostics applications (Wang et al., 2012; Zhang, Liu, Shen, & Gurunathan, 2016). Surface-modified magnetic vectors are extensively used as CAs in various imaging methods, including MRI and single-photon emission computed tomography (Xie, Liu, Eden, Ai, & Chen, 2011). The magnetoreception-a gene delivery method using magnetic vectors came into light recently and has successfully been used as a combination therapy for effective targeting and drug delivery (Reddy, Arias, Nicolas, & Couvreur, 2012) (Fig. 19.3).

19.3.5 Organic nanovectors

Organic nanovectors (ONV) are crucial in the delivery of genes and therapeutic drugs are becoming a powerful strategy to treat different genetic and nongenetic disorders. The ONVs in combination therapies provide a suitable stage to deliver genes along with conventional therapeutic drugs to monitor disease progression or therapy outcomes. This class of NPs is built from organic compounds such as cationic lipids (liposomes & polymersomes), solid lipids, micelles, dendrimers, and protein conjugates (Fig. 19.3) (Khalid et al., 2020). Some relevant examples are presented in the following section with respect to their role in drug/gene delivery and theranostic significance.

19.3.5.1 Liposomes

This class of ONPs is greatly used as nanovectors to deliver gene/DNA/drugs because; liposomes can hold both water-soluble (Doxil) or insoluble agents Ambison via encapsulation to achieve desired compositions with a particular size, shape, and surface charge, various functional ligands can be added that can target particular

tissues interest, coating with biocompatible and inert polymers (PEG/PLA/PLGA/PCL/PS) to enhance half-life in circulation or ability to cross BBB or increase reticuloendothelial clearance, forms protective sheet covering over the encapsulated drugs and prevent degradation or unwanted reactions with the external environment (Moghimi & Szebeni, 2003; Torchilin, 2005). Important to mention that liposomes can be made by following simple and easy methods keeping the toxicity within acceptable ranges and are commonly used because of their biocompatibility, nonimmunogenicity, and commercial feasibility (Zununi Vahed, Salehi, Davaran, & Sharifi, 2017). The fundamental feature of liposomes is the small size and presence of a hydrophilic inner core surrounded by a lipid bilayer (unilamellar or multilamellar) (Sahoo & Labhasetwar, 2003; Schnyder & Huwyler, 2005).

Liposome-based nanovectors have demonstrated a wide range of compatibility with existing imaging tools such as MRI, fluorescence, US, and nuclear imaging since the engineered liposomes displayed stability, bioavailability, and performance of other current CAs. Not only as effective CAs but also as nanocarriers for the detection of targets like protein, DNA, or small molecules have been developed (Iwai, Maeda, & Konno, 1984; Matsumura & Maeda, 1986). Liposomes are favored transporters to carry mixtures of therapeutics in one platform to enhance treatment performance. Based on the selected molecular pathway, the interaction of the receptor molecule can stimulate rupture of the lipid bilayer and release of the encapsulated signal molecule reporting, hence detection of the target (Davis, 2002). Liposomes and their derivates can deliver siRNAs or pDNA in the forms of lipopolyplexes or polyplexes in gene therapy (Cleaver & Melton, 2003). The tunable encapsulation efficiency of liposomes can be exploited to load and codeliver CAs and drugs allowing imaging and therapy evaluation of cancer, chronic inflammatory lung diseases, diabetes, and cardiovascular disorders (Nisini, Poerio, Mariotti, Santis, & Fraziano, 2018; Varsha & Mehra, 2020).

19.3.5.2 Polymersomes

Polymersomes are another class of liposomes composed of synthetic copolymers that are amphiphilic (Zhang & Zhang, 2017). A covalent bond keeps two or more different block copolymers attached, in which PEG, a hydrophilic polymer linked with PLA/PLGA/PCL biocompatible polymer forms liposome-like structures polymersomes (Rabanel et al., 2015; Rideau, Dimova, Schwille, Wurm, & Landfester, 2018). The new generation of synthetic polymersomes exhibits high mechanical resistance, stability, and low permeability compared to conventional liposomes (Discher et al., 1999; F, O, & S, 2011). With a wide range of combinations of block polymers, it is possible to develop NPs with desired properties that can have specific functions for targeting, drug delivery, and therapeutics (Xiao-ying & Pei-ying, 2017). Surface modulation of polymersomes with target-specific markers has improved cellular uptake of drugs since these nanovectors progressively accumulate in cancer cells (Alibolandi et al., 2019; Banerjee et al., 2016). Once injected, polymersomes can passively concentrate at the target site due to the EPR effect and deliver a high drug load (Alibolandi et al., 2019). The addition of stimuli-

responsive triggers can also regulate drug discharge from polymersomes, making them suitable agents in diagnostics (Alibolandi et al., 2019), gene delivery (Ge et al., 2014), and drug delivery (Qin, Jiang, Zhang, Deng, & Zhong, 2019) The current application of polymersomes is limited to cancer theranostics only and shortly one can expect wide applications of polymersomes based theranostics in noncancerous diseases at large (Alibolandi et al., 2019).

19.3.5.3 Micelles

Micelles are another class of lipid-based spherical-shaped amphiphilic macromolecules where hydrophobic ends are enclosed in the core and hydrophilic heads are directed towards the solvent. Micelles can hold water-insoluble drugs more efficiently in their hydrophobic core than liposomes, however, the water-soluble drugs can be chemically added or adsorbed to the outer shell, commonly derived from PEG or PVA. Micelles display increased solubility and stability of NPs in hydrodynamic environments (Adams, Lavasanifar, & Kwon, 2003; Stefano & Paolo, 2013). With the thermodynamic or kinetic stability and high drug load, micelles attracted more usage for disease treatments (Sonali et al., 2018). Polymeric micelles trapped with SPIONPs and DOX within have been used as multifunctional vectors compatible for MRI and delivery of therapeutics (Guthi et al., 2010).

19.3.5.4 Solid lipid nanovectors

Solid lipid NPs (SLNs) are droplets of lipids composed of oil, and solids at body temperature, stabilized by surfactants and can protect loaded agents from degradation via encapsulating and providing controlled release of the drug. The physicochemical properties of the NPs depend on the nature of the lipid and the type of surfactants chosen for the formulation (Mishra et al., 2018). The crystalline structure of SLNs ensures improved stability of drugs and extended-release of the drug compared to conventional liposomes since, during fabrication of SLNs, PEGylated lipids are added to pass on steric stability and functionalization. Both water-soluble and insoluble drugs can be loaded using simple and quick preparation methods (Liu, Xiao, & Allen, 2004). SLNs fabrication does not require any organic solvents hence organic solvent-associated toxicity can be avoided, thus making them less toxic. SLNs provide better stability, drug load capacity, inclusion complexes formation, and surface modification making them best fitted for designing nanovectors. Coming to drug delivery properties, SLNs exhibits improved bioavailability, drug efficacy, no off-target effects, and less immunogenic and pharmacokinetics. SLNs can be used in theranostics platform for noninvasive imaging or drug delivery. SLNs can form lipid nanosize vesicles to carry CAs, chemotherapeutic drugs, genes, or nucleic acids in cancer chemotherapy, brain targeting, pulmonary disorders, and skin diseases (Ghasemiyeh & Mohammadi-Samani, 2018).

19.3.5.5 Dendrimers

Dendrimers belong to the polymeric NPs class with a peculiar structure with a central core with one or a group and multiple branches that end with individual

terminal functional groups (E & J, 2005; Kesharwani, Jain, & Jain, 2014). The arms extend symmetrically and radially from the center to form an overall spherical shape. Dendrimers' advantage is that controlled architecture can be achieved with high precision, providing well-defined, and uniform target distribution. Moreover, their preparation is extremely exceptional since both natural and synthetic polymers can be used as fabrication materials. Hydrophilic or hydrophobic drugs can be combined with dendrimers' core, depending on the monomers' nature composing the macromolecule. Dendrimers have dragged attention to drug and gene delivery because they own unique and appealing qualities such as enclosed structures, inner hollows able to envelop drugs, and controllable multivalent functionalities in their internal and external parts (Nanjwade, Bechra, Derkar, Manvi, & Nanjwade, 2009). These attributes make dendrimers an indispensable option for the construction of nanovectors for nucleic acid delivery. Dendrimers can interact with DNA, RNA, and oligonucleotides through electrostatic interactions to make complexes, protecting the nucleic acids from degradation. The properties of dendrimers depend on various factors, such as stoichiometry, amines, nucleic acid phosphates concentration, and, solvent properties like pH and concentration of salt (Dufès, Uchegbu, & Schätzlein, 2005). However, the inherent struggle of manufacturing unique dendrimers that are suitable vectors for drug delivery has led researchers to focus primarily on modifying existing dendrimers instead of developing novel dendrimers for gene delivery systems. Poly(amidoamine) (PAMAM) dendrimers have been examined as gene carriers where modification with PEG, ligands, or amino acids enhances their gene delivery capacity. Many reports have been published detailing the use of amino-terminated PAMAM or poly-propylene imine dendrimers as gene transfer tools, enhancing DNA transfection into the cell nucleus by endocytosis (Svenson & Tomalia, 2012). Dendrimers hold a promising role for various biomedical applications for the years to come, as they possess unique features, such as a high level of branching and multivalency.

19.3.5.6 Protein-based nanovectors

The primary naturally available molecules used for the development of NPs included two proteins, albumin, and gelatin. Protein-based colloidal vectors are up-and-coming as they are safe, biodegradable, and less immunogenic. They hold excellent stability in vivo, and storage potency, it is easy to synthesize and monitor particle distribution (Elzoghby, Samy, & Elgindy, 2012). Also, because of the predetermined primary folding of proteins, protein-based NPs allow multiple surface modification opportunities and covalent attachment of the drug. For all the above reasons, several proteins have been used to generate protein-based NPs for delivering target drugs, and these include collagen, albumin, gelatin, keratin, fibroin, and sericin. Many researchers have used gelatin NPs as a gene delivery vehicle. Using solvent displacement type B gelatin can be derived, which can enclose nucleic acids at physiological pH. Furthermore, the physical encapsulation in gelatin NPs shields the supercoiled structure of plasmid DNA and

increases the transfection efficiency upon intracellular delivery (Zhang, Chua, & Lynn, 2004). An approach that has recently been used for gene delivery combines lipids and peptides to obtain high transfection efficiency with minimal toxicity, even in the presence of serum. In this procedure, NPs developed using oligoarginine−lipid complexes; the lipidic moiety was 3,5-bis-(dodecyloxy) benzamide (BDB), and the peptide was oligo-Arg spaced with a PEG between the amide group of BDB and the C-terminal of the peptide.

NPs derived from carbohydrates can also improve biocompatibility using novel immobilization procedures to engineer a new class of bio nanoparticle-derived formulations. NPs from naturally occurring polysaccharides developed for the delivery of peptides, proteins, and nucleic acids. The best example of this strategy is the use of chitosan, a natural cationic polysaccharide that is biodegradable, derived from chitin made of repeating units of D-glucosamine and N-acetyl-D-glucosamine. Chitosan, a linear polymer is biocompatible and nonimmunogenic and owns mucoadhesive properties, making it an excellent biopolymer for the preparation of NPs as vectors for DNA delivery (Nagpal, Singh, & Mishra, 2010). Chitosan can automatically bind to DNA through ionic interplay to form NPs. The biochemical properties of chitosan make it a suitable vector for gene delivery. The amino groups of chitosan confer to the molecule a high charge density and are readily available for chemical alteration and salt formation with acids. In vivo, lysozymes will degrade chitosan, and it was demonstrated to somewhat protect DNA from nuclease degradation (Mao et al., 2001). Cellular transfection with chitosan−DNA complexes has demonstrated that complexes of 100−250 nm can accumulate in the nucleus once inside the cell. Finally, chitosan−DNA nanospheres have been nontoxic in experimental animals and humans.

19.3.6 Biomimetics nanovectors

The main intention of nanovector-mediated drug delivery is to accumulate therapeutic agents at the given disease site while preventing systemic off-target effects, which demand either organic or inorganic material to carry biologics or drugs to the target location (Zhou, Kroll, Holay, Fang, & Zhang, 2020). Developing nanovectors with the above-stated biomaterials has been explored at large in the past. Given their extensive use in several disease conditions, it raises concern over the nature of biomaterial and design principles when choosing the ideal material (Yu et al., 2016). The advantages and disadvantages of tested materials have exposed three fundamental duties that nanovectors must fulfill to obtain their drug delivery goal:

1. Suitable circulation time permits them to move to the target site (Yoo, Chambers, & Mitragotri, 2010).
2. Capacity to act upon the only disease or damaged tissue (Friedman, Claypool, & Liu, 2013).
3. Must be comprised of a biodegradable material that is easy to clean from the body (Naahidi et al., 2013).

Drug delivery vectors having properties such as biological compatibility, degradability in biological environments, and prolonged circulation have significant advances in developing human-friendly theranostic platforms to treat various diseases (Fig. 19.4) (Howard et al., 2014). Recently, nanovectors, popularly known as biomimetics, gained recognition from worldwide research since these vectors can combine both natural and synthetic materials for imaging, delivery cargo and most importantly excel in their performance even in complex biological locations (Fang, Jiang, Fang, & Zhang, 2017; Kroll, Fang, & Zhang, 2017). Nanovector covered in cell membranes represents new biomimetics that can imitate their source cells' membrane activities in biological systems (Fig. 19.4). Cell membranes from red blood cells (RBC), cancer cells, immune cells, and macrophages have been used extensively in the past. RBC membrane-shielded poly(lactic-co-glycolic acid) NPs are the first studied biomimetic NPs. The RBC membrane acted as a nanoscale sponge for toxins and gave more extended circulation pharmacokinetics than uncoated NPs (Hu et al., 2011). Inspired by this study, several researchers showed interest in applying cell membrane camouflaged nanovector development where membranes from neutrophils (Kang, Zhu, et al., 2017), macrophages (Xuan, Shao, Dai, He, & Li, 2015), platelets (Diana et al., 2017), cancer cells (Chen, Chen, & He, 2019), beta cells (Chen et al., 2016), CART cells (Ma et al., 2020) tested (Fig. 19.4). The general architecture

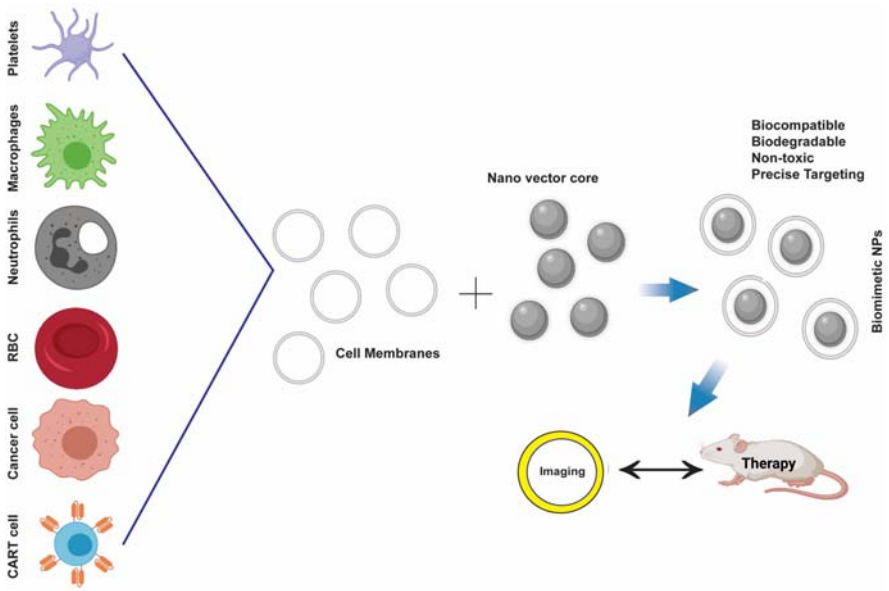

FIGURE 19.4

Illustration of biomimetic nanovector architecture and applications biomimetic membranes can be isolated from the above-depicted cells for efficient and safe delivery of drugs.

of biomimetic NPs consists of a core (imaging or therapy agents) camouflaged with a plasma membrane (Fig. 19.4). Since then, many novel biomimetic strategies have been engineered using cell ghosts (Toledano Furman et al., 2013), whole cells (Evangelopoulos et al., 2020), and the merging of cell-derived membrane proteins to copy the physiological properties and functions of source cells (Fig. 19.4). Biomimetic nanovectors exploit the natural cooperation among NPs and the biological elements of body while imitating source cells' traits and duties (Parodi et al., 2017). The most important feature of using the biomimetic nanovector is to escape immune attacks, clearance, and increased therapeutic efficacy (Perera & Coppens, 2019; Yao, Jingshan, Xiaojia, Wei, & Tongkai, 2019) (Fig. 19.4).

Triggers of Theranostic NanoVectors A variety of NPs suitable for drug delivery systems will have a stimuli-responsive property for regulated drug release and preventing premature drug delivery. The stimulus for nanotheranostics may be external or internal (Raza et al., 2019). The engineering of stimuli-responsive drug-delivery nanovectors enhances drug accumulation at target sites, responsive to external or internal stimuli, and higher penetration capacity in the pathological area. The endogenous stimuli such as pH, redox state, hypoxia, enzyme, miRNA, small biomolecules, and nucleic acid-based vectors have been introduced to develop theranostic systems for treating cancer and infectious diseases via enhanced drug release (Kim et al., 2017; Taghizadeh et al., 2015). Care must be taken while choosing the material for designing vectors responsive to endogenous stimuli and delivering encapsulated drugs. On the other hand, exogenous or physical stimuli such as photo/light, electric, magnetic, temperature, and ultrasound radiations are employed to trigger the drug's delivery in the active site (Said, Campbell, & Hoare, 2019). Hence, the current research has shifted towards developing stimuli-responsive nanocarriers using one or more vectors that are stimuli-responsive and increase targeting percentage (Said et al., 2019; Seo et al., 2016; Shin et al., 2017). However, definite limitations by exogenous stimuli-responsive materials exist. It is daunting to find exogenous stimuli that can infiltrate and precisely reach the target site. Moreover, endogenous stimuli that will regulate the drug release exactly at the target site at the relevant time and dosage are complicated to discover. In the last decade, the synthesis of combined triggers that use more than one stimuli-responsive vector has been brought to light. A combination of vectors that responds to pH-magnetic fields or redox potential-temperature, pH-temperature was considered for smart-responsive drug-delivery systems to regulate drug release precisely at target locations (Chen et al., 2013; Li et al., 2020; Wen et al., 2018). Moreover, vectors sensitive to more than two stimuli are growing increasingly; particles with more than two sensitivities combined in one single matrix are called multiresponsive systems (Chen et al., 2017; Hegazy et al., 2017; Lei et al., 2020). The multiresponsive systems act as smart vectors that discharge their cargoes on demand. These multiresponsive nanovector systems own all the benefits of the single stimuli-based methods in addition to precise control

over delivering the drug to pathological sites. Multiresponsive vectors not only react to a single motive for drug targeting, but they also exhibit increased release when simultaneously triggered by multiple stimuli.

Advanced NanoVectors For Theranostic Applications The evolution of nanotheranostics, an upcoming nanotechnology field, brought dramatic changes in the healthcare system to deliver drugs, imaging tissues or organs, and diagnosis. This new nanomedicine branch strives to combine diagnostics and therapeutics into a single platform to get more productive outcomes in curing aggressive diseases (Raza et al., 2019; Sharma et al., 2019). Nanotheranostics contains all those benefits of nanomedicine, such as drug protection, biological availability, regulated natural distribution, synchronous detection, and disease treatment through enhanced penetration of drugs in the physiological systems with a better safety profile than conventional therapies (Murthy, 2007). Nanotheranostics are mostly guided by the molecular profile of affected individuals or disease-associated biomarker, thus acting as personalized medicine where the drug release will be on-demand (Silva et al., 2019). Nanotheranostics provides an unparalleled possibility to combine various segments and customized therapeutic tools, controlled-release methods, targeting approaches, and describing functionality for treatment detection within a nanoscaled construction (Sonali et al., 2018).

A nanotheranostics vector mainly comprises three components, viz, an imaging agent, a therapeutic moiety, and a carrier system for site-specific delivery of both therapeutic and imaging agents. Though the concept of theranostics was initially focused on cancer therapy, it was later extended to various biomedical applications such as antimicrobial therapy, cardiovascular diseases, neurological disorder, inflammatory diseases, etc. Multifunctional theranostic materials are developed to aid in simultaneous diagnosis and therapy with real-time monitoring of therapeutic efficacy using multiple techniques such fluorescence aided molecular imaging, magnetic resonance imaging using nano-CAs, positron emission tomography, a CT, etc. These nanomaterials can also be surface engineered and made stimuli-responsive to factors such as pH or temperature or the presence of any targeting receptor/moiety for controlled and regulated drug release. Key requirements in developing a nanotheranostics system include prolonged blood circulation time, resistance to opsonization, reduced systemic toxicity, and enhanced targeted accumulation in the required site. The therapeutic efficacy of any nanosystem can be significantly enhanced via targeted delivery using functional active molecules such as antibodies, peptides, ligands, aptamers, etc. With the development of nanotechnology, several multifunctional nanovectors have been developed for combinatorial therapy wherein multiple therapeutic moieties (such as chemotherapeutic drug, photothermal agents, immune checkpoint inhibitors, targeting ligands, etc.) can be integrated into a single nanoplatform for synergistic therapeutic efficacy. Some of the recent studies using various types of nanovectors and their significance in nanotheranostic applications are summarized in Table 19.1.

Table 19.1 Summary of recent studies using various types of nanovectors.

Type of NV	NV conjugates	Significance
Gold	Paclitaxel and docetaxel in PEG	Solubility and high drug loading
		pH-responsive drug release
		Active targeting with anti-EGFR antibody
	Aminopterin and methotrexate	Selective targeting with foliate receptors
		Strong SERS
		Diagnosis and quantification
Iron oxide	Crystal violet and D2B antibody	94% accurate targeting
		95% toxicity upon photothermal therapy
		SERS-based detection and analysis
	PEG-PEI copolymer/siRNA/anti-GPC3 antibody	Specific binding to tumor
		Sequence-specific gene silencing
Silica	Chlorin e6 (Ce6) conjugated	Retained loading capacity for drugs
		Precise cell death up to 60% upon PDT
		Significant uptake by target cells
	Doxorubicin/capped with agarose	Drug loading
		Synergistic chemo/photothermal ablation
		Ideal SERS
Polymers	Methotrexate loaded/PAMAM-based	Minimal toxicity
		Enhanced drug loading
		Efficient uptake
		Target specific cytotoxic
	PEG/PTMC copolymer/parthenolide	Minimal toxicity
		Enhanced therapeutic efficacy
Lipids	Doxorubicin	Enhanced cellular uptake
		Minimal toxicity
		Excellent EPR effect and biodistribution
		Enhanced tumor inhibition
	Doxorubicin and GQDs	Exhibited imaging bimodality
		NIR light-mediated tumor reduction
		ROS scavenging ability
Proteins	Gemcitabine and IR780	Enhanced accumulation and retention
		Antitumor activity with minimal toxicity
	Artesunate, folic acid, and indocyanine green loaded	NIR triggered artesunate release
		Fluorescence aided bioimaging
		Efficient tumor inhibition

19.3.6.1 Antimicrobial therapy

The primary goals of antimicrobial nanotheranostics are to detect the presence of a pathogen, detect the infection site, deliver antimicrobial drugs, offer therapy of infection at the active site, and restrain transmission of disease from one place to another (Gao et al., 2018; Larrañeta, McCrudden, Courtenay, & Donnelly, 2016). Nanotheranostics can be used to treat drug resistance microbes causing cold, pneumonia, malaria, HIV, and tuberculosis that are difficult to kill with preexposed medications (Inoue & Minghui, 2017). Nanotheranostics play a crucial role in identifying drug resistance, determining susceptibility, delivering new drugs, monitoring treatment, and killing the pathogen (Burnham, Leeds, Nordmann, O'Grady, & Patel, 2017; Vangara et al., 2018; Xie et al., 2017). Nanovectors incorporated in medical devices mostly help in early detection and treatment. The likelihood of being covered with antimicrobial particles is an excellent way to avoid and control nosocomial infections (Li et al., 2017). Screening of pathology of infection site through preventing biofilms to understand pathogenic stages and study antimicrobial resistance development can be identified and reported via nanotheranostics (Gomes et al., 2018; Magana et al., 2018; Ribeiro et al., 2016). Antimicrobial susceptibility testing is a diagnostic method to discover the resistance profiles of the infecting pathogens and guide the antibiotic treatment and prognosticate the clinical behavior to the antimicrobial therapy (Syal et al., 2017). Furthermore, the implementation of innovative therapeutic alternatives such as light-triggered (photothermal or photodynamic) antimicrobial therapy to ensure sufficient treatment without unfavorable impacts has been evaluated (Wainwright et al., 2017). The photodynamic inactivation of pathogens has recently gained popularity in preventing foodborne infectious diseases (Silva, Borges, Giaouris, Graton Mikcha, & Simões, 2018). Furthermore, nanotheranostics have been employed successfully in antiinfectious therapy, understanding pharmacogenomics, antimicrobial drug delivery, and screening of multidrug-resistant microbes. Noteworthy to mention that, research is ongoing to improve the immunogenicity of existing vaccines via nanotheranostics vectors as antigen delivery tools and immunopotentiators (Pati, Shevtsov, & Sonawane, 2018; Yan et al., 2020).

19.3.6.2 Cardiovascular disease therapy

Nanotheranostics for cardiovascular diseases CVD have been engineered, and considerable successes were achieved at preclinical stages and might need further investigation in future clinical translation programs. The first nanotheranostics employed was a noninvasive screening method to validate drug therapy in atherosclerosis patients (M.E et al., 2003; Tang et al., 2009). Molecular vascular disease mechanisms were primarily investigated and presented ways to compose specific nanovectors targeted precisely to diseased tissues or organs. PEGylated, Gd-linked AuNPs, and chitosan-DNA NPs have been developed and used in the detection and therapy of atherosclerosis. The endothelial cellular biomarker or platelet-associated markers, or activated macrophages and smooth muscle

cells with a pathological, synthetic phenotype were used as targets for distinguishing CVD at early stages. Also, noncellular biomarkers such as tissue factor, fibrin, lipoproteins, annexins, ECM, and MMPs are utilized to diagnose CVDs and associated pathologies (Gupta et al., 2017). Nanovectors built with these above-stated biomarkers allow targeting specific vascular regions affected by oxidative stress, inflammation, thrombosis, atherosclerosis stenosis, peripheral artery disease, restenosis, and aortic aneurysms (Gupta et al., 2017). Treating CVD such as coronary cardiac disease, heart failure, inflammatory heart disease, myocardial infarction, hypertension, and dyslipidemia, the most prevalent difficulty for prescription guided in these diseases is their low lipid solubility and their biological availability (Okur, Karantas, Okur, & Siafaka, 2017). Nanovector-based methods are among the best help for CVD since nanovectors present regulated drug delivery for lipid-soluble molecules and enhance their bioavailability to treat most CVDs. Furthermore, several nanovectors compatible with imaging tools were developed to diagnose the macrophages linked with atherosclerotic lesions and these nanotools were helpful in the early diagnosis of atherosclerosis. However, no clinically approved procedure is available to estimate nanovectors' potency in general and or their usage for CVDs (Fitzgerald et al., 2011).

19.3.6.3 Central nervous system disease therapy

Neurodegenerative diseases like AD, Huntington's diseases, and PD affect millions of lives globally. Curing neurodegenerative disorders has not been successful because of a lack of effective active agents, limited therapeutic efficiency, reduced penetration at the target site, failure to cross BBB, and most importantly, the pathology of many neurodegenerative disorders poorly studied (Cong, Bai, Li, Wang, & Chen, 2019; Filippousi et al., 2015). Hence, significant research is ongoing to design useful delivery vectors to cross BBB and release drugs to the brain. Nanovectors can be modified with various bioagents to couple their interaction with brain-specific receptors to improve penetration efficiency. Theranostic formulation with ceria, iron oxide nanocomposites coated onto MSNs binds with tau proteins to protect neuronal cells from apoptotic cell death and enhances AD rats' cognitive capacity (Chen et al., 2018). Theranostic vectors such as manganese oxide NPs carrying l-3,4-dihydroxyphenylalanine (L-DOPA) have been applied in imaging (MRI), diagnosis, and PD therapy (McDonagh et al., 2016). The full knowledge of the pathways and pathogenesis of neurological disorders and the construction of synergies to recognize defects at early stages to prevent the emergence of adverse symptoms is a prerequisite and nanotheranostics can help find answers to such clinical problems.

19.3.7 Clinical translation of nanovectors

Two potential nanomedicine have been FDA-approved and commercialized for the treatment of cancer, namely, Doxil (Liposomal formulation of DOX) and

Abraxane (Albumin conjugated paclitaxel) for treating ovarian cancer and breast cancer respectively. Some of the nanomedicines that are either clinically approved or under preclinical or clinical trials have been listed in Table 19.2. Despite such clinical success, nanomedicines still suffer from critical challenges such as

Table 19.2 Nanomedicines under clinical/ preclinical trials.

Trade name	Nanovector	Application	Clinical trial ID
Abraxane	Albumin bound paclitaxel	Breast cancer	NCT02555696
		Lung cancer	NCT02027428
		Pancreatic cancer	NCT02555813
Doxil	Liposomal doxorubicin	Ovarian cancer	NCT03818282
Nanoplatin	Polymeric (PEG poly aspartate) + Cisplatin	Pancreatic cancer	NCT00910741
AmbiSome	Liposomal amphotericin	Antifungal	NCT00697944
Onivyde	Liposomal irinotecan	Pancreatic adenocarcinoma	NCT03207724
		Small cell lung cancer	NCT03088813
DEP docetaxel	PLL dendrimer bound docetaxel	Antiangiogenesis therapy	Starpharma.com
Patisiran/ ONPATTRO	Lipid nanoparticle encapsulating RNAi	Transthyretin (TTR)-mediated amyloidosis	NCT01960348
NBTXR3/ Hensify	Hafnium oxide nanoparticle	Radio enhancing	NCT01946867
AuroLase	Gold coated silica nanoshells	Head and Neck cancer	NCT00848042
		Lung cancer	NCT01679470
VYXEOS	Liposomal formulation of cytarabine: daunorubicin	Acute myeloid leukemia	NCT03575325
Feridex	Dextran coated SPIONs	Imaging agent	FDA approved
Pulmaquin	Inhaled ciprofloxacin loaded-liposome	Bronchiectasis and Chronic *P. Aeruginosa* Infection	NCT02104245
Silvasorb	Silver-based NPs	Antibacterial (gram-negative bacteria)	NCT00659204
Arikace	Liposomal Amikacin	Antibacterial (gram-negative bacteria)	NCT01315691
miRNA-1273 vaccine	Lipid nanoparticle (LNP) loaded with mRNA	SARS COV-2	NCT04283461
Liprostin	Prostaglandin E-1 (PGE-1) loaded liposomes	Peripheral vascular artery disease	NCT00053716
Ferumoxytol	Carbohydrate coated SPIONs	MRI of Myocardial infarction	NCT01323296
Ferumoxytol	Carbohydrate coated SPIONs	MRI of CNS disease	NCT03270059

systemic toxicity, stability, and immunological responses from the host. Clinical translation of nanovectors is majorly limited due to their scaleup production. The crucial challenge faced during large-scale production is the variation in characteristics of the developed nanosystems like size, drug loading efficiency, stability, etc., when compared with that prepared on a laboratory scale. Hence, it seeks much attention from the science workers in this aspect. Upon successful scaleup synthesis of these nanosystems being achieved, the nanoformulations would serve as a better alternative in various treatment modalities for a myriad of diseases compared to the conventional ones.

19.4 Conclusion

Nanotheranostics has been a successful strategy to overcome limitations associated with conventional therapies. Intrinsic attributes of nanovectors allow them to alleviate diagnostic and therapeutic tools into a single platform, where precise drug delivery and improved pharmacokinetic efficacy are achieved. Theranostics vectors can achieve real-time visualization of disease progression and therapy response, making it a suitable tool for diagnosing and treating several life-threatening diseases such as cancer, infectious diseases, cardiovascular diseases, and neurological disorders. Though theranostic vectors talked loud for their value and effectiveness in many applications, toxicological effects have a more significant concern over their applications. The vector size, shape, stability, and surface functionality might produce toxic results on biological systems that concurrently depend upon external factors (age, target site nature, extracellular environment, and pH) that nanovectors come into contact with. Hence, a strategic formulation and tuning of nanovectors can advance nanotheranostics towards next-generation nanomedicine. The toxicity profile of metallic nanovectors and carbon nanotubes has concerns. These particles display slow degradation and in vivo clearance—biopolymers coating to subdue toxic face and enhance their suitability for upcoming clinical applications. Furthermore, the use of biodegradable or nontoxic clinically approved materials might increase the chance of nanotheranostics and their clinical use. Though enormous discoveries made their point that nanotheranostics effectively prevent a broad range of diseases in preclinical studies, however, it has not still met the clinical standards. Apart from toxicity, complex nature and synergistic pathways raise a question in nanotheranostics application for clinical purposes. For example, systems with superior diagnostic capability fail to compensate for therapeutic ability and, in some instances, higher therapeutic efficiency coupled with low contrast imaging ability. However, biomedical engineers strive to make efforts in making nanomedicine a reality soon. To end, though an extraordinary effort is still required, the fate of nanotheranostics efficiency in clinical habit is imminent.

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CHAPTER

Nanoprobes for advanced nanotheranostic applications

20

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20.1 Introduction

The concept "theranostics" refers to a treatment strategy that integrates therapeutics with diagnostics (Sumer & Gao, 2008). Nanotheranostic is a novel and unique nanomedicine approaches for highly developed theranostics, for instance, to develop many nanocarriers such as inorganic and metal nanoparticles, polymer conjugations, micelles, carbon nanotubes, dendrimers, and liposomes for controlled, sustained, and target specific codelivery of therapeutic and diagnostic agents to improving the theranostic effects. The main purpose is to provide a bright prognosis for personalized therapy that renders the serious diseases treatable or diagnosed the illnesses at their earlier stage. Nanotheranostics may be effective for various deadly diseases, including cancer, AIDS, and cardiovascular disorders that provide the potential to make therapy much less complicated and prognosis bright, resulting in improving the patients' quality of life. However, nanomedicine is described as the use of nanotechnology for the prevention, treatment, and diagnosis of illnesses at the molecular and cellular level that may be useful in developing a theranostic agent to simultaneously combine therapeutics with diagnostics (Deveza, Choi, & Yang, 2012; Janib, Moses, & MacKay, 2010; Sumer & Gao, 2008). Emerging nanotheranostics is usually multifunctional and able to diagnose and deliver the therapeutics to the targeted sites by using targeting biomarkers and ligands (Janib et al., 2010; Xie, Lee, & Chen, 2010; Ye & Chen, 2011; Yu, Park, & Jon, 2012). Moreover, theranostic nanomedicine contains polymers or macromolecules by which the therapeutic and diagnostic molecules are conjugated, adsorbed, and entrapped for simultaneously therapy and diagnosis at the molecular and cellular levels (Feng, 2006; Muthu & Feng, 2013; Muthu & Singh, 2009). The main advantage of using theranostic nanomedicine over other theranostics is that they have highly developed capabilities in a single all-in-one network involving targeted delivery, controlled or sustained release, high efficiency of transport via endocytosis (Muthu & Singh, 2009), synergetic

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performance (such as siRNA codelivery) (Zhao, Mi, & Feng, 2013a), stimuli-responsive release (such as smart delivery) (Muthu & Singh, 2009; Muthu, Rajesh, & Mishra, 2009), and multimodality therapies and diagnosis (i.e., autophagy inhibition, and oral deliver) (Caldorera-Moore, Liechty, & Peppas, 2011; Lammers, Aime, & Hennink, 2011; Ma, Zhao, & Liang, 2011; Mei, Zhang, & Zhao, 2013; Smith & Smith, 2012; Xu & Zhao, 2013). In nanomedicine, encapsulation of a single therapeutic and diagnostic agent cannot contain high sensitivity/specificity/efficiency for various biomedical applications.

In addition, multimodality nanotheranostics may be built to take advantage of the benefits that can be obtained by coencapsulating various therapeutic and diagnostic modes in nanomedicine platforms targeting (Tan, Chandrasekharan, & Maity, 2011). Notably, siRNA may be involved in the nanotheranostics as a theranostic resistance inhibitor. It has been observed that siRNA-based theranostic nanomedicine enhanced the therapy and diagnosis as a multimodality remedy (Muthu et al., 2009). Besides, nanomedicine has also been used as a new approach for oral chemotherapy. Previous studies reported D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) based nanomedicine for oral transportation of therapeutic drugs, such as paclitaxel and docetaxel with increased oral bioavailability (Mei et al., 2013; Win & Feng, 2006; Zhao & Feng, 2010). It is proposed that researchers pay more attention to the oral delivery of theranostic nanomedicine for future research to promote the cancer theranostics practice, which will improve the patients' quality of life (Mei et al., 2013). Moreover, autophagy is a catabolic approach in which intracellular damage occurs in the lysosomes of dysfunctional cellular components. Besides, autophagy or autophagocytosis affects the nanomedicine during endocytosis and its therapeutic effects by altering the nanomedicine pharmacokinetics, such as nanomedicine metabolism, delivery, absorption, and excretion. However, the theranostic nanomedicine loaded with autophagy inhibitors may increase the delivery of therapeutic agents (Smith & Smith, 2012). Therefore, highly developed nanotheranostics has been under development for simultaneous therapy and diagnosis of illness at the molecular and cellular level (Ma et al., 2011; Xu & Zhao, 2013).

Moreover, the therapeutic agents also involve peptides, hydrophobic organic drugs, genetic materials, and proteins. Apart from therapeutic agents, diagnostic agents applied in nanomedicine include those for magnetic resonance imaging (MRI) (using superparamagnetic metals such as iron oxides), computed tomography (using heavy elements such as iodine), optical imaging (using fluorescent dyes), and nuclear imaging (using radionuclides) (Janib et al., 2010; Xie et al., 2010; Ye & Chen, 2011). In general, any disease can be treatable or curable if it is diagnosed at its early stage (Muthu & Feng, 2013). Thus, highly developed theranostic networks were used for accurate targeting, sensitive diagnosis, and controlled/sustained delivery of materials (McCarthy, Jaffer, & Weissleder, 2006; Xu & Zhao, 2013). Nowadays, nanoparticles have gained increasing attention as contrasting agents for cancer diagnosis in the latest imaging systems such as molecular imaging (MI). By using various nanoparticles, it is feasible to (1) deliver significant quantities of payload in a single dose, (2) concurrently deliver

both therapeutics and imaging agents, and (3) obtain a significant specificity towards a target. In this chapter, we have summarized the latest works on highly developed theranostic nanomedicine networks such as polymers/magnetic nanoparticles, dendrimers, gold nanoparticles, liposomes, solid lipid nanoparticles, carbon nanomaterials, and drug-polymer conjugates. The benefits of using nanotheranostics for foreseeable future research involve, (1) advanced therapy, (2) in vivo biodistribution and intracellular diagnosis (3) and therapeutic monitoring of disease (Ding & Wu, 2012; Velikyan, 2012).

20.2 Theranostic nanomedicine

Theranostic nanomedicine can be described as nanomedicine that integrates therapeutics with diagnostics (Janib et al., 2010). Table 20.1 summarizes some advanced nano theranostics platforms for concurrent integration of therapy and diagnosis. Notably, some nanomedicine such as carbon nanotubes, gold nanoparticles, and magnetic nanomaterials have unique intrinsic therapeutic/diagnostic properties. Generally, they serve as self-theranostic-based nanomedicines. Highly developed theranostic nanomedicines or platforms combined with biological moiety may identify specific target binding and possibly be internalized by endocytosis (Choi, Liu, & Chen, 2012). After intravenous administration, nanomedicine with an appropriate surface and aspect ratio may reside in blood circulation for a longer duration (Win & Feng, 2005). It was found that nanomedicine has a size range from 100 to 200 nm with hydrophilic surface functionalization using vitamin E and polyethylene glycol (PEG), which is generally applied with better efficiency for theranostic applications (Decuzzi & Ferrari, 2007; Tan, Feng, & Leong, 2014; Win & Feng, 2005; Zhang, Li, & Lykotrafitis, 2009; Zhao & Feng, 2010). Gan and colleagues reported nanomaterials to comprise poly-lactic acid-D-α-TPGS (PLA-TPGS) loaded with docetaxel containing hydrophilic surface functionalization that may obtain effective chemotherapy for 360 h as compared to Taxotere for 22 h after intravenous administration (Gan, Chien, & Feng, 2010).

Moreover, Zhao and colleagues observed the effects of PEG tethering chain length of vitamin E TPGS on cellular uptake proficiency (Zhao & Feng, 2014). They investigated that the short length of the PEG chain could have the highest cellular uptake capacity of nanomaterials because of their high surface energy to mitigate bending energy required for the detachment of cellular membrane bilayer via endocytosis mechanisms (Zhao & Feng, 2014).

20.3 Gold nanoparticles

Gold nanoparticles are used as a versatile platform that gives highly favorable properties for various theranostic systems (Chen, Zhang, & Dai, 2013b; Daniel &

Table 20.1 Some highly developed nanotheranostics platforms under development for simultaneously combining therapeutics with diagnostics.

Theranostic nanomedicine	Materials	Diagnostic agent	Therapeutic agent	Targeting agent	Advancement	Reference
Gold nanoparticles	Gold nanoparticles	Gold nanoparticles	DOX	CPLGLAGG peptide	Stimulus-responsive release of drug	Chen, Xu, and Jia (2013a)
Conjugates of drug-polymer	HPMA	64Cu	64Cu	RGD	Radiochemo-therapy and cancer imaging	Yuan, Zhang, and Kaur (2013)
Carbon nanomaterials	SWCNTs	Intrinsic property	Intrinsic property	Passive	Photothermal and self-photoluminescent activity	Robinson, Welsher, and Tabakman (2010)
Polymeric nanoparticles	PLA-TPGS	Quantum dots (QDs)	Docetaxel	Folic acid	Codelivery of nanoparticles QDs and docetaxel	Pan, Liu, and Feng (2010)
Dendrimers	Polypropylene-mine	Phthalocyanines	Phthalocyanines	LHRH	Single theranostic agent delivery	Taratula, Schumann, and Naleway (2013)
Micelles	TPGS	Iron oxide nanoparticles	Iron oxide nanoparticles	Passive	Single theranostic agent delivery	Chandrasekharan, Maity, and Yong (2011)
Liposomes	Cholesterol, TPGS, phospholipids	QDs	Docetaxel	Folic acid	Codelivery of nanoparticles QDs and docetaxel	Muthu, Kulkarni, and Raju (2012a)
Solid lipid nanoparticles	Low-density cholesterol, lipoprotein	QDs	Paclitaxel/ siRNA	cRGD	Multimodal therapy	Shuhendler, Prasad, and Leung (2012)

Astruc, 2004; Rengan, Jagtap, & De, 2013; Xiao, Hong, & Matson, 2012). Gold nanoparticles are synthesized with a size range from 1.5 to 10 nm, offering a large surface area for proficient ligand and therapeutic drug conjugation (Link & El-Sayed, 2002). Generally, the chemical treatment of hydrogen tetrachloroaurate is applied for the synthesis of gold nanoparticles. To recognize the specific receptor for active targeting, they may be combined with targeting ligand or drug as highly developed theranostic (Connor, Mwamuka, & Gole, 2005; Kumar, Korideck, & Ngwa, 2013). Two approaches can be used for therapeutic drug loadings, such as either covalent chemical conjugation of organic drug or noncovalent electrostatic interaction. However, the intrinsic properties of gold nanoparticles depend on certain factors, such as low toxicity, surface plasmon absorption, light-scattering, and diagnostic properties, tunable core size, and large surface area to volume ratio (Connor et al., 2005; Han, Martin, & Rotello, 2006; Kumar et al., 2013). Currently, a study conducted by Lee and colleagues demonstrated the potential to treat multidrug resistance tumors through photothermal therapy in conjunction with a therapeutic drug (Lee, Kim, & Kim, 2014). In another study, Heo and colleagues reported the surface modification of gold nanoparticles with paclitaxel, PEG, rhodamine B linked beta-cyclodextrin (beta-CD), and biotin as a single theranostic platform (Heo, Yang, & Moon, 2012). Paclitaxel was found to form an inclusion complex with beta-cyclodextrin (beta-CD) adsorbed rhodamine B that was covalently linked with gold nanoparticles.

In addition, in vitro findings proposed that gold nanoparticles have a strong affinity to various cancerous cells, including MG63 and A549 compared to NIH3T3. Also, gold nanoparticles exhibited a considerable cytotoxic effect against HeLa cancerous cells. Earlier researches show that gold nanoparticle is the best candidate to be used as theranostic nanomedicine. Smart theranostic gold nanoparticles were formed in another study such as the therapeutic drug doxorubicin (DOX) covalently linked with gold nanoparticles using a peptide substrate that is, Cys-Pro-Leu-Gly-Leu-Ala-Gly-Gly (CPLGLAGG) that is particularly cleaved via protease as shown in Fig. 20.1A. Previous animal studies demonstrated that overexpressed protease in intracellular glutathione and cancer cells resulted in the quick discharge of DOX from the modified gold nanoparticles upon administration in mice. Thus, gold nanoparticles may be conjugated with various therapeutic and diagnostic agents for simultaneous tumor therapy and diagnosis (Chen, Xu, 2013).

20.4 Drug-polymer conjugates

Chemical pathways are used for the synthesis of drug-polymer conjugates through covalent interactions that primarily depend on the therapeutic drug functional group and a polymeric carrier. There are two types of conjugates, such as drug-polymer conjugates and protein-polymer conjugates (Lammers & Ulbrich, 2010).

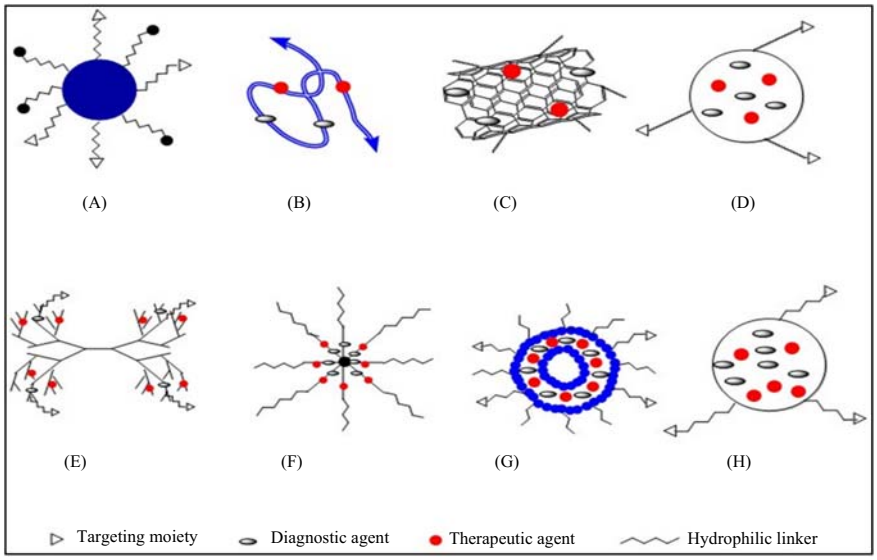

FIGURE 20.1

Schematic illustration of theranostic (A) gold nanoparticles; (B) Drug polymer conjugates; (C) Carbon nanomaterials; (D) Polymeric nanoparticles; (E) Dendrimers; (F) Micelles; (G) Liposomes; (H) Solid lipid nanoparticles.

The most widely used polymer for the theranostic drug-polymer conjugates is N-(2-hydroxypropyl) methacrylamide (HPMA) (Lammers & Ulbrich, 2010). Moreover, copolymerization and chemical conjugation is the successful approaches for HPMA copolymers modification with therapeutic and diagnostic agents (Allmeroth, Moderegger, & Gündel, 2013; Lammers & Ulbrich, 2010). Previous studies show that HPMA-based conjugates are usually applied for theranostics due to their nontoxic in nature, biocompatibility, and stability for in vivo applications (Kopecek & Kopeckova, 2010; Lammers & Ulbrich, 2010; Nakamura, Etrych, & Chytil, 2014). Besides, various studies have been conducted on HPMA polymer applications in association with several diagnostic molecules, such as I-131-labeled HPMA-DOX conjugate (Vasey, Kaye, & Morrision, 1999). Borgman and colleagues prepared the targeted HPMA therapeutic drug-conjugate by copolymerization method as theranostic nanomedicine (Borgman, Coleman, & Kolhatkar, 2008). Another study conducted by Yuan and colleagues synthesized the poly(HPMA) based theranostic copolymers loaded with RGD and Cu-64 for targeting tumor angiogenesis (Fig. 20.1B).

Moreover, positron emission tomography (PET) was used to evaluate the tumor localization of therapeutic drug-polymer conjugate in mice carrying xenografts of human prostate cancer. A quantitative analysis of PET showed that radioactivity of tumor Cu-64 in mice 3 h after intravenous administration of

therapeutic drug-polymer conjugate was considerably higher compared to the radioactivity of tumor Cu-64 in mice administered with the nontargeted conjugate of drug-polymer (Yuan et al., 2013).

20.5 Carbon nanomaterials

Carbon nanomaterials have been well-studied for theranostic purposes due to their remarkable physicochemical properties (Mei et al., 2013). Nanocarbon or carbon nanomaterials are categorized into sp^2 hybridization, such as two-dimensional (2D) graphene, carbon nanotubes (CNTs), fullerene, and carbon dots (Baughman, Zakhidov, De, & Heer, 2002; Liu & Liang, 2012). The functionalization of CNTs rendered them ideal candidates for theranostic application due to their capability to encapsulate therapeutic/diagnostic agents (Fubini, Ghiazza, & Fenoglio, 2010; McDevitt, Chattopadhyay, & Kappel, 2007; Porter, Gass, & Muller, 2007). Carbon nanotubes are mainly categorized into three types, such as single-wall nanotubes, double-wall nanotubes, and multiwall nanotubes. They also contain a cylindrical structure based on their graphene sheets layer. Various methods can be used for the fabrication of CNTs, for instance, chemical vapor deposition method, laser ablation, arc discharge, and ball milling methods (Boncel, Müller, & Skepper, 2011; Chen, Ma, & Liu, 2012; Fubini et al., 2010; McDevitt et al., 2007; Nerl, Cheng, & Goode, 2011; Porter et al., 2007; Shen, Zhang, & Liu, 2012; Yang, Feng, & Shi, 2013). Various studies were performed on the synthetic processes of CNTs modification, which introduced CNTs-based platforms for theranostic biomedical applications. A study conducted by Robinson and colleagues reported on the inherent theranostic application of intravenously administered SWCNTs. They found that SWCNTs acted as photoluminescent agents in case of in vivo tumor imaging within the 1.0−1.4 μm emission range, and were used as a heater or near-infrared (NIR) absorbers at 808 nm for the removal of a photothermal tumor at low doses as shown in (Fig. 20.1C) (Robinson et al., 2010). The distribution of SWCNTs within the tumors was observed by ex vivo resonance Raman imaging. Moreover, fluorescence images show evidence of SWCNTs absorption by the tumors. A hundred percent of tumor destruction was observed in photothermally treated mice after 6 months of post-treatment, showing no cytotoxicity. The elimination of effective tumor along with SWCNTs was achieved at 10 times fewer irradiation powers and injected dose as compared to control preparation. All these studies propose the benefits of using inherent physicochemical properties of CNTs, such as SWCNTs for theranostic biomedical applications (Robinson et al., 2010).

In addition, Das and colleagues successfully developed theranostic MWCNTs with the help of acid-oxidized MWCNTs and using four different functional moieties, such as a targeting agent (folic acid), methotrexate, fluorochrome (Alexa-fluor), and radionuclide (Technitium-99m) (Das, Datir, & Singh, 2013). Many

studies demonstrated the selective theranostic MWCNTs internalization through folate receptor-mediated endocytosis using various cell lines. Tumor-specific theranostic MWCNTs accumulation in xenografted mice after 24 h was 8.62 and 19.14 times higher than nontargeted and free theranostic MWCNTs. This work indicates the receptor-mediated delivery, radio-traceability of ^{99m}TC, controlled distribution of methotrexate, and optical detachability of fluorochromes (Das et al., 2013).

In conclusion, CNTs can be used as an efficient multimodal theranostic platform for cancer therapy and diagnosis. A study was conducted by Qin and colleagues who designed a graphene oxide theranostic nanohybrid to diagnose and treat the tumor (Qin, Feng, & Rong, 2014). Thus, CNTs have shown effectiveness during diagnostic and therapeutic drug delivery at the preclinical stage for future use (Saleemi, Kong, Yong, & Wong, 2020).

20.6 Polymeric nanoparticles

Polymeric nanoparticles have been widely studied for several therapies (Mu & Feng, 2003; Muthu & Singh, 2009; Zhang, Tan, & Feng, 2012). Due to lower phagocytic uptake, polymeric blocks were utilized to enhance the blood circulation half-life (Hu, Fang, & Luk, 2014; Luk, Fang, & Zhang, 2012; Moore, Chen, & Morrison, 2014). Polymeric nanoparticles are generally applied for the targeted codelivery of therapeutic and diagnostic agents in various theranostic applications due to their storage stability, controlled/sustained release, biocompatibility, and protection of loaded diagnostic/therapeutic agents (Luk et al., 2012). Moreover, biodegradable polymers have been applied for the fabrication of nanoparticles to give biocompatibility with less cytotoxicity. Polymer nanoparticles from biodegradable polymers demonstrated their benefit over liposomes and micelles because of their remarkable storage stability (Hu et al., 2014). For instance, poly (D, L-lactide-co-glycolide)-polyethylene glycol (PLGA-PEG), PLA-TPGS, and poly(D, L-lactide-co-glycolide) (PLGA) are widely investigated copolymers to prepare the theranostic based polymeric nanoparticles (Soppimath, Aminabhavi, & Kulkarni, 2001; Vijayakumar, Muthu, & Singh, 2013). However, PLGA and PLA can easily hydrolyze into their monomers, such as glycolic acid, and be removed from the human body through metabolic pathways. Preliminary reports showed that biodegradable polymeric nanoparticles synthesized by either polymer dispersion (i.e., solvent evaporation, and salting out) or monomers polymerization (i.e., emulsion method) (Moghimi, Hunter, & Murray, 2001; Vijayakumar et al., 2013).

However, magnetic nanoparticles are usually prepared from hematite or magnetite (Gupta & Gupta, 2005). Magnetic nanoparticles have been extensively used because of their acceptable biocompatibility and superparamagnetic effects. Two methods can be used to produce iron oxide nanoparticles, such as thermal

decomposition and coprecipitation approaches. Generally, they serve as theranostic in the treatment of cancer alone or combined with nanoplatforms such as liposomes, polymeric nanoparticles, etc. (Gupta & Gupta, 2005; Huang, Chen, & Dong, 2013; Li, Jiang, & Luo, 2013; Yen, Padmanabhan, & Selvan, 2013; Yoo, Lee, & Shin, 2011). They also play a significant role in cancer treatment as an immunotherapeutic platform and hyperthermia agent for various autoimmune disorders (Clemente-Casares & Santamaria, 2014; Yoo et al., 2011). The iron oxide nanoparticles' surface coating is very important by using oleic acid, dextran, PEG, and other surfactants to improve their water dispersibility and stability (Laurent, Forge, & Port, 2008; Mornet & Vasseur, 2004).

Zhang and colleagues synthesized nanoparticles comprised of cholic acid conjugated PLGA with coinstillation of autophagy inhibitor (i.e., chloroquine and 3-methyladenine) (Zhang, Dong, & Zeng, 2014). The half maximal inhibitory concentration (IC50) values of therapeutic drug docetaxel prepared in PLGA nanoparticles along with chloroquine (30 mM) or 3-methyladenine (10 mM) demonstrated 8.0 or 5.7-fold higher proficiency for nanoparticle-based drug delivery. After treatment with PLGA nanoparticles in combination with chloroquine or 3-methyladenine, the xenograft tumor volume of severe combined immunodeficiency (SCID) in mice is found to be only half compared to PLGA nanoparticles formulation only (Zhang et al., 2014). Therefore, it is important to combine autophagy inhibitors to improve the coadministration of therapeutic and diagnostic agents. Moreover, they also investigated a novel cancer cell mechanism by capturing biodegradable polymer nanoparticles and eliminating them by autolysosomes via the autophagy pathway (Zhang et al., 2014). Earlier research showed that polymeric nanoparticles were internalized within the cells by endocytosis and may be delivered to the lysosomes for degradation via the endosome pathway, as shown in Fig. 20.2 (Smith & Smith, 2012; Zhang et al., 2014).

Pan and colleagues reported on the PLA-TPGS-based theranostic platform (Pan et al., 2010). The poly(lactide)-tocopheryl polyethylene glycol succinate (PLA-TPGS) and acid-modified D-α-TPGS (TPGS-COOH) copolymer synthesized with folate targeting is the best example of PLA-TPGS based theranostic (Fig. 20.1D). The polymeric nanoparticles can be incorporated with QDs as well as docetaxel. The QDs targeting effects after incorporation with PLA-TPGS were observed in breast cancerous cells (MCF-7) that expressed a high quantity of folate receptors compared to fibroblast cells (NIH-3T3). Was observed the lower cytotoxicity or internalization of QDs after incorporation with PLA-TPGS in fibroblast cells (NIH-3T3) as compared to breast cancerous cells (MCF-7) (Pan et al., 2010). Thus, TPGS copolymer incorporated with specific targeting ligands can be a promising theranostic strategy for targeted therapy and diagnosis. The PLA-TPGS nanoparticles have been manufactured to integrate their benefits to facilitate controlled and sustained imaging with selective cancer cell targeting. This new approach improved their cellular uptake and biocompatibility and minimized contrasting agents' cytotoxicity. The model of xenograft was also carried out among various organs for the biodistribution of iron oxides and QDs

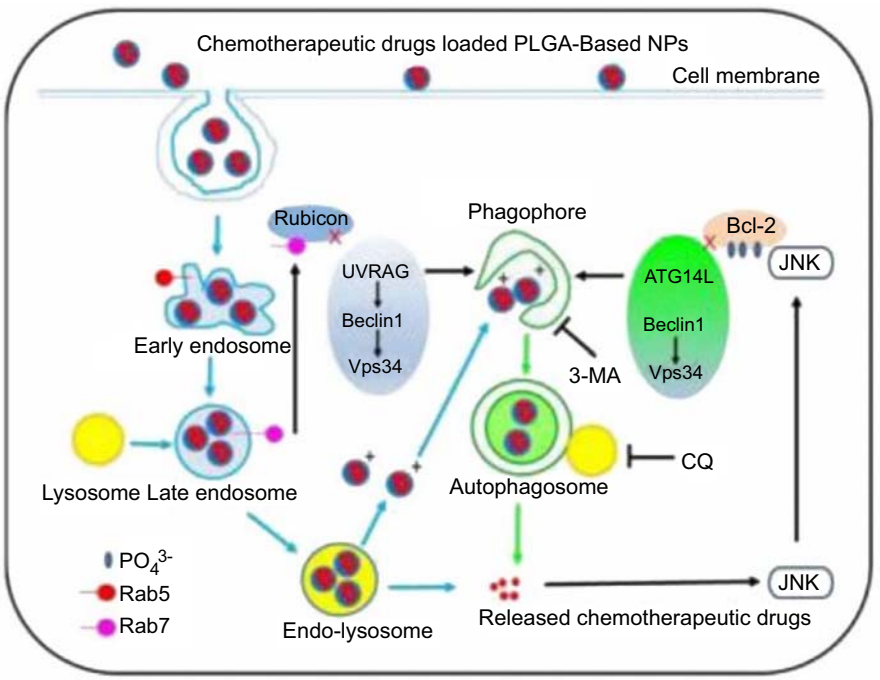

FIGURE 20.2

Schematic illustration of PLGA-based nanoparticles degradation pathway in cancer cells.

Reproduced with permission from Zhang, X., Dong, Y., Zeng, X. et al. (2014). The effect of autophagy inhibitors on drug delivery using biodegradable polymer nanoparticles in cancer treatment. Biomaterials, 35 *(6), 1932–1943. Copyright 2014 Elsevier Ltd.*

incorporated PLA-TPGS showed improved tumor imaging. In addition, ex vivo fluorescent image evaluation demonstrated a significant fluorescent percentage rise of 152.8% in the tumor, 51.5% in the kidney, and 67.1% in the liver. The surface adsorption of nanoparticles in the blood-brain barrier (BBB) exhibited more fluorescent signals for brain cells compared to other body organs. Besides, fluorescent percentage improved in the brain cells that were found less for the already treated group due to less development in the biodistribution of iron oxides and QDs conjugated PLA-TPGS. The imaging system demonstrates more benefits of contrasting agents and renders the probe susceptible for a longer duration (6 h) that confirms each diagnosis by imaging (Tan et al., 2011). Various studies also suggested the ligand conjugation and theranostic encapsulation by this system to develop unique multimodal nanotheranostics. For instance, Medarova and colleagues prepared iron oxide nanoparticles for concurrent in vivo distribution and imaging of siRNA into cancer cells through NIR and resolution MRI in vivo optical imaging. The animated dextran particles were applied to couple siRNA by

using N-succinimidyl-3-(2-pyridyldithio) propionate to act as a bridging compound. Moreover, NIR dye (Cy5.5), a membrane translocation peptide, and myristoylated polyarginine peptide were used to conjugate with the iron oxide nanoparticles. To monitor these probes by NIR and MRI imaging in vivo, the transportation of siRNA and its silencing effectiveness was assessed through dextran coupled iron oxide for 48 h, as shown in (Fig. 20.3) (Medarova, Pham, & Farrar, 2007).

20.7 Dendrimers

Dendrimers are nanosized, radially symmetric molecules with a well-defined monodisperse and homogeneous structure. Dendrimers are used as synthetic nanomedicine that contains a branched spherical polymer. The size of dendrimers used in the theranostic nanomedicines is 10−100 nm (Fahmy, Fong, & Park, 2007). Various approaches have been used for the preparation of dendrimers such as either divergent synthesis or convergent synthesis. A repeated branching around the center is the fundamental structure of dendrimers, as a result of an early-optimal three-dimensional geometric pattern. Because of the positive charge on the surface, dendrimers exhibited cytotoxicity through the disruption of the cell membrane. In certain cases, dendrimers used for target-specific therapeutic drug distribution tend to discharge encapsulated drugs quickly, before reaching a targeted site (Bosman, Janssen, & Meijer, 1999; Frechet, 1994). In addition, dendrimers can be prepared in different chemical compositions, sizes, and molecular weights by regulating the polymerization degree (Jansen, de Brabander-van den Berg, & Meijer, 1994; Li, Cheng, & Xu, 2007). However, higher-generation dendrimers are closely resembled spherical forms, having several branches and cavities to hold diagnostic and therapeutic molecules for various theranostic applications. The fifth-generation dendrimers are generally preferred due to their increased stability of the drug in dendrimers and hydrophobicity (Bosman et al., 1999; Frechet, 1994; Jansen et al., 1994; Li et al., 2007). One study reported the sodium-iodide symporter (hNIS) gene carried by a replication-defective adenovirus serotype 5 (ADV5) that was incorporated with poly(amidoamine) dendrimers generation 5 and assessed the transduction effectiveness in the xenograft mouse model by 1−123 scintigraphy. After coating of dendrimers, a replication-defective ADV5 demonstrated less safety from antibodies and increased the transduction effectiveness in coxsackie-adenovirus receptor-negative cells. While it was found that a significant reduction in hepatic transgene expression after intravenous administration of ADV5 coated with dendrimers (Grünwald, Vetter, & Klutz, 2013). Moreover, Saad and colleagues characterized, designed, and assessed a nano theranostics dendrimer for in vivo and in vitro studies (Saad, Garbuzenko, & Ber, 2008). These nanocarriers transported the therapeutic—paclitaxel and diagnostic—Cy5.5 agent. The dendrimers were attached to the synthetic analog of

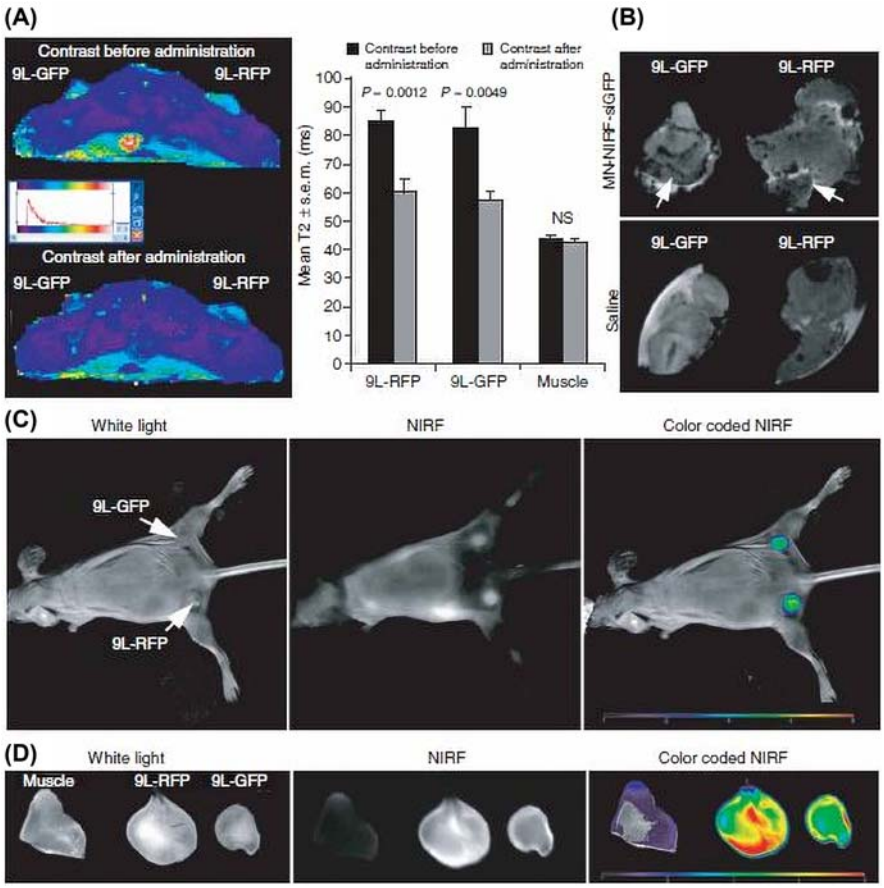

FIGURE 20.3

Theranostic procedure using dextran-coated iron oxide nanoparticles (A) In vivo MRI was performed on mice bearing bilateral green fluorescent protein (9L-GFP) and red fluorescent protein (9L-RFP) tumors before and 24 h after nanoparticles administration. After injection of the probe, there was a significant drop in T2 relativity association with the tumors. Note that the T2 relaxation times of muscle tissue remained unchanged. (B) Ex vivo high-resolution MRIs of excised tumors (78 m isotropic). Distinct foci of signal loss (arrows), reflecting probe accumulation, were easily identifiable in tumors derived from mice injected with the probe but not from saline-injected controls. (C) In vivo, NIR optical imaging of the same mice as in a produced a high-intensity NIR signal associated with the tumors. This confirmed the delivery of the nanoparticles probe to these tissues. (D) Ex vivo NIR optical imaging demonstrated a significantly higher fluorescence in tumors than in muscle tissue ($P = .0058$).

luteinizing hormone-releasing hormone (LHRH) peptide targeted to the receptors overexpressed on the cancerous cells' membrane. A considerable difference was observed between several studied no-targeted vectors in their anticancer efficacy, cellular toxicity, organ, and tissue distribution as well as cellular internalization. The targeted paclitaxel accumulation was associated with elevated cellular absorption of targeted specific nanotheranostic dendrimer that enhanced the sensitivity and specificity of cancer imaging by using fluorescent probes and minimized the side effect on normal tissues. It demonstrated the theranostic nature of targeted dendrimer for LHRH peptide (Saad et al., 2008).

However, Taratula and colleagues synthesized a new theranostic dendrimer platform for the purpose of tumor-targeted phthalocyanines (Pc) delivery as shown in (Fig. 20.1E) (Taratula et al., 2013). The process of synthesis included the Pc molecules modification with a specific hydrophobic linker that considerably increases the hydrophobic therapeutic drug encapsulation into a 4th generation dendrimer. Besides, the Pc surface was modified with LHRH peptide and PEG surfactant to modify the tumor-targeted distribution and biocompatibility. A distinct fluorescence emission (710−815 nm) and NIR absorption (700 nm) of the fabricated phthalocyanine derivative trapped in the dendrimer nanocarrier are needed for effective fluorescence imaging and photodynamic treatment. It has been shown that in vivo organ distribution and in vitro subcellular localization of advanced nanocarrier may be assessed based on the inherent fluorescence emission of encapsulated phthalocyanine. This formulation demonstrated high toxicity and considerable photodynamic therapy for 24 h. However, in vivo and in vitro imaging results showed that theranostic dendrimer targeted by LHRH can efficiently internalize into the cancerous cells. The study exhibited that dendrimers have significant potential as effective NIR theranostic nanoparticles (Taratula et al., 2013). Thus, dendrimers were successfully used as a theranostic carrier in a variety of preclinical studies.

20.8 Micelles

Micelles are lipid molecules containing a hydrophilic shell and hydrophobic core with a self-assembling colloidal structure. The size of micelles is less than 100 nm (Mahmud, Xiong, & Aliabadi, 2007). Micelles are another better option for parenteral instillation of poor water-soluble particles (Kataoka, Harada, & Nagasaki, 2001; Sawant, Jhaveri, & Koshkaryev, 2014). The micelles' stability depends on a strong cohesive force between the therapeutic drug and polymer core. Generally, they are produced by two approaches, such as the organic solvent method and the direct dissolution method (Vriezema, Comellas, & Elemans, 2005). Moreover, the diagnostic or therapeutic agent may be loaded into the outer hydrophilic layer and the hydrophobic core of micelles with a targeting molecule that can be injected intravenously (Kumar, Kulkarni, & Nagesha, 2012; Liu,

Yong, & Roy, 2012; Mi, Liu, & Feng, 2011; Torchilin, Lukyanov, & Gao, 2003). In certain cases, premature drug release from micelles nanomedicine inhibits targeted drug delivery by micelles before reaching the molecular target. Theranostic micelles less than 50 nm in diameter prevent reticuloendothelial system as well as renal exclusion and increased the endothelial cell permeability in tumors (Moghimi, Hunter, & Murray, 2004; Savic, Luo, & Eisenberg, 2003). Micelles are an effective vehicle for drug distribution, such as Genexol-PM that is a micellar formulation loaded with paclitaxel using poly(ethylene glycol)-block-poly(D, L-lactide) (Kim, Kim, & Shim, 2001). Few preclinical trials have already been conducted that assessed the efficacy and safety of Genexol-PM (Kim, Kim, & Kim, 2007; Lee, Chung, & Im, 2008; Lim, Tan, & Toh, 2010).

However, another alternative material such as TPGS used for the synthesis of diagnostic or therapeutic drugs loaded with targeted and nontargeted micelles (Kutty & Feng, 2013; Muthu, Kulkarni, & Liu, 2012b; Zhao, Mi, & Feng, 2013b). Theranostic TPGS micelles comprising superparamagnetic iron oxides can be used for the treatment and diagnosis of cancer as shown in (Fig. 20.1F). It contains certain properties, such as reduced cytotoxicity and improved magnetic and thermal properties and in vitro cellular uptake capacity as compared to commercial Pluronic F127 and Resovist micelles. Moreover, the synthesized TPGS micelles are highly stable, monodisperse, and suitable in size. The cellular uptake capacity and toxicity were observed using breast cancer cells (MCF-7) after 24 h. The viability of cells was reduced following hyperthermia therapy with micelles as shown in (Fig. 20.4). Besides, the T2-mapped xenograft images of SCID mice demonstrated that TPGS micelle loaded with iron oxide nanoparticles had a 1.05- and 1.7-times decrease in T2 at tumor site than Pluronic F127 and Resovist micelle composition, respectively (Chandrasekharan et al., 2011). A study conducted by Kim and colleagues synthesized a conjugate of hyaluronic acid-DOX through the formulation of amide bonds between hyaluronic acid carboxylic groups and DOX amine groups (Kim, Park, & Lee, 2012). They found that hyaluronic acid-DOX conjugates self-assembled to form a micelle-like structure. While toxicity study conducted on the cancerous cells showed that hyaluronic acid-DOX conjugate had an anticancer effect. In addition, the thermal vapor deposition method coated the micelles with a gold half-self that could lead to the residence time prolongation by applying the gold surface. Notably, hyaluronic acid-DOX conjugate micelles are the best platform to be used in potential applications for cancer theranostic nanomedicine (Kim et al., 2012). Thus, a micelle is a novel platform as a theranostic nanomedicine delivery system due to its hydrophobic encapsulation, ease of fabrication, stability, and success in clinical studies.

20.9 Liposomes

Liposomes are spherical-shaped vesicles that may be generated by using cholesterol and amphiphilic phospholipids (Lasic, 1998). The size of liposomes is

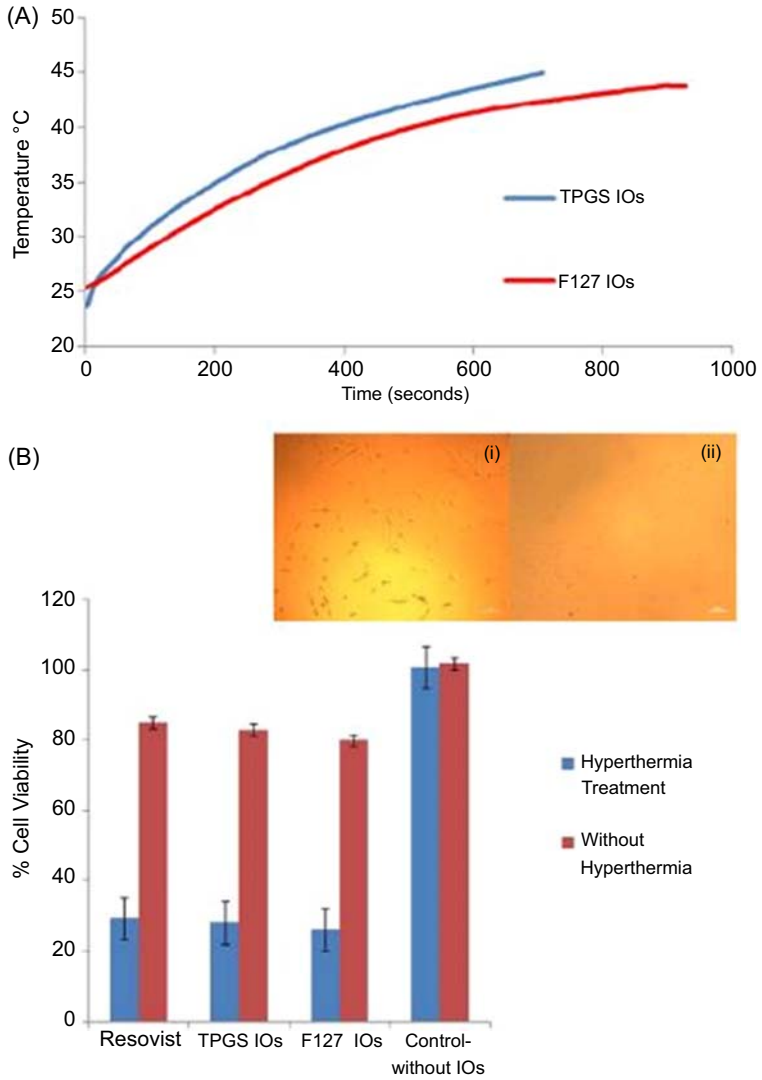

FIGURE 20.4

In vitro effect of the theranostic procedure using micelles coated with iron oxide (A) Hyperthermia study shows that the time-dependent temperature rise of 1 mg/mL of the iron oxide nanoparticles (IQs)-loaded TPGS and F127 micelles on exposure to 89 KA/m altering current field at 240 KHz frequency. The specific absorption rate value for the IQs-loaded TPGS and F127 micelles was found to be 51.4 and 25.5 Watt/g. (B) The cytotoxic assay was performed after hyperthermia treatment of MCF-7 cancer cells incubated with the IQs-loaded TPGS and F127 micelles and Resovist. Hyperthermia treatment in all cases leads to significant cell death. Insert image (i) shows the cells incubated with the IQs-loaded TPGS micelles but no AC field applied and insert image (ii) shows the cells incubated with the IQs-loaded TPGS micelles and hyperthermia treatment using an AC field. From (iii), it can be seen that after hyperthermia treatment, the cell loses viability and does not attach.

400 nm or less. Generally, liposomes provide the best platform for therapeutic drug and diagnostic delivery due to their low toxicity and immunogenicity, size, biocompatibility, hydrophilic and hydrophobic characteristics, and biodegradability. Liposomes with a small diameter ranging from 80 to 200 nm and negative or neutral charge are mostly used for the theranostic purpose (Grange, Geninatti-Crich, & Esposito, 2010; Torchilin, 2005; Voinea & Simionescu, 2002). There are three different methods applied for the preparation of liposomes, such as detergent removal, solvent dispersion, and mechanical dispersion. However, there are also certain drawbacks of liposomes, for instance, poor stability, short half-life, low solubility, and leakage of an encapsulated drug (Grange et al., 2010; Torchilin, 2005; Voinea & Simionescu, 2002). Moreover, nanosized diagnostic agents (i.e., gold and iron oxide nanoparticles) can be loaded into the therapeutic agents and theranostic liposomes, which may either be embedded in the shell of the lipophilic bilayer or encapsulated in the core (Al-Jamal & Kostarelos, 2011; Al-Jamal, Al-Jamal, & Tian, 2008; Leung & Romanowski, 2012; Nie, Ji, & Ding, 2012; Papahadjopoulos, Allen, & Gabizon, 1991).

In addition, stealth liposomes such as PEG-coated liposomes have been developed to resolve opsonization by the immune system and quickly remove it from the blood circulation (Al-Jamal & Kostarelos, 2011; Muthu & Feng, 2010). Muthu and colleagues synthesized liposomes surface-coated TPGS loaded with docetaxel and then assessed in vitro PEGylated liposomes, such as liposomes coated with or without TPGS. They found that PEGylated liposomes demonstrated lower efficacy as compared to TPGS-coated liposomes. In another study, the same group synthesized TPGS-coated liposomes containing QDs and docetaxel with/without targeting moieties, as shown in (Fig. 20.1G). Besides, folic acid was also applied as a targeting probe to trigger the folate receptor of breast cancerous cells (MCF-7). The highest cytotoxicity and cellular uptake capacity of targeted theranostic liposomes were found as compared to nontargeted liposomes. However, Wen and colleagues prepared apomorphine and QDs coated with theranostic liposomes to enhance brain targeting and eliminate uptake by the liver (Wen, Zhang, & Al-Suwayeh, 2012). The liposome distribution by the endocytosis pathway was confirmed by an in vitro cellular uptake study. Theranostic liposomes were substantially distributed in contrast to free QDs. The uptake of QDs was decreased in the liver and heart by liposome delivery as compared to the brain where their uptake was increased by 2.5-fold.

Moreover, the brain fluorescence was observed for 1 h in the case of the liposome targeted group. Also, the free QDs were quickly removed from the brain cells, while they were retained in the liver for 35 min. The proposed mechanisms for cellular uptake were caveola- and clathrin-mediated endocytosis (Wen et al., 2012). Wen and colleagues synthesized QDs loaded theranostic liposomes with irinotecan and camptothecin for concurrent drug delivery and bioimaging (Wen, Sung, & Aljuffali, 2013). Various formulations, such as deformable, cationic, and PEGylated liposomes were compared to determine their theranostic efficacy. The cellular migration and cytotoxicity assay showed the highest cationic liposome

activity relative to other carriers. Besides, cationic liposomes were more effective in accumulating fluorescent signals in solid tumors than in control samples. Furthermore, cationic liposomes demonstrated the strongest fluorescence in tumors as compared to control liposomes (Wen et al., 2013).

20.10 Solid lipid nanoparticles

Solid lipid nanoparticles are another very attractive and promising carrier of the colloidal matrix for traditional polymeric nanoparticles, emulsion, and liposomes for intravenous delivery (Muthu & Singh, 2009). Solid lipid nanoparticles consist of a hydrophobic core comprising dispersed or dissolved therapeutic drugs. They have usually produced nanomedicine from biocompatible lipids (such as triglycerides) that remain solid at ambient temperature. There are two approaches used for solid lipid nanoparticle synthesis such as cold and hot homogenization. They can easily enter the blood circulation due to their lipophilic surface and small size. Solid lipid nanoparticles may also cross the tight-endothelial cells of the BBB for brain targeting. The high capacity of therapeutic drug loading keeps therapeutic molecules safe in the matrix and allows controlled delivery for many weeks (Wissing, Kayser, & Müller, 2004). Like the other nanomedicines, they can be applied for the codelivery of therapeutic and diagnostic agents as a theranostic platform (Muller, Rühl, & Runge, 1997; Wissing et al., 2004). Currently, a new technology such as solid lipid nanoparticles lymphatic distribution is used to provide better lymphatics transportation, resulting in increased therapeutic agent's oral bioavailability (Singh, Swami, & Khan, 2014). For instance, Shuhendler and colleagues prepared a new targeted specific NIR light-emitting nanoparticles by combining cyclic Arg-Gly-Asp (cRGD) and incorporating them with NIR QDs for the imaging of live animals (Shuhendler et al., 2012). Moreover, they were administered this to the mice carrying xenograft breast tumors. The optimal imaging of formulations was used to study the whole animal biodistribution and tumor micropharmacokinetics. The active cRGD tumor targeting demonstrated considerably higher distribution in the kidney, spleen, and liver as compared to nontargeted solid lipid nanoparticles. Increased tumor sensitivity and persistence of RGD-solid lipid nanoparticles proposed their possible use in theranostic applications, such as neovascular imaging.

Bae and colleagues studied QDs incorporated solid lipid nanoparticles for theranostic nanomedicine of cancer with synergistic effects of siRNA and paclitaxel (Fig. 20.1H) (Bae, Lee, & Lee, 2013). Besides, they were prepared low-density lipoprotein (LDL)-mimetic solid lipid nanoparticles conjugating paclitaxel and QDs inside the lipid shell, but the anionic siRNA molecules are electrostatically crosslinked with solid lipid nanoparticles surface. The siRNA/solid lipid nanoparticles complex effectively transported the Bcl-2 and paclitaxel targeting siRNA into the human lung carcinoma cells and showed synergistic anticancer

activities via caspase-mediated apoptosis. The high fluorescence strength of QDs inside solid lipid nanoparticles enables them to be visualized in situ and translocated intracellularly in cancer cells. Moreover, this study demonstrated that LDL-mimetic solid lipid nanoparticles are a multimodal system to be used as an optically traceable nanocarrier for successful anticancer theranostics (Bae et al., 2013).

20.11 Modular development of hybrid nanoplatforms

The newly developed cancer nanomedicine domain has been shifted to theranostic nanoplatforms multimodality with unified functions, such as imaging or diagnosis and controlled chemotherapeutic distribution of tumors therapy, after exhibiting the surface modifications, geometries, and bioconjugation approaches of different hybrid nanostructures (HNCs). Previous preparation methods and procedures for the synthesis of HNCs restrict the large-scale assembly of highly homogenous nanostructures (Thorat, Otari, & Patil, 2013). Recent advancements in synthetic processes and material science have shown potential for a more precisely specified homogeneous assembly of theranostics nanostructures. To date, the technical information transfer provides HNCs synthesis protocols that confirm good manufacturing practice (GMP). The introduction of GMP compliant for the development of HNCs increases the efficiency of nanoparticles (NPs) based on pharmaceutical regulations. While some heterogeneity is inevitable across various nanoformulations, new processing methods allow HNCs to be engineered and regulated with respect to geometry and particle size distribution (Türeli, 2018; Wicki, Ritschard, & Loesch, 2015). The modular (such as scalable, standard, and flexible) development of multifunctional HNCs, which combines inorganic and organic chemistry engineering, results in the codelivery of therapeutics and diagnostics (Doan-Nguyen, Natsathaporn, & Jenjob, 2019).

After recognizing the value of HNCs modular design principles, researchers focused on research studies as the design of a logical nanoparticle to accomplish passive accumulation in tumors with an adequate amount. Scientists are trying to establish a method for preparing tuned-size NPs to make it possible to hasten free entry between endothelial cell barriers in the tumor blood vessel. Even after three decades, this was a persistent problem because of the restricted clinical translation of nanomedicine cancer. Recently, this problem has been solved using a nanotheranostic method focused on the analysis of different models of mouse and human tumors as well as mathematical modeling and simulation (Sindhwani, Syed, & Ngai, 2020). It is important to address this question to resolve the weak clinical translation of nanomedicine cancer and to take a step forward toward newly developed cancer nanotheranostics. The highly dynamic architecture of HNCs resulted in increased demands for multifunctional efficiency and adjunct therapy approaches. The molecular heterogeneity of tumors and the precise chemistry of

materials used to design HNCs are equally essential in the HNCs' complex design. In the past, the missing connection between these two distinct banks, cancer nanomedicine development and research has been enabled to obtain a clinical translation. In the next generation nanomedicine translation roadmap, the design connected with the clinical requirements over quality-oriented procedure enhancement is currently suggested (Tahara, 2020). However, quality by design is the key pillar of this approach and plays an important part in the clinical translation of nanomedicine.

Nanotheranostic platforms have become relevant in cancer therapies that are attracted a lot of researchers' interest. Although, the idea of different nanoplatforms modular design had not attained much attention. The idea of modular design has currently been extended as an advantage of high nanostructure loading power, and prompt and intelligent response to several internal and external stimuli. Moreover, hybrid nanocarriers modular designs are generally categorized based on standardization, extendibility, and reproducibility for targeted delivery and synergistic therapy (Wang, Xiao, & Chen, 2018). Besides, modular HNCs design is beneficial for the release of localized chemotherapeutic agents, resulting in reduced tumor cell exposure and chemoresistance (Thorat, Bauer, & Tofail, 2020). Specifically, scientists are working to obtain a high level of reproducible HNCs that may be used in the models of clinical cancer. The direction of this research study is encouraging, but also demanding for researchers working in the nanotheranostics field (Rosenblum, Joshi, & Tao, 2018). Modular nanoplatforms should be optimized in the foreseeable future to observe a clear view of HNCs functions.

20.12 Newly developed hybrid nanoplatforms

Hybrid nanoplatforms have demonstrated higher therapeutic outcomes and therapy efficiency in cancer theranostics compared to earlier designed drug-NPs formulations. The production of newly developed HNCs that combine different chemical properties tackles existing challenges in cancer therapy (Fan, Yung, Huang, & Chen, 2017). The novelty of nanoplatforms using HNCs is developed to resolve main strategic directions for cancer therapies, these main strategies are; (1) surface modification using RBC, viruses, or polymer to conjugate therapeutic cargos, (2) general but efficient production protocols on large-scale fabrication, (3) rational therapeutic outcomes for patient stratification, (4) balancing and supportive interactions among different therapeutic payloads, (5) therapeutic drug synergy of molecular and chemical cotreatment using HNCs, (6) HNCs provide the opportunity to revolutionize the cancer therapy in modular nanocarrier. Notably, these hybrid nanoplatforms techniques facilitate the production of cancer nanomedicine for the next decade in highly developed clinical settings.

Hybrid systems have the potential for multifunctionality as compared to their existing counterparts. Moreover, HNCs have many novel properties that render them an ideal candidate for advanced chemotherapeutics. However, a new hybrid nanomicelle incorporating therapeutic drug DOX, PTA, and NIR fluorescence dye (IR780) is being developed (Song, Li, & Li, 2020). This hybrid nanomicelle demonstrates excellent blood circulation, damage the deep-seated cancer tumor, and improves the sequential release of therapeutic drug. The pristine hybrid nanoplatform is designed to resolve pathological heterogeneity at the advanced clinical level of long-acting amplified cancer therapy. In addition, the stimuli-responsive HNCs also promote the delivery of tumor-specific drugs. In addition to the multifunctionality of various therapeutic approaches and successful targeting of tumor lesions, supramolecular HNCs may also be effective in enhancing chemotherapy. The nanodrug reservoir of folic acid incorporated PEG molecules, and hollow mesoporous silica NPs exhibit a surprising coherent fashion for synergistic chemo-PTT (Yang, Dai, & Lou, 2020). Similarly, the dual-mode fluorescence-Raman active DNA HNCs approach is designed to improve the chemo-PTT and also provides noninvasive and endoscopic imaging accompanied by cancer surgery (Pal, Ray, & Andreou, 2019).

The chemodynamic therapy (CDT) can be fulfilled by a typical new hybrid nanoplatform class with a mesoporous copper and biodegradable cancer cell membrane (Liu, Wang, & Zhang, 2019). CDT is a recently suggested cancer therapeutic technique that integrates ROS-dominated PDT into a single nanoplatform. The HNCs distribution into tumor sites triggers GHS fatigue and tumor hypoxia that disturbs the tumor microenvironment. HNCs composed of a newly invented smart Pt-CuS Janus have high optical absorbance that enables dual modal NIR and PA thermal imaging (Liang, Deng, & Chang, 2019). A multipurpose nanoplatform opens by this Janus HNCs that enhances therapeutic drug effect and improves cancer nanotheranostics.

20.13 Conclusion and outlook

In this chapter, we discussed the current advancement of several theranostic nanomedicines and their preclinical and clinical success. We found that results of nanotheranostics in previous literature are only accessible for in vitro research in most cases. The researchers are facing more challenges with respect to their preclinical trials. Most of the previously published work is focused on the synthesis, physicochemical properties, and in vitro study of theranostic nanomedicines, while very less information is available on in vivo studies. The available in vivo reports are either diagnostic or therapeutic, but not in a combined form. To date, only a few studies have shown some in vivo evidence of nano theranostics that are listed in Table 20.2. Hence, a highly developed theranostic nanomedicine becomes a future generation approach with a wide range of nanomedicine

Table 20.2 In vivo effectiveness evaluation of theranostic nanomedicine.

Theranostic nanomedicine	Targeting/ diagnostic/ therapeutic agent	Duration of therapy	Duration of diagnosis	Administration site	Fold of efficiency enhancement	Reference
Conjugates of drug-polymer	RGD/64Cu	3 h	3 h	Intravenously (i.v.)	Nearly onefold in tumor	Yuan et al. (2013)
Magnetic nanoparticles	Iron oxide nanoparticles/ siRNA/Cy 5.5	48 h	48 h	Intravenously (i.v.)	Observed the in vivo silencing in tumor	Medarova et al. (2007)
Carbon nanomaterials	MWCNTs/ Technitium-99m/Folic acid	1 to 15 days	24 h	Intravenously (i.v.)	Nearly 8.5-folds greater as compared to nontargeted MWCNTs and at least twofolds greater inhibition of tumor	Das et al. (2013)
Polymeric nanoparticles	Iron oxide nanoparticles/ quantum dots	–	6 h	Intravenously (i.v.)	Nearly 1.5-folds in tumor	Tan et al. (2011)
Dendrimers	LHRH/ phthalocyanines	24 h	10 h	Intravenously (i.v.)	A significant fluorescent was found in tumors with toxicity	Taratula et al. (2013)
Micelles	Passive/iron oxide nanoparticles	24 h	24 h	Intravenously (i.v.)	Reduction in the onefold T2 value with considerable cell death	Chandrasekharan et al. (2011)
Liposomes	Passive/ cholesterol, irinotecan/ camptothecin	24 h	24 h	Intratumoral	More concentration of drug at the tumor site and sixfolds longer duration of detection	Wen et al. (2013)
Solid lipid nanoparticles	RGD/lead selenide	–	120 h	Intravenously (i.v.)	Nearly onefold in tumor vasculature	Shuhendler et al. (2012)

characteristics, such as synergistic performance (i.e., combination therapy, siRNA deliver), multimodal/multifunctional approach, and quality performance (i.e., autophagy inhibition, oral delivery), and stimulus-responsive therapeutic drug release (i.e., ultrasound, pH, temperature). More research are required to further investigate these ideas on available nanomedicine clinical platform for the development of smart and versatile theranostic applications (Muthu & Feng, 2011). However, theranostic nanomedicine loaded with iron oxide, QDs, and gold nanoparticles would be an effective carrier to discuss these problems in the future. Moreover, iron oxide nanoparticles have demonstrated a remarkable biosafety profile since they can be easily metabolized and degraded to produce hemoglobin (Ho, Sun, & Sun, 2011). Notably, the QDs loaded with heavy metals as theranostic nanomedicine probes for human use are a big concern at present. Studying the QDs cytotoxicity and in vivo biodistribution is also another challenge in an animal system. In the future, it is highly anticipated to develop nonimmunogenic, biocompatible QDs for loading of nanomedicine that could be secreted via renal elimination mechanism (Zhu, Hong, & Xu, 2013).

However, carbon nanotubes (CNTs) have a great potential to produce in vitro and in vivo cytotoxicity. They are generally produced by oxidative stress such as reactive oxygen species by lipid peroxidation that causes inflammation and cellular destruction (Yang, Luo, & Zhou, 2012). Besides, another study conducted by Mateo and colleagues reported the cytotoxicity of gold nanoparticles (Mateo, Morales, & Avalos, 2014). Thus, more studies will need to be performed on the biosafety/toxicity profile of both gold nanoparticles and carbon nanotubes before they can be applied in theranostic applications. Multimodal/multifunctional nanomedicine such as the combination of therapeutic drugs and diagnostics may be beneficial for theranostic purposes (Muthu, Leong, Mei, & Feng, 2014). The theranostic nanomedicine approach such as nanotheranostics that targets advanced molecular biomarkers is likely to participate in improving cancer therapy accuracy.

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CHAPTER

Nanorobots for improved theranostic applications 21

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21.1 Introduction

Nanotechnology is the development and implementation of resources, structures, and processes by modification of substance on a nanometer-length scale, that is, at the chemical, molecular, and supra-molecular stages (Jain, 2011). Nanotechnology has advanced tremendously over the last few decades. Novel nanomaterials have been exposed and discovered for a diversity of purposes such as these are commonly employed as parts of much bigger assemblies or as nanofunctional components. Nanomaterial-based devices have discovered use in a range of applications like biosensing, drug delivery, and triggering devices (Sanchez & Pumera, 2009). Nanorobotics is a modern discipline that emerged between the late 1990s and early 2000s as a result of the integration of robotics and nanotechnology. Nanorobotics is the study of nanoscale devices' layout, manufacturing, programming, control, and locomotion. Nanorobotics adds another dimension to robotics, resulting in the expansion of nanorobots, also known as "nanobots." Instead of operating from the outside, nanobots would be miniaturized and incorporated into the skin by the vascular system or at the ends of valves into various vessels and other hollows in the human psyche (Jain, 2011).

Nanorobots are promising devices that could be used in drug distribution, sensing, environmental remediation, and small object manipulation. They move by extracting energy from exterior bases for example light, ultrasound, electrical or magnetic fields, or a combination of all of these (Chen et al., 2017a,b). The robotic system has significantly extended humans' ability to sense, communicate with, control, and transform the world around us. The integration of numerous technologies, in particular, has permitted a breakthrough in medical robotics applications aimed at improving health care. Medical robotic systems are engineered for very diverse conditions and activities related to the handling, treatment, and anticipation of diseases, whereas industrial robotics were built mainly to automate repetitive and hazardous macroscale manufacturing activities. For multifaceted and detailed

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operations and coupling with the human body, medical robots need remotely controlled and advanced miniaturized parts and smart materials like Nanomaterials (Li et al., 2017). Nanorobotics is a wide field that includes nanofabrication methods for fabricating nanoscale robots, nanosensors, nanoactuators, and physical modeling at nanometer scales (Rahul, 2017).

Nanorobotic manipulation system involves the arrangement of nanometer-sized elements, the modification of bacterial structures or molecules, and the robots that perform functions are all examples of nanotechnology. Designing advanced, miniaturized, and flexible robots with a diameter of a few microns or less will enable control over the entire human body, coming up with innovative treatments down to the molecular level, as well as more effective and accurate targeted diagnostic and treatment. Robotic device miniaturization at the nano- and microscales grips significant potential for improving the treatment and detection of an extensive range of diseases. Medical nanorobotics has the potential to develop novel technologies for treating a variety of human illnesses and for strengthening the human biological system (Li, Xi, Wang, & Liu, 2020).

21.2 What are nanorobots?

Nanorobots are self-contained devices with overall dimensions in the micrometer range or less, made up of nanoscale materials of at minimum 1D varying from 1 to 100 nm. Nanorobots are described by at least 1D in the micrometer (1000 m = 1 mm) or nanometer (1000 nm = 1 m) scale. These are normally controllable devices made up of nanosized element assemblies that can interact with or even penetrate the cell membranes due to their small size and weight, having a clear path to the cellular level. At the nanoscale level, nanorobots are capable of performing at least one of these three basic functions: actuation, acceleration, sensing, signaling, information processing, cognition, and swarm-like behavior. They could be used for drug delivery, surgery, diagnostic testing, genetic manipulation, dental care, and plenty of other things (Shetty, Swati, & David, 2013). Drugs typically pass across the whole body before reaching the diseased region. The medicine may be tailored to a particular location using nanotechnology, making it much more effective and reducing the risk of side effects (Nistor & Rusu, 2019). Some enthusiasts of this field refer to nanorobots as nanobots or nanites, despite the fact that all these words do not accurately characterize the engineering features of the device (Singh, Ansari, Laux, & Luch, 2019). Though nanorobots are realized as potential bionanomachines that will overlay way for much more promising medical therapies, nothing is flawless, and this subject will still have advantages and disadvantages. Nanorobots have a number of advantages over larger competitors, including increased agility, increased longevity, the ability to self-replicate to restore worn-out devices, and the ability to provide targeted therapy straight to the diseased area. Some of the drawbacks include high construction costs, a complicated architecture, and susceptibility to electrical disturbances

like radio frequency (RF) or electric fields, as well as electromagnetic waves produced by outdoor causes (e.g., in vivo electrical devices like pacemakers) (Wang et al., 2018).

Various types of nanorobots based upon different actuation concepts have been produced over the last decade. Externally driven motors are propelled by magnetic and ultrasound forces, as well as optical, thermal, and electrical energies. Externally powered motors mainly use optical, thermal, and electrical energy to accelerate their motion, while chemically operated motors transform locally supplied fuels to force and movement. Surface reactions enable chemically driven motors to propel themselves through aqueous solutions by creating local concentration, electrical potential, and gas bubble gradients. Magnetic swimmers mimic the movements of normal aquatic microorganisms with helical or flexible flagella by using magnetic actuation. Synthetic nanodevices may also be combined with motile species to create hybrid nanorobots that run on renewable energy. All of these diverse propulsion concepts have resulted in many micro/nanorobotic experiments, comprising fuel-powered tubular micro rockets, magnetically driven radial swimmers, ultrasound-controlled nanowire (NW) engines, and a biohybrid microrobot powered by sperm (Li et al., 2017; Mishra, Dash, & Kumar, 2012) (Fig. 21.1).

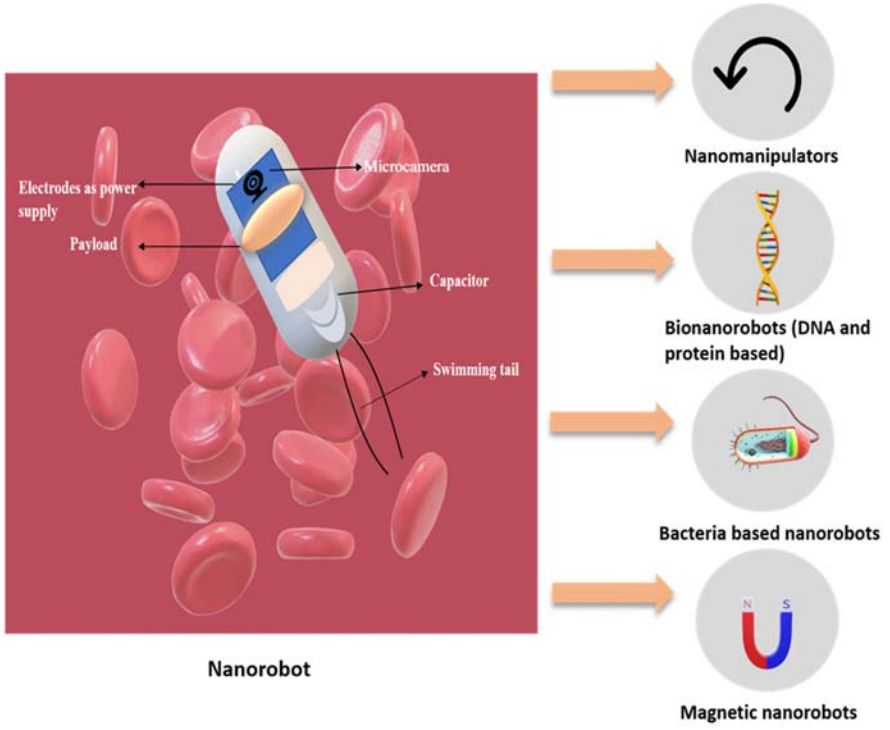

FIGURE 21.1

Components and classification of nanorobotic system.

21.3 Fabrication/design of nanorobots

The design of nanorobots is based on biological modeling techniques, primarily bacterial behavior. Nanorobots must travel across small channels a few hundred nanometers in diameter in biological fluids. The main properties of deceptively amplified viscosity and low Reynold numbers should be considered when designing a nanorobotic propulsion mechanism. The positional assembly, as well as self-assembly, remains the two utmost common methods for creating nanorobots. Molecules are manually picked and stored using a miniature robot's arm or a microscopic array in self-assembly. The researcher's determination collects billions of molecules and leases them mechanically accumulate into the expected shape using their inherent empathies in positional assembly (Sharma, Rege, Budil, Yarmush, & Mavroidis, 2008).

Carbon nanocomposites made of diamond/fullerene is the primary element used in the construction of the exterior body of nanorobots because it has various inert properties and strength. Certain light elements, including oxygen and nitrogen, could be used in a range of applications. To prevent recipient immune system resistance reactions, the outermost layer including its nanomaterials is coated with an inactive diamond coating or another nonallergic lipid or protein. The less the immune system reacts to the coating, the smoother and more flawless it is. The less the immune system reacts to a coating that is clean and flawless, the better and stronger (Dolev, Narayanan, & Rosenblit, 2019).

Power source, sensors, a fuel buffer container, engines, actuators, exploiters/manipulators, onboard processors, Pumps, Pressure containers, and structural reinforcement are all things that need to be considered for the manufacturing of a nanorobotic device (Venkatesan & Jolad, 2010). The nanorobot would be able to drive owing to motors and manipulator arms or a mechanical leg. Additionally, to achieve biomedical application, an ideal nanorobot also has some substructures including a payload section that holds the drug/medication and dispenses the drug at a particular site of infection; a micro camera that can direct the nanorobot when actively traveling around the body; batteries that use electrolytes found in the body fluid, such batteries/electrodes may detect and destroy cancerous cells as well by creating an electrical charge and burning them to death; lasers that may melt harmful material such as plaque present in the arteries, blisters in the blood, or cancerous cells; ultrasound wave producers, that are often used to monitor and eliminate kidney stones using nanorobots, and a flowing antenna, which enables them to get into the body as they move. All of the above requirements must be addressed and decoded at the nanoscale stage to build a fully functional nanorobot (Manjunath & Kishore, 2014; Sokolov et al., 2017).

The final design of a practical nanorobot often necessitates the deliberation of several dissimilar mechanisms, like actuation approaches, locomotion strategies, control over the motion of nanorobots, their navigation system, etc. (Manjunath & Kishore, 2014). Each of these mechanisms is being studied to improve the performance of the nanorobot in relation to its application environment.

21.4 Actuation methods

Nanorobot actuation methods are classified as physical and chemical methods. In general, magnetism, electricity, light, acoustics, and piezoelectricity are used to actuate nanorobotic physical motion. When selecting an actuator configuration, the exchange between a number of movements, torque, acceleration (rate of actuation), power usage, control efficiency, power efficiency, durability, payload size, and so on must be weighed (Cavalcanti, Shirinzadeh, Freitas, & Hogg, 2007). This section discusses fundamental actuation technologies and possible applications at the nanometer scale.

21.4.1 Physical actuation

Magnetism, electricity, light, acoustics, and piezoelectricity are all used to actuate physical motion. Magnetic actuation, amongst all mechanisms, is popularly used for nanorobot actuation because of its advantages of powerful penetration and distant drive behavior. Magnetic actuation, which is nontoxic, can be used to drive the acceleration of live biological samples (Sanchez & Pumera, 2009). Magnetic field actuated nanorobots can convert magnetic energy to mechanical energy by way of magnetic field gradient and magnetic torque, allowing them on the way to realize their precise applications. Cargo handling, transportation, targeted drug delivery, as well as environmental cleanup due to magnetic fields of lower intensities are supposed to be safer for living organisms. Nanorobots triggered and directed by the exterior-based magnetic fields hold countless potential for in situ simulations. According to the literature, various nanorobot designs using different forms of actuation have been reported to swim in vitro in physiological environments. Hoop et al. employed magnetic nanorobots, a hybrid NW for chemotherapeutic treatments, and Magnetically actuated Au and Ni−Ni oxide NWs were used for controlled targeting and inducing cancer cell death by Serra et al. (Hoop et al., 2018; Serrà, Vázquez-Mariño, García-Torres, Bosch, & Valles, 2018). Through remotely manipulating the magnetization direction of each microgripper arm, Diller et al. demonstrated a grasping action that can be combined with locomotion, resulting in a magnetic-field-actuated handheld microgripper used for 3D micro assembling (Diller & Sitti, 2014). Chen et al. also developed an advanced system capable of wireless locomotion and onsite activated therapeutics, both are controlled by a particular magnetic field from the outside. The system can be wirelessly guided to a specific location by spinning magnetic fields, and it can perform on-demand magneto-electrically assisted drug delivery to kill cancer cells (Chen et al., 2017a,b).

After the magnetic field, the electric field is the preferred system for nanorobot propulsion. Electric charge is caused by an accumulation or deficiency of free electrons in a substance, and it may attract or repel oppositely charged objects. Due to the rapid appearance and disappearance of electrostatic fields, such

devices can have extremely high operating speeds and will be unaffected by ambient temperatures. Electrostatic fields can exert significant forces over short distances, but only on a small scale. Electric fields work over longer distances, necessitating a higher voltage to sustain a given force (Sanchez & Pumera, 2009). Kopperger et al. (2018) applied an electrical field for the actuation of a 55 nm by 55 nm DNA-based molecular assembly with a combined robotic armrest of length 25 nm, that could remain protracted for about 400 nm and powered through an external electric field (Kopperger et al., 2018). A tunable and precise method for altering catalytic nanomotors in three dimensions (3D) by strategically attributing electric fields was also reported. The catalytic nanomotors' high controllability established their adaptability in collecting, distributing as well as discharging loads to specific sites, and in vivo incorporation with the nanomechanical devices (nanoelectromechanical systems, NEMS) for chemical powering (Guo, Gallegos, Tom, & Fan, 2018).

Piezoelectric propulsion systems are a novel and appealing method of propulsion generation. When crystalline materials are exposed to an electric field or an electric charge, they undergo permanent deformation that induces piezoelectric motion. Piezoelectric materials comprise quartz (SiO_2), lead zirconate titanate, lithium niobate, and polymers including polyvinylidene fluoride. Piezoelectric materials can retort to voltage changes rapidly and consistently. Quartz timing crystals, which are used in a number of electronic instruments, could be used to produce accurate signals of repeatable oscillations. Tension and compression strains can be converted to voltages using piezoelectric materials as sensors (Nelson, Dong, & Arai, 2016). Jain et al. defined the construction of microgrippers by employing a piezoelectric actuator for demonstrating the micro-assembly (Jain, Majumder, & Ghosh, 2015). A group of scientists proposed and tested a piezoelectric actuator based on the parasitic motion principle. The presented parasitic-type piezoelectric actuator had a maximum speed of 7.95 mm/s and tenacity of about 10 nm. The findings validated the viability of the dual-servo nanopositioning system based on piezoelectric stacks and flexure hinges (J li et al., 2017). Boudaoud et al. described an amplified voltage/frequency modeling for a multi-DOF sequential nanorobotic device depending on a piezoelectric inertial actuator (Boudaoud, Lu, Liang, Oubellil, & Régnier, 2018).

Light, as a sensory input, has the potential to drive the motion of nanorobots reversibly and wirelessly with high spatial and temporal resolution. In the last decade, there has been a lot of curiosity about light-powered nanorobots (Xu et al., 2017). Huang and coworkers reported a remotely driven and controlled two-finger microgripper employing light-induced distortion smart material to make one of the two fingers (Huang et al., 2016). Wang and coworkers described a light-actuated nanomotor grounded on a single silicon NW. The silicon nanomotor derives energy from light and moves through a process known as self-electrophoresis. Light controlling, as compared to others, theoretically allows

greater independence and versatility because light can be moderated in terms of intensity, direction, frequency, and polarities (Wang et al., 2017).

Acoustics offers a novel and attractive method to produce significant propulsive forces, which could be useful in medical applications (Chen et al., 2018). Acoustic fields were used by Xu and colleagues to trigger the reversible assembly of catalytic nanomotors, coordinated swarm movement, and separation of separate nanomotors. Changing the frequency of the acoustic field demonstrated the controlled movement of the resulting swarm. As a result, the ability of acoustic fields to control the aggregate behavior of catalytic nanomotors holds great promise for a variety of practical applications (Xu et al., 2015). Esteban et al. described acoustically propelled NWs modified with an interfering RNA's (siRNA) payload (Esteban-Fernández de Ávila et al., 2016). An ultrasound field can achieve noninvasive and on-demand motion modulation with a long lifetime and strong biocompatibility when used as an outside energy input to nanobots. Uygun et al. functionalized asparaginase enzyme-propelled NWs made up of Au/Ni/Au/PEDOT-PPy-COOH segments as an important anticancer agent (Uygun et al., 2017).

Several groups have stated that by integrating some approaches for extracting energy from diverse power sources, small-scale robots' propellant thrust could be improved. Li and colleagues developed a magneto-acoustic hybrid nanomotor that can move without the use of chemical fuel in the company of either magnetic or acoustic fields (Li et al., 2015). Both acoustic, as well as magnetic fields, were used to validate the bioinspired rolling motion, a mixture of both fields was capable of circumventing the boundaries of single actuation techniques (Ahmed et al., 2017).

21.4.2 Chemical actuation

Nanorobots employ chemical methods for power generation, which is a common actuation method. Chemically driven nanorobots propel by chemical energy formed by the reaction between fuel and the motor. Depending upon the type of reaction between the fuels [such as hydrogen peroxide (H_2O_2), water, acids, bases, glucose, etc.] and the motor, the force could be generated via the generation of bubbles, concentration and other gradients, etc (Moo & Pumera, 2015). Due to the ease with which H_2O_2 decomposes to oxygen and water, it is widely used in self-propelled systems. The catalytic decomposition of H_2O2 is the subject of much research in the literature on self-propelled nanobots. By adding suitable catalysts such as Au, Pt, and MnO_2 onto the nanorobots, H_2O_2 can be decomposed into water and oxygen for gas propulsion (Chen et al., 2018). Orozco and colleagues described a powerful method for high yielding exacerbated oxidative fumigation of chemical threats which require small peroxide concentrations but no external anxiety (Orozco et al., 2013). Kagan and colleagues used an electrolyte gradient activated by the addition of hydrazine to a hydrogen peroxide (H_2O_2) solution to organize Au microparticles (Au MPs) in separate areas.

The study demonstrated that by altering the catalytic gold surfaces, the shape and size of the Au MP swarms, as well as the formation rate, can be suited (Kagan, Balasubramanian, & Wang, 2011). The use of a cost-effective silver catalyst for bubble propulsion resulted in the development of Pt-free tubular micromotors, as described by Teo et al. (2016). While nanorobots powered by hydrogen peroxide could reach far higher acceleration rates than other motors, hydrogen peroxide is poisonous to organs and tissues in vivo conditions, restricting their use in biomedical applications (Xing et al., 2015).

The use of enzymes opens a completely new approach for promoting biocompatible self-propelled nanorobots for applications in biomedicine because of their relatively higher reaction rate and the variety of enzyme/fuel combinations available. Ma, Hortelao, Miguel-López, and Sánchez (2016) demonstrated the construction and analysis of bubble-free powered cylindrical nanojets (as small as 220 nm in diameter) propelled by an enzyme-triggered biosynthetic method using urea as a source. Because of their one-dimensional (1D) nanosize, longitudinal self-propulsion, and biocompatibility, tubular nanojets are promising for potential biomedical applications (Ma et al., 2016). Gao and colleagues reported catalyzed micromotors that ran on incredibly low chemical fuel concentrations, as low as 0.0000001%. The micromotor is self-propelled at 20 body lengths per second and is based on an iridium hemispheric sheet for catalytic hydrazine decomposition in combination with SiO_2 spherical particles (Gao, Pei, Dong, & Wang, 2014). Chemically actuated nanorobots have a higher swimming speed when the fuel supply is passable and is less expensive than others, but they require a continuous fuel supply in the microenvironment to power (Chen et al., 2018). Wan et al. described a hyperbranched polyamide/L-arginine (HLA) nanomotor. The presented nanomotor uses L-arginine as fuel for the manufacture of nitric oxide, which serves as a driving force as well as providing beneficial effects such as promoting endothelialization and anticancer effects, amongst many additional beneficial by-products (Wan et al., 2019) (Table 21.1).

21.5 Locomotion

If the nanorobot isn't tethered or designed to travel actively around the bloodstream, it'll have to find some way to get about. For its size, the propulsion system must be relatively strong, and it must fly in the direction of blood flow. The locomotion of nanorobots represents the first challenge for robot miniaturization into micro- and nanoscales. As the device's dimensions are reduced, Locomotion is challenged by the low Reynolds number atmosphere and Brownian motion. Inertial and viscous forces are two main forces that a swimmer encounters while swimming in fluids. The Reynold number, that is, the ratio of inertial to viscous forces, is used to determine their relative importance.

$$\mathrm{Re} = \rho.V.L/n$$

Table 21.1 Summary of various actuation system advantages and disadvantages towards theragnostic applications

Actuation system	Advantages	Disadvantages	References
Magnetic field	• Magnetic fields with low strengths are harmless to cells and tissues. • Have powerful penetration as well as they drive from afar • Nontoxic and can also be used to propel biological materials that are alive.	Since these are medical applications involving humans, the protection of high-intensity magnetic fields must be addressed.	Qiu and Nelson (2015); Wang et al. (2021)
Electric field	• Contactless • Less expensive setup	Can exert enormous forces, but only over very short distances	Kopperger et al. (2018)
Light	• Multiple agents may be concentrated and scattered for collective tasks.	When compared to other chemically driven ones, light-driven actuation has slower speeds.	Safdar, Simmchen, and Jänis (2017)
Acoustic field	• Tissue and body penetration are high. • Fast response and long lifetime.	Does not function in the presence of gases.	Diller and Sitti (2013)
Piezoelectricity	• Rapid response • High resolution, natural frequency, and driving force.	Requires the use of on-board elevated power sources	Diller and Sitti (2013); Zhang et al. (2012)
Chemical actuation	• Rapid response with good speed • Less expensive than others	Requires continuous fuel supply in the microenvironment to power.	Chen et al. (2018)

where ρ is the specific mass, V is the characteristic velocity, L is the characteristic length, and n is the particular viscosity.

For the design of an efficient nanomachine, a swimming approach that operates under such low Reynolds number constraints, as well as a navigation strategy to overcome Brownian motion, is needed. Since conventional power supply modules and batteries are impractical at these limited sizes, bioinspired architecture concepts must be used to satisfy the challenging powering and locomotion requirements (Requicha, 2003; Luo, Feng, Wang, & Guan, 2018).

21.6 Motion control and navigation

Controlling nanorobot movement is a vital feature that must be accomplished for realistic applications. Integrating ferromagnetic parts like nickel metal between the platinum and gold segments and thus attaining power by a magnetic field is the easiest method for achieving wireless control (Sanchez & Pumera, 2009). An operator normally directs and guides externally guided nanorobots to their target site by detecting the produced magnetic field, ultrasound imaging, radio waves, X-rays, microwaves, or heat. Multiple ultrasound probes can be used to obtain images from diverse focus planes to monitor nanorobots in numerous planes or three-dimensional (3D) space. Ultrasonic signals are typically used to detect the position of nanorobots and guide them to their intended destination. Ultrasonic waves can be used by physicians to relay information to the patient's body. The signals will either pass through the body or reflect the source of the signal, or both. Doctors may detect ultrasonic signal pulses emitted by the nanorobot using ultrasonic sensors and special equipment. Doctors could keep an eye on the nanorobot's location and guide it to the right spot on the patient's body. Chen et al. recently suggested using two probes to map the location of a two-dimensional (2D) navigated microrobot. Since the 1D ultrasound array probe only captures a cross-sectional image, two US probes are used in tandem to capture the entire 2D motion of the microrobot as it travels along the tank's bottom surface. This multiprobe technique demonstrated low tracking error and can be used to navigate untethered objects in 3D (Chen et al., 2019; Luo et al., 2018). Ultrasound has many drawbacks, including a poor signal-to-noise level, heavy scattering from bones and air pockets, a narrow scanning area for the patient, a limited penetration depth in the human body (approximately a few cm), and low special resolution in consumer instruments (Pané et al., 2019).

By detecting a nanorobot's magnetic field with a Magnetic Resonance Imaging (MRI) system, physicians could trace and monitor it. With this combination of imaging and motion monitoring, vision-based control allows for targeted delivery/therapy in a particular area (Bhore, 2016). According to various scientists, MRI can also be used to wirelessly direct and control magnetic nano-objects within the body, taking advantage of the magnetic field gradients often used for imaging (Belharet, Folio, & Ferreira, 2010; Belharet, Folio, & Ferreira, 2011; Dahmen, Belharet, Folio, Ferreira, & Fatikow, 2016; Tabatabaei et al., 2012). The method faces many difficulties, including the need for different magnetic field gradients for imaging and directing, necessitating the use of a time-dependent multiplexed series. The amplitude of magnetic field gradients in traditional MRI devices is also reduced (Pané et al., 2019).

A radioactive dye may also be injected into the patient's bloodstream to monitor nanorobots. The radioactive dye may be detected using a fluoroscope or other similar instrument as it passes through the circulatory system. The location of the nanorobot will be indicated by complex 3D images. Alternatively, as the nanorobot

passes around the body, it could release the radioactive dye, forming a pathway behind it (Senthilnathan, Bejoy, & Robertson, 2016; Tabatabaei et al., 2016). Nanorobot visualization has become a new method for directing precise sites with high accuracy and performing such therapeutic procedures in a negligibly invasive manner appreciating the enormous advancements in biomedical imaging (Saadeh & Vyas, 2014). Nanorobots can also have a tiny video camera that can view the device's live movement on a screen, making physical navigation by an operative at a console easier. These control and navigation approaches are still in the works (Nistor & Rusu, 2019).

21.7 Classification of nanorobots

For biomedical applications, multiple nanoscale robots with various architectures, types of functionalization, actuation modes, and imaging techniques for localization and response have been described. The key grouping is defined in the following paragraphs.

21.7.1 Nanomanipulators

The most common nanorobotic systems were large manipulator-like structures with nanopositioning capabilities. A nanomanipulator device consists of a massive microscopic probe known as a scanned-probe microscope (SPM), which can expand an image up to 1,000,000 times the size of the original specimen. This interface helps users to manipulate the nanoscale material under investigation by performing operations, movements, and manipulations. A manipulator's basic components are actuations, weighing forces, real-time imaging, and examination under AFM, SEM, or TEM (Mekid & Bashmal, 2019). In general, any microscope can be converted into a manipulator. SPMs like AFMs, is the most commonly used nanomanipulators in practice. SPMs have been employed to allow atom-by-atom modifications to matter. A light microscope can be turned into optical (laser) tweezers to treat a single DNA molecule (Du, Cui, & Zhu, 2006). Eigler and Schweizer demonstrated atom nanomanipulation using an STM for the first time in 1990 (Eigler & Schweizer, 1990). A nanomanipulation device is a robotic technique combined with nanotechnology. The perfect nanomanipulation method will provide high-resolution image feedback and a modular multimanipulator that could accomplish the ultimate objective of automated 3D assembly of nanodevices. Wang et al. suggested the dual-nanomanipulator, an innovative dexterous coordination modulation method for 3D system assembly (Wang et al., 2013). Furthermore, dual nanomanipulators attached to a scanning electron microscope were used to controllably manipulate a single NW (Yu et al., 2013). Carbon Nanotubes (CNTs) have gained a lot of publicity because they have a lot of interesting properties like definite morphologies (Single-Walled Nanotubes or

Multiwalled Nanotubes) and powerful mechanical properties that are unrivaled (Anzar, Hasan, Tyagi, Yadav, & Narang, 2020). CNTs have a variety of uses in nanoelectromechanical systems (NEMS), particularly nanomanipulation systems, due to their unique properties. Wei et al. demonstrated a new approach for conducting in situ physical, electromagnetic, and electro-mechanical experiments on specific single thin CNTs using nanomanipulators within a scanning electron microscope (SEM) (Wei et al., 2010).

21.7.2 Bio nanorobots (DNA and protein-based robotic systems)

In 2003, the word "bionanorobots" was coined to describe all the nanorobotic systems containing nanocomponents founded on biological fundamentals like DNA and proteins, which act at the cellular levels to produce force, motion, or a signal as a nanorobotic component. In an artificially created environment, these nanorobotic components play their intended biological purpose in response to complex physiochemical stimuli. Scientists in the discipline of physics, chemistry, biology, chemical engineering, and biomedicine engineering have already developed molecular methods and materials which could be used in bio nanorobotics. The following are a few well-known references from the literature. Sherman et al. discovered a DNA-based bipedal walking platform and discovered DNA-based nanomachine modules (Sherman & Seeman, 2004). Yurke and coworkers described DNA-based nanotweezers (Yurke et al., 2000). Nanogrippers can grasp and manipulate nanoparticles and can be used within an SEM; but, due to their limited size, nanogrippers still have a high level of difficulty in handling and grasping nanoparticles (Sharma et al., 2008). Prefoldin beta subunit from Thermococcus strain KS-1 (prefoldin 1) was identified by Askarian et al. as a new bionanogripper with applications in capturing and transferring bionanocargos of various shapes and sizes. Despite the fact that such bionanorobots and modules represent major technical advances, they all lack one crucial component: robotics science and architectural engineering, construction, regulation, and preparation (Askarian, Moavenian, & Ghasemi, 2013).

Douglas and colleagues discovered a DNA nanorobot that can deliver cell-borne molecular payloads, since cell surface contributions for contingent, active initiation, and reconfigure its construction to deliver payloads (Douglas, Bachelet, & Church, 2012). Li and his colleagues identified a DNA nanorobot that could hold payloads and send them directly to cancers. According to the researchers, injected subcutaneously DNA nanorobots transfer thrombin to tumor-associated blood vessels and trigger intra-arterial thrombosis, which results in tumor necrosis and cancer progression inhibition. As a result, it seems to be a promising technique for cancer therapy drug delivery accuracy (Li et al., 2018). A group using DNA origami technology proposed a DNA nanorobot for tumor vascular occlusion. DNA molecules are folded to create patterns and shapes (Zhang et al., 2019). Yang and colleagues designed a smart DNA nanorobot that can self-anticoagulate blood in human plasma. The computing heart of the DNA nanorobot appears to be embedded in

molecular reaction cascades, with the model of a barrel-shaped DNA nanostructure. Where there is so much thrombin in the environment, this nanorobot will intelligently sense it and activate an anticoagulation reaction on its own. The development of bionanorobotic systems is still in progress. However, it is obvious that much further work is required to get the two research groups together to collaborate on bio nanorobots (Yang et al., 2020).

21.7.3 Bacteria based nanorobots

Unicellular species, like *Escherichia coli*, *Streptococcus*, etc., hold a unique approach to fluidity. Bacteria-based nanorobotic systems are inspired by how bacteria move in a fluidic atmosphere. Propulsion is provided by a flagellum in these models. The motion of this type of biologically integrated system is usually regulated by electromagnetic fields (Modi et al., 2013). The development of bacterial-based nanorobotic systems can be approached in two ways. The first approach employs live bacteria as a nanorobotic device capable of moving and controlling objects in a fluidic setting. Another method involves making completely artificial bacteria-like nanorobots that are driven by an external magnetic field (Mavroidis & Ferreira, 2013). The first method attempts to exploit the biological engineering that is already present in live bacteria, especially their propulsion flagella motors. In terms of robotics, the aim is to use a swarm of bacteria to passage a slight entity (such as a miniature droplet/bead) forward in a fluidic environment while monitoring the method's speed, course, displacement, and on-demand break and recommence. The goal of the biomimetic approach to designing bacterial-based nanorobotic systems is to build artificial nanoswimmers by copying bacteria's architecture (Martel et al., 2011). By adding a shrill paramagnetic strand to a blood cell, Dreyfus and coworkers stated a microswimmer founded upon the motion of spermatozoa. The swimmer used an oscillating magnetic field to propel the cell by continuous filament deformation, analogous to a eukaryotic flagellum (Dreyfus et al., 2005).

21.7.4 Magnetic nanorobots

Magnetic nanorobots are small robots that use magnetic fields for propulsion and actuation. They are essentially simple nanoparticles made of ferromagnetic content. In magnetically driven nanorobots, an externally employed magnetic field and its inclines can be used to apply a six degree of freedom magnetic force to nanoparticles for actuation and propulsion. Exterior imaging modalities like microscopes or MRI scanners could be used to track and record nanoparticle motion. The MRI method is preferred because it is noninvasive, allows for immediate actuation and trailing of nanoparticles, and can provide accurate magnetic particle localization (Vartholomeos et al., 2011).

Magnetic fields have been widely acknowledged among the numerous techniques; it is recognized as one of the many techniques available for remote

actuation of nanorobots. Because of their versatility and accuracy in monitoring magnetic structure locomotion, as well as their exceptional biocompatibility. A closed loop control algorithm can be used to steer the nanoparticle/nanorobot to the target spot after it has been actuated and detected using external magnetic fields and imaging modalities (Chen et al., 2017a,b). Magnetically guided nanorobots have been reported extensively in the literature. Hamdi et al. developed superparamagnetic nanocapsules (spherical or rod-like) that use magnetic forces to move across the brain-blood barrier (BBB) (Hamdi & Ferreira, 2013). Andhari et al. introduced a multimagnetically driven nanobot by chemically combining magnetic Nanoparticles Fe_3O_4, antiepithelial adhesion molecules antibodies (anti-EpCAM mAb), and multiwalled CNTs (MWCNTs) armed with the chemotherapeutic drug doxorubicin hydrochloride. This multicomponent nanorobotic device architecture reflects a much more popular form of tumor targeting of self-assisted delivery of anticancer drugs for healthcare in rural places (Andhari et al., 2020). In drug research, Bu et al. discovered a new form of cell membrane-cloaked coated magnetic nanogripper with high consistency (Bu et al., 2020).

21.7.5 Applications of nanorobots in therapeutics

Nanorobots have provided an effective platform in the medical sector for humans by enhancing their lifespan. Nanorobotics is a significant component of human health. They help in the diagnosis of diseases in the acute stage. Nanorobotics has been used for the acute diagnosis of diabetes and site-specific delivery of therapeutics for cancer treatment. Furthermore, they are also used in Nanorobots are used in brain aneurysm diagnosis by tracking vessel endothelial injury. They exhibit nonreplicating behavior to reduce reliability and the risk of interfering components. Herein we have explained various applications of nanorobots in medicine:

1. Nanorobots in dentistry

   Nanorobots are used to treat posttraumatic abnormalities, mandibular joint arthroscopy, holes, and operations, in addition to maxillofacial surgery. Nanomachines are used to deliver medications for medical treatment of hypersensitivity, enhancing the lifespan of teeth, and orthodontic treatments to change the tissue for the realignment and flatten an uneven group of teeth (Dalai et al., 2014). Nanorobots in dentistry exhibit several benefits including reliability, safety, and efficient detection and medication of dental ailments (Mitthra et al., 2016). The principal application of nanorobots includes anesthesia and osseointegration for carrying dentine tubules to block tooth hypersensitivity. Moreover, they are also used in the form of vehicles in a solution containing bonding agents, impression materials, nanofillers, and bone repair materials, for nanoencapsulation of drug components into polymeric nanocapsules to produce nanocomposites with sterile and blanching mediators to enhance the polishing of tooth. Polymeric nanomaterials are used in many dental applications. These nanomaterials are

used to form nanocomposites like silk, gelatin, poly (lactic acid), collagen, poly (glycolic acid), poly (lactic-co-glycolic acid), and poly (lactic-co-glycolic acid) (caprolactone). Bioactive glass nanomaterials, electromagnetic nanomaterials, CNTs, hydroxyapatite, silver or gold nanomaterials, graphene oxide, titanium oxide, and silica nanomaterials are examples of inorganic materials used in medicine.

2. Nanorobots in cancer detection and treatment

   Although, cancer has been treated by chemotherapies and various biomedical technologies nanorobots have also reduced the negative consequences of chemotherapies by site-specific delivery of drugs. They work as blood-borne devices and can detect cancerous cells in the acute phase (Freitas, 2005). Researchers have programmed nanorobots for the identification of different types of cancers. Organic, inorganic, and hybrid nanorobots are used in cancer treatment. Nanorobots are constructed from a range of materials, including lipids, resins, microemulsions, quantum dots, metal nanoshells, AuNPs, and CNTs. Chemical nanorobots have a plethora of features, including unique consistency, solubility, and biocompatibility in a variety of environments. Vartholomeos et al.(2011) have reported the use of MRI-guided nanocapsules in designing nanorobots. Magnetic worms have been used in invasive therapy. These nanomachines are inserted into the patient's body as nanoseeds, which are then transmitted to cancerous cells (Rahmer et al., 2017). A helical form of nanorobots is used in adaptive radiation cancer therapeutics. Laser-based nanorobots are used in the detection of cancerous cells.

3. Nanorobots in surgery

   Nanorobots have been used in surgery by inserting them inside the body through the vascular system. Human physicians control the implanted nanorobots, which act as semiautonomous targeted surgeons within the human body (Cavalcanti & Chairman, 2009). Such devices are used in the monitoring of pathology, diagnosis, and treatment of diseases by nanomanipulation (Vanderheiden, 2006).

4. Nanorobots in diagnosis and testing

   Nanorobots have been used to diagnose, test, and analyze tissue and circulatory system functions. They gather and record vital data from various parts of the body, including temperature, strain, and chemical constituents. To diagnose a stomach infection, nanorobots were used.

5. Nanorobots in gene therapy

   By corresponding to the molecular structure of DNA and proteins present in a cell to a reference structure, nanorobots are used to treat genetic diseases. Any anomalies can be corrected by making the desired improvements.

6. Nanorobots in arteriosclerosis

   Nanorobots help in reducing infection by removing rubbish from wounds (Hamdi & Ferreira, 2009). They are more efficient for puncture wounds that

are difficult to treat by conventional systems. Microrobots prepared from calcium carbonate are used in the delivery of thrombin to interrupt the bleeding of lesions in mouse models. Locomotive microrobots are used in wound sealing using a laser. On increasing the temperature, laser-microrobots generate site-specific collagen denaturation and melting. Furthermore, the resulting drop in temperature allows for wound healing and condensation (He et al., 2016)

7. Nanorobots in real-time imaging

   Nanorobots are used in real-time imaging. Approximately, 75% of biohybrid nanorobots integrated with fluorescence are used in real-time imaging (Akin et al., 2007; Din et al., 2016; Park et al., 2013). Furthermore, physical nanorobots are associated with various imaging approaches; however, about 60% of the literature supports real-time imaging. Techniques like endoscopy and X-rays are used in the detection of microgrippers in the gastrointestinal tract of the digestive system (Gultepe et al., 2013), application of a visual camera in the visualization of drive in the eyes (Priya et al., 2016; Ullrich et al., 2013) while fluorescence imagery approaches are used for tracking the location of microrobotics in the peritoneal pit of the mouse (Servant et al., 2015). Besides these, dual imaging techniques are used in the detection of biodegradation of magnetic microhelix nanorobots in mice. Microgrippers in the gastrointestinal tract is examined utilizing endoscopy and X-rays (Gultepe et al., 2013).

8. Nanorobots in respiratory diseases as artificial oxygen carrier

   Nanorobots are used as respirocytes. These are synthetic mechanical red cells used as imaginary nanorobots which are present in the bloodstream. They are small pressure tanks that pump respiratory gases that is oxygen and carbon dioxide (Soto and Chrostowski, 2018). These gases are emitted in a controlled manner from the small tank. Externally, respiocytes are connected with gas sensors. So, nanorobots move towards lung capillaries where the partial pressure of oxygen is more than carbon dioxide. Therefore, the computer sends the signal to the sorting rotors for loading the tank with oxygen and releasing carbon dioxide. On the other hand, when the partial pressure of carbon dioxide is more than the computer indicates rotors release oxygen and absorption of carbon dioxide. Thus, respirocytes enhance the functioning of natural hemoglobin-filled blood cells and transport More oxygen per unit volume by 236 times as likened to normal red blood cells.

9. Nanorobots in kidney disease

   Nanorobots have been employed in the breakdown of kidney-stones using shocks caused by ultrasonic waves. Internally, kidney-stones are broken by the ultrasonic frequency however sometimes they show inefficiency. Nanorobots use a laser for breaking kidney stones into small pieces (Wang, 2009).

10. Nanorobots in inflammatory response

    Microscopic robots have been used in the healing of injured tissues. They show quick response and work efficiently with white blood cells for healing injured tissues without leaving marks (Casal, Hogg, & Cavalcanti, 2003). However, nanorobots have shown high susceptibility to digestion with phagocytic cells. Nanorobots are programmed to avoid digestion with macrophages, phagocytes, and lymphocytes (Freitas, 1999).

11. Nanorobots in atherosclerosis

    Nanorobots have been used for the treatment of atherosclerosis (Nistor & Rusu, 2019). Nanomachines are located in stenotic vessels. Nanorobots loaded with the therapeutic agent are used to prevent the infection and healing of injured tissues by releasing therapeutic components at the target site. Nistor and Rusu (2019) have reported the significance of polymeric nanoengineered particles of collagen type IV for releasing anti-inflammatory drugs. Moreover, in vivo investigation in mice revealed the repairing of damaged arteries (Fredman et al., 2015).

12. Nanorobots in the treatment of parasitic diseases

    Nanorobots have provided an effective platform for the treatment of bacteria and small-scale parasitic organisms. Berry and Saraf (2005) have developed nanorobots using spherical-shaped nanomachines for killing parasites by electrical shock. Synthetic micromotors were developed using poly(3,4-ethylenedioxythiophene)/zinc for dissolving gastric acid to release loaded therapeutics viz. antiparasitic drugs, antibiotics, and many more (Gao et al., 2015).

13. Nanorobots in ophthalmology

    Nanorobots have shown tremendous several applications in ophthalmology for monitoring, control, repairing, and defense to improve the function of the eye. They are also used to prevent wound formation after glaucoma surgery, regulate oxidative stress, evaluate intraocular compression, prognostic, drug delivery for choroidal new vessel treatment, and promote healing processes. Furthermore, employing the use of DNA and ocular prosthetics, are used in the treatment of retinal degenerative disease. Treatment of the eye by means of nanorobots is similar to other organs by injecting the therapeutic agents into any part of the body however released at the target site that is, the eye (Nikalje, 2015). Many intravitreal implants are developed and clinically investigated. These nanorobots show similarity to reservoir implants of silicon, poly(vinyl alcohol), poly(vinyl alcohol, or ethylene vinyl acetate that are injected into the ending part of the eye. These reservoirs show solubility with the drug's lipid contents (Nistor & Rusu, 2019).

14. Nanorobots in tissue engineering

    Nanorobots have shown many advantages in tissue engineering such as replacing the damaged cells, rapid healing, minimizing complications, preventing offensive surgery, and site-specific delivery of target therapeutic components (Nistor & Rusu, 2019). Depending on the custom, Nanorobots

can also be used in their natural state or by adding proteins, medications, or biomolecules to the board. These modified nanorobots have a wide surface area and have been used as analytes for various agents. Furthermore, they demonstrate enhanced rapid absorption and diffusion skills.

15. Nanorobots in cardiovascular diseases

    Nanorobots are used for the prevention of heart attacks. They are used in the elimination of yellow fat bodies in blood vessels. Consequently, increase the flexibility in the walls of blood vessels. Nanorobots have been used in the treatment of cardiac ailments and have shown futuristic promises in the treatment of defective heart valves, detection of arterial plaques, and understanding of the process of the heart in both healthy and damaged organs at the subcellular level (Nistor & Rusu, 2019). Research is going on for the development of such nanorobots that show movement similar to the microorganisms in the bloodstream. Moreover, nanorobots integrated with sensors can be used to assess the factors like heat and chemical concentration in the blood (Palagi et al., 2017; Priya et al., 2016). Nanobots are used in the targeted delivery of therapeutics like lovastatin, simvastatin, etc., for the treatment of cardiovascular diseases (Wang, 2009).

16. Nanorobots in drug delivery

    Nanorobots have shown proven applications in drug delivery. They help in the precise and site-specific delivery of drug components. Moreover, they also increase the surface area and minimum adverse effects (Sokolov et al., 2017). Nanorobots use DNA nanostructures as drug vehicles because they exhibit unique biocompatibility, improved permeability, and release of drugs in a regulated manner. These drug vehicles can be inorganic or polymeric shells. Magnetic helical robots, which can navigate in 3D in liquid, are among the inorganic mobile nanorobots. Site-specific therapeutics, invasive surgeries, remote sensing, and cell recombination and analysis are some of the applications for these inorganic nanorobots (Qiu & Nelson, 2015). On the other hand, polymeric nanorobots are biodegradable, subtle to several limits like heat, pH, enzymatic attack, acidity, and many more (Vasile, 2019). Polymeric nanovehicles with several applications in drug delivery comprising poly (ethylene oxide), poly (aspartic acid), poly (ethylene glycol), poly (aspartate-hydrazone)-based copolymers, sensitize particles with pH and/or temperature-responsive polymers such as poly (acrylic acid), poly(N-isopropylacrylamide), and block porosity. [poly(styrene)-co-poly(vinyl benzaldehyde)]-block temperature-dependent amphiphiles, block polymers based on silk-elastin protein, poly(lactide-co-glycolic acid) nanoparticles covered with sgc8 aptamer, sphere micelles based upon poly [oligo (ethylene glycol) methacrylate]-block-[poly(styrene)-co-poly(vinyl benzaldehyde)] block polymers that could show alteration in shape to rods as the degree of polymerization blocks is increased, hybrid polymer_lipid amphiphiles such as one based on hydrophilic poly(N-methyldietheneamine sebacate) attached with N-(2-bromoethyl) carbamoyl cholesterol, etc (Yu et al., 2016).

21.8 Conclusion and future prospects

Nanorobots have shown exemplary applications in medicine for the betterment of human life. They are innovative and compatible for handling and analysis of vital diseases. Respirocytes have shown 236 times more efficient than normal red blood cells. Moreover, nanorobots have been used in the cure and diagnosis of different diseases including Cancer, heart attack, asthma, arteriosclerosis, kidney stones, and other diseases are among the most common. They are also used in the site-specific delivery of drugs and tissue. Despite the tremendous applications of nanorobots, there is a further need to resolve various issues of nanorobots in practical clinical applications. There is an incongruity between the aims of therapeutic nanorobots and actuality. Moreover, micro/nanostructure engines should be improved in terms of material biocompatibility and degradation, to address toxicity issues. Future efforts should also be focused on proper standardization to elucidate the merits of nanorobotics over conventional and modern therapeutics that at present satisfy FDA criteria. In addition, these futuristic nanorobots must be cost-effective, societal, and ethical insinuations for using nanorobots in the clinical sector.

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Conflict of interest

The authors of this chapter declare no conflict of interest.

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CHAPTER

Nanoghosts for therapeutic applications 22

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22.1 Introduction

There are many therapeutics having significant pharmacological actions against specific diseases or pathologies but their administration in the native form is usually accompanied by lots of drawbacks such as instability, restricted distribution, and biological barriers to penetration with limited targeting capabilities.

Effective, less iatrogenic, and safe therapies became an urgency in targeting drugs and a motivation to evaluate the setup of different therapeutic carriers. Most novel conceived carriers are designed to owe better functional specifications including; better biodistribution, biocompatibility, and selective targeting (Gagliardi, 2017).

Administration of nanodrugs or therapeutic molecules in their free form was found to face some obstacles such as rapid clearance or breaking down into inactive metabolites or even toxic ones. The therapeutic action of free drugs may also be hindered due to the inability to permeate through the biological barriers as the blood-brain barrier and its random distribution all over the body tissues which may cause serious hazardous effects, especially in the case of drugs with narrow therapeutic index. The results of all these hurdles facing free drugs led to their poor bioavailability, especially in the case of chemotherapy where the location of the tumor and the mechanism of resistance that may arise against the drug when systematically administrated are considered to be critical issues that must be carefully addressed (Mitragotri, Burke, & Langer, 2014). The need for targeted delivery of therapeutic drugs became an urge to overcome all these challenges facing efficient therapeutic outcomes. Active targeting methodology was suggested as a solution by modifying the surface of the nanoparticles with some targeting ligands including oligonucleotides, antibodies, aptamers, and peptides (Valencia et al., 2011). Active targeting using these targeting ligands is a little bit complicated and could lose their reproducibility and efficiency with the progress of tumors (Rosenblum, Joshi, Tao, Karp, & Peer, 2018). The nanovesicles or nanocarriers delivery platform presents another approach for

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drug-targeted delivery due to their features that could be fine-tuned or customized to enhance permeability, retention, and accumulation in the desired sites without any adverse effect on the payload stability.

The synthetic nanovesicles obtained for example by coating or modifying the surface of nanoparticles with polyethylene glycol coating have some advantages including longer circulation time due to particles stabilization and protection against phagocytosis (Alexis, Pridgen, Molnar, & Farokhzad, 2008; Luk & Zhang, 2015) to obtain sustained systemic effects and enhanced targeting through active and passive mechanisms, unfortunately, PEGylation process was not able to completely protect the synthetic nanovesicles from opsonization by the reticuloendothelial system (Saadati, Dadashzadeh, Abbasian, & Soleimanjahi, 2013). Because of several synthetic vesicles draw backs, efforts have been recently directed towards miming nature through generating cell-based nanocarriers also known as biomimetic nanovesicles or nanoghosts (NG).

Nanoparticle-based systems for drug delivery were recently thoroughly studied and widely used as homing systems to preserve the pharmacological actions of unstable therapeutically effective molecules and protect them against physiological environments. Nanoparticles delivery systems are also associated with enhanced bioavailability and decreased disposition in healthy tissues owing to functional ability and customizability (Gagliardi, 2017). However, the foreign nature of synthetic nanoparticles makes them easy prey to the immune system facilitating their recognition and clearance (Li et al., 2020). The biomimetic drug delivery approach was also found to be one of the best approaches to improving most of the functional properties. In this approach using different cells, biological molecules, organisms, and pathogens (bacteria/viruses) or vesicles inspired by them is considered to be a progressive step toward the fabrication of optimum drug carriers. Thus, for the sake of achieving drug delivery with maximum efficiency and least rate of clearance, more prolonged circulation time, and homotypic targeting, designing, and fabrication of different biomimetic nanoparticles offered the best solutions (Gagliardi, 2017; Li et al., 2020). Camouflaging nanoparticles with membranes derived from natural cells or biomimetic nanotechnology was widely applied recently in delivery systems of different therapeutics where they combine the superior physicochemical properties of nanoparticles and intrinsic functionalities of cell membranes resulting in the most efficient delivery and targeting for both diagnostic and therapeutic purposes (Chen et al., 2020; Li et al., 2020). These Biological therapeutic carriers and/or different cell-based platforms for delivery and targeting are known as NG. NG are not only restricted to drug delivery but have been also used for genetic materials and enzymatic systems delivery to specific tissues and organs for treatment of various diseases including malignancies, HIV, inflammations, infections, cardiovascular and Parkinson's diseases, and in gene therapy.

Therapeutics based on cells or cell membranes are considered to be promising methodologies to address or approach complicated medical conditions and needs, not only drug delivery but also genetic engineering, regenerative medicines, and

bioimaging and theranostic applications. To enhance the efficacy and improve the functionality of therapeutics based on cells, different strategies were also developed for modification of the cell membrane surface through surface engineering either genetically or nongenetically via utilizing some artificial receptors, multifunctional nanoparticles, and a variety of therapeutic moieties. Due to sophisticated steps and procedures in addition to possible associated hazards that may accompany the genetic engineering strategy, the nongenetic one has gained more popularity for potentiating the functionality of cell-derived or inspired therapeutics. The nongenetic engineering modification methodologies include both Covalent chemical conjugation and physical noncovalent bioconjugation (Liu, He, & Liu, 2019a).

22.2 Formation of nanoghosts

The first step to obtaining cell membranes in a purified form is collecting the source cells.

1. culture cells are detached through trypsinization or scraping from their culture plates or flasks and then cycles of sequential centrifugations are performed.
2. blood cells are collected through centrifugation directly.

The second step is breaking the cell membrane which could be achieved through different methods either alone or in combination

1. with hypnotic lysing
2. cycles of freeze-thaw
3. mechanical forces.

The cellular debris is then removed through ultracentrifugation cycles.

It is worthy to mention that collecting cell membranes of cells with internal organelles and nuclei is much harder and requires additional steps of separation in comparison with cells without internal organelles (Fig. 22.1).

The separated cell membranes could be utilized directly or used to form vesicles. The coating is then performed either through sonication, coextrusion with the core nanoparticles or drugs, or through microfluidics electroporation which exhibited colloidal stability improvement.

22.3 Characterization of nanoghosts

For the characterization of biomimetic nanoparticles, various techniques could be used.

1. Transmission electron microscope imaging: detecting the size and shape of NG and visualizing the lipid bilayer is considered to be a sign of successful coating.

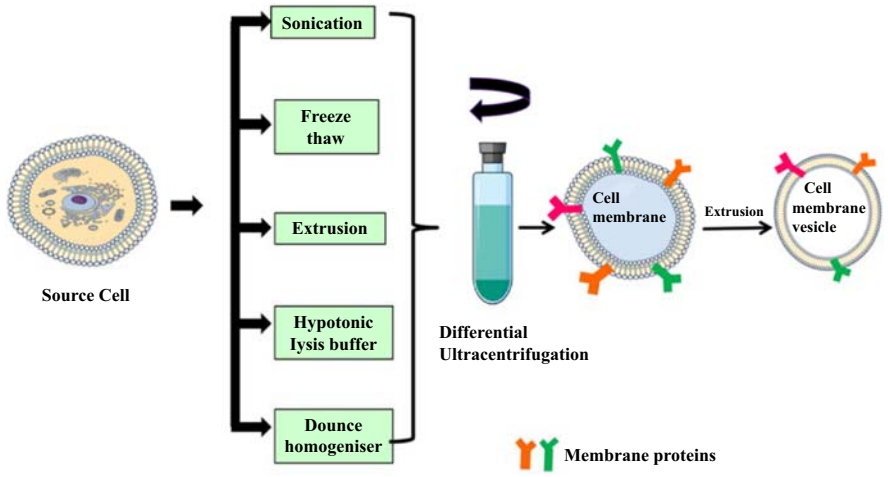

FIGURE 22.1

A diagram of different methods for isolation of the cell membrane from natural cell sources (Vijayan, Uthaman, & Park, 2018).

2. fluorescent microscopes: investigating fluorescently labeled lipid bilayers, fluorescent-labeled nanocores, and their coatings.
3. Dynamic light scattering: predicting size and distribution of sizes
4. Zeta potential: demonstrating the surface charge of NG.

The assessment of protein retention in cell membrane after the coating process could be performed via

1. Bradford protein assay: quantifying proteins
2. Gel electrophoresis: predicting proteins type
3. Western plot (with specific antibodies): detection of certain proteins.

22.4 Source of cell membranes

The cell membranes may be extracted from cells with or without nuclei, the latter is used on a wider scale where obtaining their cell membranes is much easier as the amount of debris is lesser than in the case of cells containing nuclei. A variety of cells could be utilized as a natural source for extracting membranes to be used as a coating for different kinds of nanoparticles. Each type of cell membrane can employ unique features to present desirable functionalities to coated cargos or nanoparticle cores whose composition could also be varied according to the intended application (Fig. 22.2).

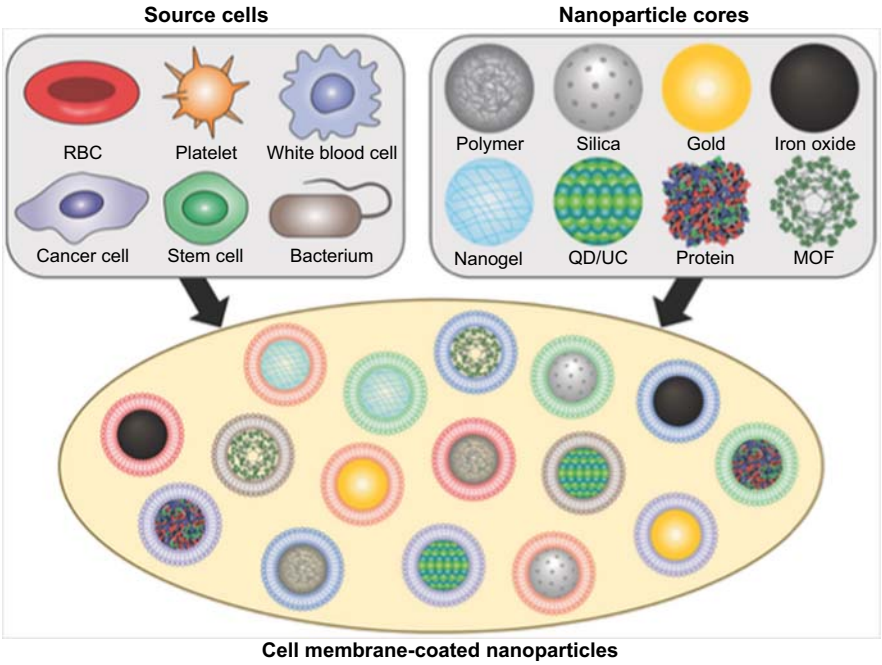

FIGURE 22.2

Different types of nanoghost according to the source of the cell membrane (Fang, Kroll, Gao, & Zhang, 2018).

22.4.1 Nuclei noncontaining cells

There are two predominant types of cells without nuclei originating from blood, erythrocytes (ERT), and platelets (PT). The membranes of these two cells, when used for coating, introduce some unique properties to the biomimetic nanoparticles. For example, membranes of RBC can increase the times of blood circulation while those of PT enhance the targeting of malignant and inflamed tissues.

1. ERT could act as carriers for drug delivery (Gupta, Patel, & Ahsan, 2014) and have different privileges as biocompatibility, biodegradability, protection its cargo, or bioactive substances from proteases in the plasma, and escape immune system. The desirable therapeutic effects of coated or encapsulated drugs could be increased while eliminating undesirable ones by preventing the occurrence of allergic reactions and controlling blood levels of the free drugs. The therapeutic action is usually extended because of the gradual drug release. ERT is also capable of operating as bioreactors when loaded with enzymes. The incorporated enzyme acts on a specific substrate and eliminates it from the blood which may open the door in front of managing disorders of inborn

enzyme deficiencies. ERT NG are considered to be promising tools for diagnosis as contrast agents for magnetic resonance imaging where their life span in the bloodstream may reach 12 days due to the prevention of phagocytosis and opsonization. The long circulation time of carrier ERT allows for long-term imaging and monitoring of spleen, liver, and cardiovascular disorders. They were also used as noninvasive biosensors to track different blood analytes and parameters upon their loading with fluorescent dyes which give proportional responses in relation to the concentration of the studied analyte. Recently a new technology named red cell therapeutics has evolved, combining the advantages of erythrocyte NG and genetic engineering, which could be used in enzyme replacement therapy, cancer immunotherapy, and autoimmune disorders. A new method was developed for the synthesis of nanoerythrocyte ghost utilizing shear force. Membranes of hemoglobin-depleted ERT nanoghost were produced through disruption using mechanical shear forces in conjugation with a tissue homogenizer based on the rotor-stator. The novel proposed method has advantages of easy setup, good control of sterility control, time-saving, and decreased inter-batch variability or reproducibility in comparison to the extrusion conventional method. Nanoerythrocyte ghosts produced by the shear force disruption method were found to be stable with respect to size, count, morphology, and functionality. The ERT NG produced by the novel proposed method displayed less influence on centrifugal stress, colloidal stability, hemolytic potential, and turbulence shock. In addition, the newly suggested setup permits the production of sterile ERT NG simultaneously with extended shelf storage life, homogenous distribution of size, and better stability. The processing of highly concentrated samples allows the shear force technology to be a promising and reliable approach for the production of nanoERT ghosts on large scale for industrial applications (Capossela et al., 2020).

2. PT inspired NG or platelet membrane biomimetic delivery systems exhibit enhanced in vivo retention due to their ability to escape macrophage uptake and minimized immunogenicity (Wang et al., 2019). They also have an intrinsic affinity toward inflamed tissues and homing affinity to ischemic heart tissues, thus could improve targeting and enhancing the regeneration in these sites. It was also found that platelet NG could contribute to enhancing the efficacy of photodynamic therapy in tumors and eliminate damage to the skin by reducing the dose of irradiation (Xu, Gao, Fan, & Yang, 2018). It was demonstrated that carrier PT inherently release their load faster in acidic media enabling them to be more selective to malignant tissue (more acidic) than normal ones. In addition, loading coating antitumor therapeutic with platelet-derived membranes was found to hinder metastasis (Du & Chen, 2020; Lutz, Hu, Dinh, & Cheng, 2019; Zhang, Liu, Wei, & Nie, 2018). Several technologies were developed and used for characterization and loading different bioactive substances and therapeutic agents to ERT and PT (Du & Chen, 2019).

22.4.2 Nuclei containing cells

They have the advantage of keeping their genetic data base preserving their ability to divide and proliferate. Thus they could be easily cultivated and produced on large scale but the other hand separating and purifying their cell membranes are more difficult and require additional steps due to the extra cellular constituents. Nuclei-containing cells include stem cells, cancer cells, and white blood cells.

White blood cells or leukocytes are considered to be an important part of the immunity system and have a significant role in the inflammatory cascade and clearance of foreign invaders. They also share some physical properties with malignant cells. Thus they could be related to various infections, diseases, and tumors. Leukocytes and their membranes are considered to be promising candidates for encapsulation and coating for delivery and targeting specific sites (Yu, Yang, Li, Xu, & Sun, 2020).

Macrophages: Examples of White blood cells used in the fabrication of NG are macrophages where they have a unique ability to cross the blood-brain barrier as well as homing capabilities allowing them to be promising candidates for targeting neurodegenerative diseases, inflammatory conditions, and malignancy (Liu, Luo, Chen, Liu, & Chen, 2019b; Lutz et al., 2019). Reprogramming of macrophages via nanoghost synthesis from the membrane of polarized macrophages, via cytokines and chemokines bound to the surface, was recently investigated and found to be helpful in wound healing (Hwang et al., 2020).

T-cells: Another type of leukocyte used as the carrier for delivery and targeting is the *T-cells* that owe a peculiar inherited property of specificity as each T-cell presents receptors to only one kind of antigen enhancing targeting to specific sites. Their specificity and receptors could also be modified via repurposing or reprogramming and have access to different types of tumors (Lutz et al., 2019).

Neutrophils: naturally originate from the bone marrow and are the most abundant among the leukocytes. They are characterized by their fast transmigration to inflamed tissues, high abundancy compensates for their short life span, and rapid increase in number in short duration in response to inflammation. Cytomembranes of the activated neutrophils have a chemotaxis behavior which helps them in exerting the antiphlogistics function by tracking chemoattractants and cytokines released in case of inflammation or injuries (Xuan, Shao, & Li, 2019). All these features enhance the ability of carrier neutrophils to be promising delivery and targeting systems. In addition, neutrophils have a natural response towards released chemokines at places where surgical removal of tumors occurs, this inherent feature enabled neutrophil NG to be ideal transporters to nanochemotherapeutics aiming at suppression of recurrence of tumors after their surgical removal (Huang, Gao, & Chen, 2018).

Dendritic cells are considered to be the messenger between the innate and adaptive immune system functionally, their natural habitat is the tissues usually subjected to the outside environment as the intestines, stomach, lungs, nose, and skin. Dendritic cells' action is accomplished via expressing foreign antigens on their outside surfaces to attract lymphocytes.

Stem cells: are known to be universal self-renewable cells capable of differentiating into different types of cells to perform a predetermined biological mission and are necessary for the regeneration and repair of tissues in addition to tumor tropism properties. Its cell membrane exhibits hypo immunogenicity giving them the power to migrate to and target inflammatory sites and malignant cells at different stages of development (Huang et al., 2018). The membranes of adult stem cells as mesenchymal stem cells are most used widely used in designing and fabricating NG with a unique delivery and targeting capabilities. Stem cells could be easily collected from patients directly or cultured, expanded, and harvested in vitro. Under controlled conditions, their differentiation could be induced into a specialized type of cells. Stem cell nanoghosts are currently used in the management and treatment of different diseases including cardiovascular, neurological, and malignancy (Yu et al., 2020). Recently a small cancerous cells subpopulation was explored and known under the name of cancer tumor-initiating cells or cancer stem-like cells which possess properties similar to those possessed by stem cells as self-renewing and differentiating capabilities. This subpopulation was believed to cause the overall cancer cells heterogeneity and its enrichment leads to regrowth of tumors as well as they exhibit cytotoxic agents as chemotherapy resistance. It was suggested that there is a link between mesenchymal stem cells and tumor-initiating cells enrichment, especially in desmoplastic pancreatic malignancy and lung cancers where drug accumulation is difficult to be achieved. Efforts have been exerted to synthesize NG produced from mesenchymal stem cells based on the fabrication of nanovesicles derived from their cytoplasmic membranes to preserve the targeted delivery abilities of bioactive therapeutics to different kinds of tumors with optimum safety and effectiveness (Timaner et al., 2018). Stem cell nanoghost-based therapy could also be administered concurrently with conventional chemotherapeutic agents for tumor recurrence delay with improved pharmacokinetics and specificity towards anticancer drugs. Mesenchymal NG were also approved for the management of degenerative arthritis due to immune evasiveness and anti-inflammatory capabilities (Gao et al., 2016a).

Cancer cells: The wide use of cell membranes from various sources of cancer cells for the synthesis of nanoghost is due to homologous targeting allowing them to be successful in the treatment and imaging of different types of tumors. The retaining ability of cancerous cell membrane to the antigens required for immune system activation enables them to be candidates for cancer vaccination. Besides homologous binding ability, cancerous cell membrane owes various unique properties, such as their potential for limitless replication, death resistance, long time of circulation and escaping immune system as well as antigenic diversity, allowing them to be a promising therapeutic and theranostic platform (Xuan et al., 2019).

Bacterial cells: bacterial nanoghost synthesis and using the bacterial protein shells in conjugation with antibodies and nanoparticles with different functionality or applying coating strategy with bacterial membrane offers a novel perspective as a system for bio hybrid therapy in delivery and targeting of drugs genes, RNA,

cancer imaging and treatment and immunization. Bacterial ghosts can retain the bacterial immunogenic properties as the inner proteins of the membrane and lipopolysaccharide. The retention of these immunogenic properties stimulates both humoral and cellular immunity through initiating the pro-inflammatory cytokines production and antimicrobial peptides expression. The bacterial ghosts also possess intrinsic bioadhesive features and could enclose biomolecular payloads within their membranes making them promising delivery and targeting vehicles for different therapeutic cargos. In addition, some bacterial ghosts can be used as vehicles for immunization (Farjadian et al., 2018). They have the advantages of easy production on large scale via a fermentation process, lyophilized bacterial nanoghost could be stored for a long time at ambient temperature and regarding enzymatic reaction, they are capable of serving as bioreactors. Bacterium-based nanosystems have the potential of surviving anaerobic media and travel across oxygen taxis heading to anoxic tumors. They also display noticeable stability in the physiological habitat and the immunogenic antigen bound to the membrane potentiates the action as an antibacterial vaccine promoting the response of adaptive immunity against invasion (Xuan et al., 2019).

Beta cells: constitute more than half the cells of the pancreas and are responsible for the secretion of insulin, thus the use of membranes extracted from Beta cells is mainly under study for the management of hyperglycemia. Coating therapeutic nanofibers with their membranes and the preserved membrane proteins contributed to prolonged survival, enhanced proliferation, and increased insulin secretion in comparison to noncoated nanofibers (Jiménez-Jiménez, Manzano, & Vallet-Regí, 2020).

Fibroblasts: the membranes extracted from fibroblasts are utilized for coating nanoparticles aiming at treating different diseases and were recently used for managing diabetes mellitus (Tan et al., 2019). In addition, coating nanoparticles with a semiconducting polymeric nature permit tumor-associated fibroblast targeting (Tan et al., 2019).

Endothelial cells: play an important role in maintaining the health of vasculature. Nanoparticles with different functionalities covered with membranes extracted from endothelial cells have wide applications as were internalized well and the release was controlled by starvation. Targeted delivery could be achieved through coating magnetic nanoparticles that are directed to specific sites using magnetic fields (Jiménez-Jiménez et al., 2020).

22.5 Approaches for surface chemistry

The surface membranes surrounding the biological cells are highly complicated consisting of complex lipid bilayers and well-organized surface glycated proteins that can send and receive signals which are critical and essential for the regulation and direction of cell functions in healthy and diseased conditions. In general,

there two main approaches could be adopted for developing biomimetic therapeutic nanoparticles, especially those that mimic or simulate the cell surfaces. The first approach is the Bottomup approach which commences by separating molecular components and putting them together and building them up into bigger structures through chemical and physical methods. The bottomup approaches involve different methodologies and techniques such as self-assembly and microfluidics to construct the essential molecular cell components including the lipids and glycated surface proteins. This approach is adopted for designing and fabricating synthetic cell membrane vesicles where the lipid bilayer is incorporated synthetically via surface functionalization technique or through the formulation of the proteins with the aid of lipid linkers and putting them together during the step of particle coating. On the other hand, the second Top-down approach includes print technology or the most widely used methodology depending on the utilization and reformulation of macroscale biological entities. For example, NG from natural sources are fabricated by harvesting the cell membranes from the biological cells followed by their addition with the therapeutic core particles or cargoes. Since this chapter is dealing with the fabrication of nanoghost and biomimetic nanovesicles from natural cell sources, the Top-down approach will be discussed in short (Meyer, Sunshine, & Green, 2015).

22.5.1 Top-down approaches

NG or natural cell membrane coated nanoparticles are being recently studied and explored rather than the synthesis of fully artificial membranes from synthetic constituents. The main concept is based on harvesting the biological surface cell membranes followed by their purification. The purified cell membrane is used to coat the therapeutic cargos, nanodrugs, genes, RNA, and various other nanoparticles (Meyer et al., 2015) (Fig. 22.3).

Red blood cell NG: present many privileges as their extreme prolonged circulation time, can squeeze through the capillaries where their discoid shape offers them flexibility. The surface membranes of red blood cells are obtained through hypnotic lysis followed by centrifugation for purification. The vesicles are then created through physical extrusion or sonication for sealing using defined pore size filters. The obtained red blood cell vesicles known as resealed ERT can then be directly loaded or used for coating therapeutic drugs, usually those hydrophilic (Hirlekar, Patel, Dand, & Kadam, 2008; Zarrin, Foroozesh, & Hamidi, 2014). In another fabrication method, red blood cells camouflaged nanoparticles with longer half-life are generated by mixing the preformed vesicles with nanoparticle cores allowing for their fusion together. The fusion is followed by repeated extrusion of the mixture using polycarbonate filters. The latter method offers a uniform coating to the cargo and keeps nearly all the surface proteins presenting them in the correct orientation (Hu et al., 2013) (Fig. 22.4).

Leukocyte NG utilize their surface interactions for responding to tissue infections or inflamed endothelial tissue. White blood cells surface membrane was

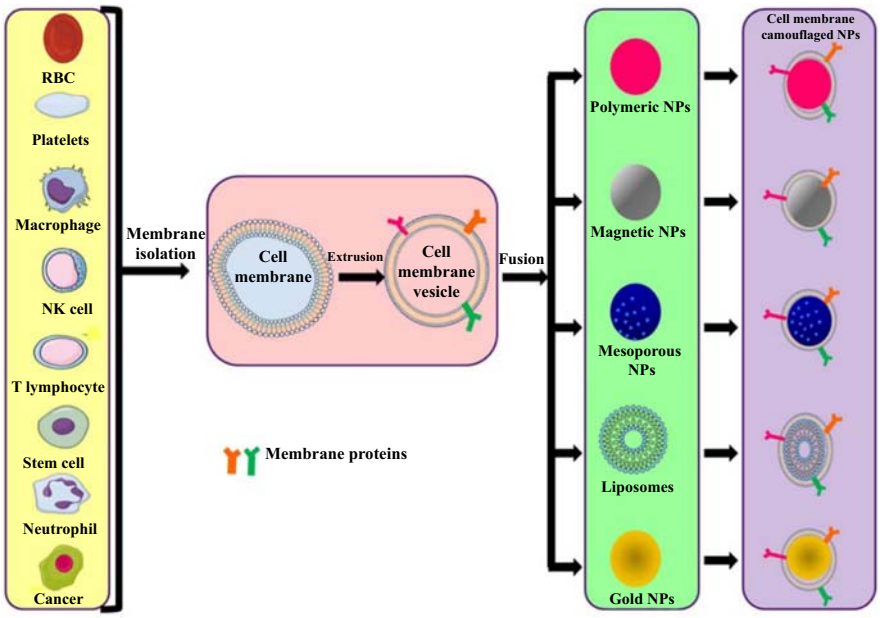

FIGURE 22.3

Isolation and extrusion of cell membranes from different cell sources to create vesicles that could fuse with various core nanoparticles to form nanoghosts (Vijayan et al., 2018).

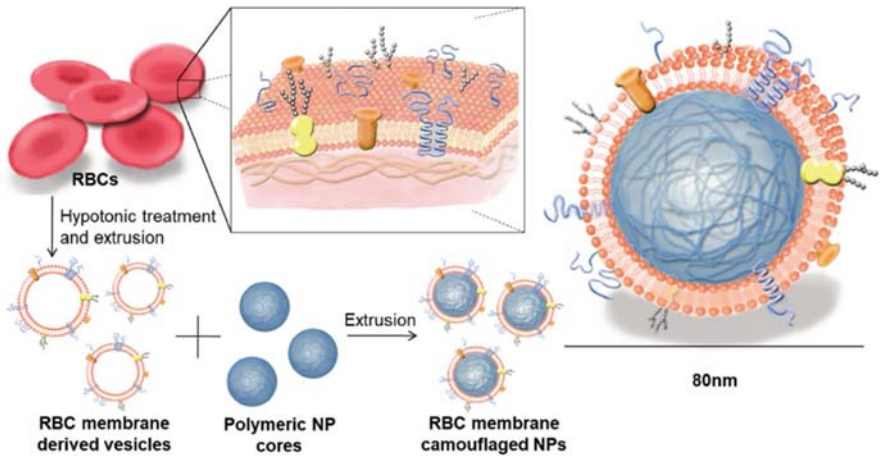

FIGURE 22.4

Red blood cell membrane-based-nanocarriers for nanoparticles coating (Fang et al., 2018).

used for encapsulation of nonporous silica also termed leukolike vectors, as discussed above, have the same behavior and could perform the same functions of leukocytes as binding to and transporting nanotherapeutics through inflamed endothelial tissue, escape clearance by the immune system (Parodi et al., 2013). The same concept applies to NG obtained from other cell sources such as PT, mesenchymal stem cells, and cancer cells.

22.6 Application of nanoghosts

NG have different biomedical applications starting from delivery and targeting going through immune induction or vaccination and biosensing applications (Fang, Jiang, Fang, & Zhang, 2017).

22.6.1 Delivery and targeting

The most widely explored and studied application for biomimetic nanocarriers or NG is targeted drug delivery. Owing to their natural origin, they display low immunogenicity and inherited biocompatibility and are best suited to different biological applications since they are tailored from the plasma membrane of cells originally present in mammals. The membrane surface proteins are crucial for their intended functionality and their absence may abort the accomplishment of their planned mission (Jang et al., 2013). For example, NG derived from ERT via the extrusion process, also called nanoerythrosomes (Désilets, Lejeune, Mercer, & Gicquaud, 2001; Lejeune, Moorjani, Gicquaud, & Lacroix, 1994) was employed for photoactivatable agents delivery for photo-thermal therapy and bioimaging, perfluorocarbons for theranostic applications and blood pressure lowering drugs (Bahmani, Bacon, & Anvari, 2013; Gupta et al., 2014; Hsieh et al., 2015) as well as tumor targeting. Nanoerythrosomes also could acquire additional targeting specifications upon functionalization of their surfaces with antibodies (Mac et al., 2016). Stem cells are a famous source for NG synthesis due to their prominent targeting capabilities to malignant sites. the mesenchymal stem cell-based NG were used to encapsulate a cargo of pro-apoptotic protein to be employed in the treatment of prostate cancer and gene delivery (Kaneti et al., 2016). In addition, Machluf and her team are trying to create a new therapy for corona virus, and trials are made to attract and entrap the virus resulting in infection with less severity.

Different leukocytes were also recruited to generate NG with peculiar functions as nanodelivery vehicles. One application is based on fusing the leukocytes plasma membrane with some synthetic lipids to create nanocarriers termed leukosomes having the capabilities of delivering dexamethasone to cure inflammation of vasculature (Molinaro et al., 2016). The ability of neutrophils to express integrin β2 that can interact with endothelial cells through ICAM-1 molecules was

exploited where activated neutrophils have been used as a source for cell membrane to create nanovesicles with targeted delivery to sites of vasculature inflammation to aid in treating lung injuries and acute inflammation (Gao, Chu, & Wang, 2016b). While Macrophage membrane-based nanovehicle was recently introduced as a selective carrier for encapsulating Amphotericin-B, where it was demonstrated that macrophage surface membrane proteins is involved and has a critical role in the progress of visceral leishmaniosis infection through the communication between infected neutrophil/ macrophage system and noninfected macrophages. Thus the membrane proteins were inspired to tailor a specific nanocarrier based on the macrophage membranes to specifically target the infected sites with pronounced improvement in the toxicity profile in comparison to the traditional method of direct drug administration (Kumar & Bose, 2019). Where the delivery of macrophage NG is controlled the recognition of antigens in the infected cells, leading to accumulation of the drug in the infected tissues preferentially, proved by the secretion of protective cytokines and generation of ROS-RNS.

The bacterial outer membrane vesicles were also studied as nanocarriers but their use is not widely extended due to their inherited immunogenicity. Engineering some bacterial strains via modification with lipopolysaccharides was an approach trying to overcome their potential toxicity (Kim et al., 2009). Acceptable levels of endotoxicity could be compromised with the high capacity to modify bacterial cells genetically making them promising candidates for the creation of targeted delivery systems. A methodology was adopted for the incorporation of human epidermal growth factor receptor-2 specific affibody to be displayed on the bacterial membrane and act as a targeting ligand. The bioengineered outer membrane vesicles of the *Escherichia coli* bacteria strain exhibited lower immunogenicity, decreased endotoxicity, and specifically target tumor cells and aid in their irradiation via targeting the kinesin spindle protein using small interfering RNA (Gujrati et al., 2014). Injecting of small interfering RNA packed with outer membrane vesicles systematically also induced pronounced regression in the growth of the tumor with selective targeted genetic silencing. The outer membrane vesicles of bacterial cells can deliver other cargos as enzymes and proteins, preserving them from the immune system and hard biological and physiological conditions. Phosphodiesterase when encapsulated within the bacterial membrane vesicles was protected significantly and found to be stable when subjected to variable conditions which may lead to better detoxification methods (Alves, Turner, Medintz, & Walper, 2015). It could be concluded that advancement in tools of genetic engineering has contributed to the safe and effective use of biological or bacterial-based carriers as cell-selective delivery systems. The strategies applied for the development and modification of bacterial outer membrane vesicles could be well fashioned to be suited for specific missions with the least adverse effects and could be used for the treatment of different tumors.

Bacterial NG were recently investigated thoroughly to generate different vaccines with effectiveness against various pathogens due to the presence of stimulating immune molecules on the surface of the bacterial outer membrane (Acevedo et al., 2014; Price et al., 2016). A vaccine based on outer bacterial membrane vesicle for

immunization against serogroup-B meningococcal infection. The vaccination potency was enhanced by genetic engineering to overexpress some targeting biomarkers. Furthermore, the attenuation of bacterial endotoxin can also aid in the reduction of potential toxicity and enhance the safety of bacterial-based vaccination (Koeberling, Seubert, & Granoff, 2008). Vaccination against different pathogens was explored as *Bordetella pertussis*, and *Burkholderia psedomallei*. The outer membrane vesicles of *E. coli* were genetically engineered to express exogenously an antigen from *Chlamydia muridarum* to induce immunization and production of antibodies against the expressed antigen (Bartolini et al., 2013). They were also explored to be utilized as adjuvants for human safe use, where their adjuvant activity was demonstrated with ovalbumin, a glycoprotein of herpes virus type 2, and with different allergens (Acevedo et al., 2014). In addition, it could be used as adjuvants in some vaccines against parasitic and viral pathogens such as malaria and HIV-1, respectively (Pritsch et al., 2016; Reza Aghasadeghi et al., 2011).

22.6.2 Biosensing

NG or biomimetic nanovesicles are considered to be one of the elements that could be employed in recognition purposes in some specific cases as well as they were recruited successfully in some applications of bioelectronics tongue and nose. The main idea behind their use lies in the employment and coupling of taste and smell receptors based on proteins with transducers of different natures including quartz microbalances, microelectrode arrays, field effect transistors, or optical detectors (Oh, Song, & Park, 2011; Wasilewski, Gębicki, & Kamysz, 2017). Upon binding the receptors with the investigated molecules even in small concentrations, a signal change is obtained which could be detected. It was demonstrated that employing biomimetic nanovesicles to be the source and express the receptors contributed to preserving the mechanisms of calcium influx and thus amplifying the obtained signals to obtain lower limits of detection (Park et al., 2012). This proposed strategy was utilized for the detection of different odors and tastants such as umami and sweet ones (Ahn et al., 2016). NG-based biosensors utilities extended beyond sensing odors and tastes, they were also used in detecting cancer biomarkers, and contamination with fungi and neurotransmitters (Jin et al., 2012; Lim et al., 2014).

22.6.3 Cancer phototheranostics

The used nanoparticle cores are made of synthetic materials, they can be recruited for use as theranostic materials to be used as vehicles that can serve imaging and therapeutic purposes. Phototheranostics platform based on cancer nanoghost was recently studied and reported. for example, a nanoparticle core made of poly(lactic-co-glycolic acid) and conjugated with indocyanine green which owes very good fluorescence and photoacoustic specifications for dual-mode imaging and tumor eradicating effects upon using NIR (Chen et al., 2016). It was also reported

the use of super magnetic iron oxide core nanoparticles, in conjugation with photosensitizer Ce6, camouflaged with cancer cell surface membrane from hepatocellular carcinoma for the aim of imaging and photodynamic therapy (Li et al., 2018). Another example is the use of breast cancer cell membrane for coating where it demonstrated selective targeting and good penetration in addition, the tumors were eradicated with a single dose in combination with treatment with laser (Jin & Bhujwalla, 2020). Xu Zhen et al. (Zhen, Cheng, & Pu, 2019) summarized the use of NG with different coated nanoparticles and their application in cancer phototherapy in Table 22.1, while the advantages and disadvantages of cell membrane coated nanoparticles using different natural cell sources are summarized in Table 22.2.

22.6.4 Cancer nanovaccination

Because the cancer cell membranes preserve the protein reservoir of their mother cancer cells, they were explored as promising candidates for preparing cancer vaccines to promote specific-immune responses against cancer. Subunit vaccines including cancer-specific or associated neoantigens and molecular antigens were found to induce potential anticancer immunity (Zhu, Zhang, Ni, Niu, & Chen, 2017). The nanovaccines demonstrate effective The nanovaccine also displays extended therapeutic effects in the combination with other immunotherapies as immune checkpoint blockades.

22.6.5 Use of nanoghost in specific diseases

The use of nanoghost as targeted delivery systems and in the treatment of tumors since cancer is considered to be the leading cause of mortality all over the world. Nanocarriers based on cell membrane coating aided in overcoming some limitations of conventional methods of treatment and direct use of drugs in their nanoform. NG as mentioned before can easily escape and evade the immune system providing longer circulation time, specifically targeting and accumulating in the tumor and thus enhancing the efficiency of therapy and the in vivo imaging of the tumor more facile. The use of NG or biomimetic therapeutic nanocarriers, from different sources, as previously discussed, are considered to be Trojan horses and could be simultaneously used with photodynamic and photothermal cancer therapies as well as in diagnosis, monitoring of progress and recurrence and to resist metastasis and drug resistance (Fig. 22.5).

The mechanism by which NG from using cell membranes from different cell sources (Bose, Paulmurugan, Moon, Lee, & Park, 2018; He, Zhang, & Feng, 2020) will be briefly discussed;

Normally circulating tumor cells bind and activate the *PT* through upregulating the adhesion molecules and tumor factors, in addition, the interaction needs the presence of some important mediators on the surface of PT as glycoproteins, integrins, and P-selectin which is rapidly expressed upon platelet activation. It is also known that PT

Table 22.1 Summary of cell membrane sources and nanoparticle cores for cancer phototherapy (Zhen et al., 2019).

Source of cell membranes	Phototherapy type	Nanoparticle cores
RBC	PTT	Gold nanoparticles
		Fe_3O_4 magnetic nanoparticles
		Fe_3O_4 magnetic nanoclusters
		Gold nanocages
		Melanin nanoparticles
		Polypyrrole nanoparticles
	CPTT	DiR-loaded membranes/PTX-loaded polymeric nanoparticles
		DOX-loaded Prussian blue/manganese dioxide nanoparticles
		DOX-loaded mesoporous Prussian blue nanoparticles
	PDT	Hypocrellin B-loaded magnetic mesoporous silica nanoparticles
		ICG- and perfluorotributylamine-loaded HSA nanoparticles
		Methylene blue-loaded hemoglobin and polydopamine complexes
	CPDT	Methylene blue- and cisplatin-loaded gelatin nanogel
		PTX- and 5,10,15,20-tetraphenylchlorin-loaded polymeric nanoparticles
Cancer cell	PTT	ICG-loaded PLGA nanoparticles
	CPTT	DOX-loaded gold nanoclusters
		DOX-loaded CuS nanoparticles
	PDT	Al(III) phthalocyanine chloride tetrasulfonic acid loaded MOF
		Catalase- and glucose oxidase-loaded porphyrin MOF
Platelet	PTT	Fe_3O_4 magnetic nanoparticles
		Gold nanorods
Macrophage	PTT	Gold nanoshells
Stem cell	PDT	Zinc phthalocyanine (ZnPC)- and merocyanine 540 (MC540)-loaded silica shell–coated UCNP
Fibroblast cell	PTT and PDT	SPNs
MDSC	PTT	Fe_3O_4 magnetic nanoparticles

CPDT, chemo-photodynamic therapy; *CPTT,* chemo-photothermal therapy.

have an important role in the metastasis of tumors and are responsible for the formation of hetero-aggregates around the circulating tumor cells supporting tumor survival and protection against immune clearance allowing prolonged circulation time in the blood-stream. Based on such interactions and affinity of PT to tumor cells, platelet NG were

Table 22.2 Summary of advantages and disadvantages of different camouflaged nanoparticles in cancer phototherapy using cell membranes from different sources (Zhen et al., 2019).

A different source of cell membranes	Common features	Unique advantages	Disadvantages
RBC membrane	Abundant availability; Facile extraction	Long circulation time; Lo and coating to nanoparticles; In vivo immune evasion	W ability for active targeting of tumor
Cancer cell membrane	Homologous targeting to source cancer cells		Shorter circulation time than RBC
Platelet membrane	Targeting specific tumors with overexpression of surface markers "CD62p, CD47, and CD44 receptors"; Specific adhesion to injured vessels or tumor tissues		Only targeting limited types of tumor;
Macrophage membrane	Targeting specific tumors with overexpression of surface marker "CD49d," such as MCF-7 tumor and 4T1 tumor		Only targeting limited types of tumor
Stem cell membrane	Tumor tropism	Shorter circulation time than RBC; Low specificity	
Fibroblast cell membrane	Homologous targeting of cancer-	Part targeting normal fibroblast-associated fibroblasts	
MDSC membrane	Active targeting to MDSC-riched tumor Immunosuppression towards tumor		

inspired and fabricated for selective targeting and delivery and their use was recently studied and applied for the management of some types of tumors. For example, upon functionalization with silica nanoparticles, they were proposed to eliminate lung metastasis in breast adenocarcinoma (Li et al., 2016). Platelet membrane was also used in coating and delivery of both active TRAIL protein extracellularly and Doxorubicin intracellularly for reducing the size of the tumor and decreasing metastasis (Hu et al., 2015). The thrombogenic influence and capabilities of bone homing were also enhanced upon conjugation with alendronate and tissue plasminogen activator (Hu et al., 2016). It is worthy to mention that biomimetic nanovehicles could be synthetized from two different cell sources where a hybrid erythrocyte and platelet membranes were used together to combine the functionality of both cells, tumor targeting, and affinity to tumors with the long ERT half-life (Dehaini et al., 2017).

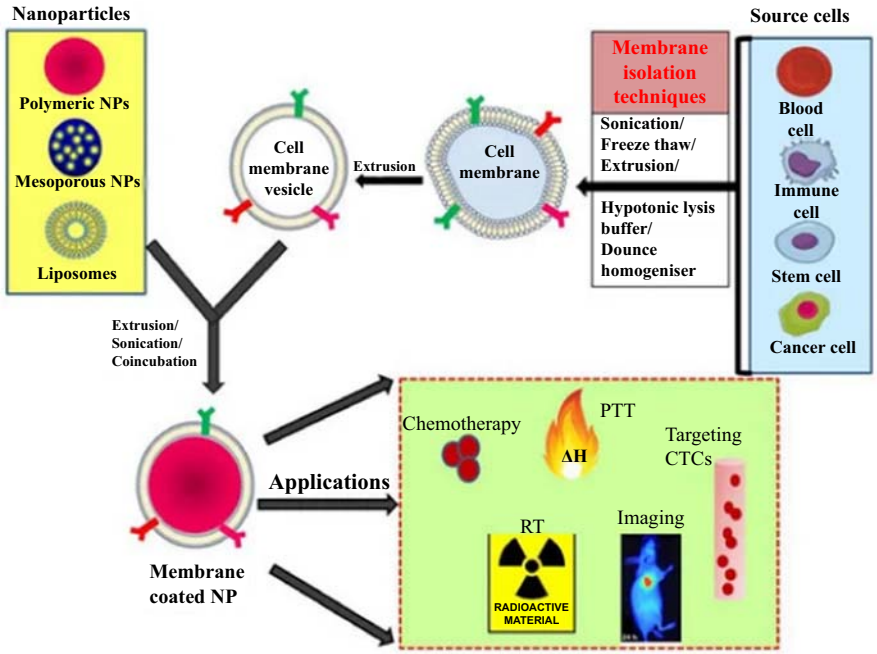

FIGURE 22.5

Preparation of nanoghosts from different natural cell sources and their biomedical application in malignancy (Vijayan et al., 2018).

Different Immunocytes-based NG have been employed as natural coating biomimetic material for tumor targeting. *Leukocytes* can recognize the endothelium of tumors and interact with the inflamed endothelium tissue selectively, it was taken advantage of this feature where membranes of leukocytes have been utilized to target the chronic endothelial in case of inflammation and tumors while escaping hepatic elimination and lysosomal sequestration (He, Frueh, Wu, & He, 2016a). The leukocyte membrane-coated nanoparticles exhibited better penetration to the endothelium monolayer and improved accessibility to the underlying tumor leading to decreased viability to the cancerous cells due to the tumoritopic accumulation abilities in comparison to naked nanoparticles. The microsized capsule cushioned leukocyte membrane vehicles displayed less accumulation in the liver promoted increased, better retention in blood and tumor, improved deformability, and enhanced tunability in comparison to bare vehicles. Generally speaking, leukocyte nanoghost was reported as a promising tumor therapeutic delivery and targeting platform with prolonged or enhanced blood circulation and better accumulation in malignant tissues (Gao, Wu, Lin, Lin, & He, 2016c; He, Frueh, Wu, & He, 2016b). Another type of immunocyte cell whose membranes could be used in the synthesis of NG for targeting tumors is the macrophages.

The macrophage's active targeting capabilities are mainly attributed to its intrinsic proteins on the surface of their cell membranes as interleukin-1 and the Toll-like receptors which can recognize and bind to the endothelium of the tumor tissue (Xuan, Shao, Dai, Li, & He, 2016). Macrophage cells can also identify or recognize foreign harmful particles and unhealthy cells as cancerous cells and phagocytose them. Upon coating nanoparticles with macrophage membranes to camouflage them and transformed into ghosts with prolonged time of circulation and enhance cell-to-cell adhesion for targeting tumors. The macrophages also have a strong affinity to metastatic cells and are naturally enriched in the microenvironment of the tumors where the α4integrins due to the expression of CD49d on the membranes of macrophages bind with the vascular cell adhesion molecule-1 on cancerous cells. This strong affinity facilitates the formation and survival of metastatic lesions but at the same time could be the reason behind macrophage NG specific targeting the metastatic cells and enhanced cellular uptakes of drugs from them (Krishnamurthy et al., 2016). Nanocapsules of mesoporous silica camouflaged with macrophage membrane resulted in decreased clearance by the organs of a reticuloendothelial system allowing prolonged circulation time, and more drug accumulation in the tumor cells in comparison to bare nanomesoporous capsules. The same effects were observed in the case of coating liposomes of Emtansine used in the treatment of metastatic breast tumors where the viability of tumor cells was preferentially inhibited compared to uncoated liposomes due to better targeting and metastatic suppression capabilities (Cao et al., 2016; Xuan, Shao, Dai, He, & Li, 2015). Macrophage NG could also be employed for camouflaging nanoparticles to promote photothermal therapy and chemotherapy while eliminating their adverse side effects (Wu et al., 2018). *Neutrophils* are the third type of immunocyte cells that have pronounced participation in the process of tumor metastasis. The mechanism by which neutrophils exert their function depends on the stimulation of cellular niche formation by the myeloid cells, the neutrophils precursors. The formation of a niche is followed by the recruitment of circulating tumor cells and neutrophils by the tumor cells via granulocyte colony-stimulating factor expression. The inflammatory neutrophils could interact and promote the circulating tumor cells towards gravitation to the premetastatic niche. This interaction or binding is achieved through both, the adhesion moieties and extracellular neutrophil trap generation (Park et al., 2012). The same mechanism by which neutrophils act and target the niche and circulating tumor cells encouraged the synthesis of neutrophil NG or neutrophil mimicking or inspired nanoparticles by using their cell membranes in coating and camouflaging nanomolecules to prevent metastasis of tumors. Neutrophil NG have an inherited affinity and selectively target circulating tumor cells either in vivo or in vitro and accelerate their apoptosis. Neutrophil NG exhibit increased accumulation in premetastatic niches and prevent or delay tumor metastasis especially at its early stages by deterring nodules formation and eliminating the circulating tumor cells (Kang et al., 2017).

The *natural killer cells*, which constitute the first defense line against pathogens and tumors, are considered to be an integral component and play a critical role in the innate immune system. They have the power to differentiate between

normal and malignant cells through recognizing some inhibitory receptors expressed on their surface, such as the killer cell immunoglobulin-like ones as well as the CD94-NKG2A, malignant cells could also be identified due to the absence of the major histocompatibility complex which is expressed and present in the normal healthy cells (Campbell & Hasegawa, 2013). The natural killer cells possess an inherent antitumor potential and can induce the death ligands on the surface of tumor cells as TRAIL and FAS ligands, therefore, stimulating tumor cells' apoptosis. Their efficacy is also enhanced when combined with gene manipulation methodologies. The natural killer cells 92 are the most widely analyzed and used due to their prominent anticancer effects whether in preclinical or clinical phases. The activated natural killer cells membranes were successfully merged with cationic liposomes to generate a novel delivery system (NKsomes) with improved efficacy against tumors through enhanced tumor-selective targeting of the natural killer cell membranes receptors together with the prolonged blood circulation time due to the stability and nonimmunogenic features of the newly generated NKsomes under physiological conditions. Camouflaged polymeric biomimetic nanoparticles using natural killer cells membranes could also be employed in bioimaging of tumors via cellular interaction in vitro, Anti imaging ex vivo, and NIR fluorescent imaging of tumors in vivo because of increased retention in circulation and accumulation in tumor cells (Daher & Rezvani, 2018; Pitchaimani et al., 2019; Rezvani, Rouce, Liu, & Shpall, 2017).

The *mesenchymal stem cells*-based NG have a significant role and are considered to be a successful candidate in fighting malignancy. They are a basic component in various solid tumor microenvironments. The tumor-associated fibroblasts are counted to be one form of the mesenchymal stem cells. When administrated systematically mesenchymal stem cells display selective homing to tumor tissues and very limited homing to normal tissues. The active inflammatory action is responsible for recruiting the mesenchymal stem cells to the malignant sites, which was harnessed to design a new delivery and targeting platform by employing the cell membranes of mesenchymal stem cells to camouflage and coat nanoparticles to form NG with enhanced targeting capacity to tumors and superposing therapeutic effects and efficiency as reported for treatment of prostate cancer in humans. Gelatin nanogels loaded with doxorubicin and coated with mesenchymal cell membranes were also studied and displayed in vitro specific targeting and homing capabilities to malignant sites and increased accumulation in the malignant sites in vivo. The same concept was applied for wrapping Poly lactic-co-glycolic acid biodegradable polymeric nanoparticles loaded with doxorubicin with umbilical cord mesenchymal cell membranes. Stem cells derived NG two prominent privileges including escaping the elimination by the reticuloendothelial system and its inherited tumor-specific targeting mechanism, not species specific mechanism allowing one cell membrane type to target different tumors (Karnoub et al., 2007; Kidd et al., 2009; Spaeth, Klopp, Dembinski, Andreeff, & Marini, 2008; Yang et al., 2018).

Bacterial cell membranes-based ghosts are considered an important category of nanocarriers that were applied successfully for targeting tumors to selectivity

deliver therapeutically active drugs to malignant tissues. Due to more facile extraction methods, the gram-negative bacterial cells are more widely used where outer membrane vehicles could be harvested directly from the bacterial filtered culture medium through the ultrafiltration process. The cell membranes of the bacteria could be engineered easily aiming at toxicity attenuation (Gao et al., 2015). Genetic engineering can be employed for modification of the bacterial membranes to perform specific functions such as toxicity attenuation. Human epidermal growth factor receptor-2 was expressed on bacterial bioengineered outer membrane vesicles as a targeting ligand specific to the tumor and was employed to deliver siRNA to tumor cells selectively for tumor therapy. The selective delivery and targeting allowed significant growth inhibition with acceptable tolerance, with no adverse effects due to endotoxins or undesirable side effects due to low specificity (Gujrati et al., 2014).

22.6.6 The use of nanoghost in targeting and treating inflammation

There are different cells related to the inflammatory process including PT, leukocytes as macrophages, neutrophils, neutrophils, and stem cells (Yan et al., 2019). Recruiting process of the inflammatory cells is achieved in double stages controlled in spatial and temporal manners. The cells are activated or reset by pathogenic products in the circulation like cytokines and chemokines and then migrate to the infected or inflammatory sites and attach to the endothelial tissue guided by endothelium adhesion molecules as integrins and selectins. After adhesion, the cells migrate either para or tans-cellular from the endothelium wall to the sites of inflammation to take control by heading to the resident cells and regulating the inflammatory microenvironments, the mechanism by which cells perform their functions is briefly discussed to demonstrate the importance of recruiting their cell membranes in tailoring selective targeting systems to deliver nanotherapeutics to regulate and manipulate the infectious and inflammatory sites (Anselmo & Mitragotri, 2014). Recently attention has been paid to the inherited properties of the cell membrane surface receptors related to inflammatory diseases. The camouflaged nanoparticles or NG are employed to neutralize the harmful effects of pathogenic agents and in detoxification.

PTs are considered to be significant effectors in inflammatory reactions, adaptive response to injury, and host immune defense mechanism. One of the well-known specifications in the progression and triggering of the inflammatory cascade is the activation of PTs, this applies to different cardiac pathologies, arthritis, sepsis, and inflammatory bowel disorder. The actions of PTs are mediated via a unique group of mediators on their surfaces responsible for adhesion to endothelium, interaction with pathogens, and evasion of the immune system. During circulation in the blood, PTs are reset and activated resulting in the release of cytokines which in turn enhance other inflammatory cells in the circulatory system to perform their

functions. The activated PTs attach to the inflamed endothelial wall and interact with immune cells simultaneously owing to their glycoproteins (αIIbβ3 and Ibα). In addition, the adhesion receptor αIIbβ3 which is the most expressed glycoprotein promotes the aggregation of PTs through binding to fibrin and fibrinogen. While thrombosis and hemostasis are triggered and propagated through another platelet receptor, LLR motifs, are responsible for interaction with inflammatory conjugated factors. PTs can interact directly with pathogens, adhere to and activate different cells existing in the microenvironment of inflammation, these processes need the P-selectin to be translocated to the platelet surface membrane from α-granules.to strengthen platelet connections and modulate their interaction with immune complexes and collagen at vascular injury locations, C1q receptors should be expressed. These are some unique features among many others displayed by PTs making them peculiar candidates for designing and synthesizing NG for targeted delivery to infected and inflamed tissues with prolonged circulating time. Recently reported studies demonstrated that therapy based on PTs is effective in the management of sepsis owing to their activation during disease development and subsequent resulting inflammatory cascade and thrombocytopenia. While in inflammatory bowel disorder, PTs activate the endothelial cells and direct the immune cells to inflamed locations (Bambace & Holmes, 2011; Becker, Sexton, & Smyth, 2018; Costa-Filho & Bozza, 2017; Dewitte et al., 2017; Tamagawa-Mineoka, 2015).

The progenitor or stem cells display significant contribution in the process of repairing and regeneration of damaged tissues via different mechanisms including remodeling regulation, effects of paracrine, ability to migrate to the damaged tissue, or sites of injury and differentiation. The most widely used type is due to its abundance in different human organs such as lungs, brain, skeletal muscles, adipose tissues, bone marrow, and peripheral blood. Stem cells are considered the inflammation guardians where they are responsible for the secretion of a set of molecules regulating the ardent immune system acting as a barrier or one of the first defense lines versus the host chronic autoimmune response or reactions. Mesenchymal stem cells express various cytokines and chemokines receptors such as CXCR-1, CXCR-2, CCR-1, and CCR2 deriving their migration to inflamed sites to locally control the inflammatory reactions. The overexpression of some homing receptors such as CCR-1 and CXCR-4 could enhance the homing and migratory behavior of mesenchymal stem cells toward inflamed tissues or injured sites as well as the tumor microenvironment. It was also demonstrated that a huge number of integrins are expressed on the surface of mesenchymal stem cell membrane surface as (α1, α2, α3, α4, α5, α6, α v, and β1, β2,). Bioengineering technology contributed to the improvement of some stem cells' functionalities as proliferation, homing, and migration via modifying some molecules and increasing the expression of some receptors. In addition, mesenchymal stem cell interacts with other inflammatory-involved cells in the localized inflammatory microenvironment, such as natural killer cells, dendritic cells, B cells, and T cells to control their functionalities affecting both the adaptive and innate immune reactions. The potent mesenchymal stem cells' immune modulation

specifications in addition to their differentiation and self-renewal potential allow them to be the most significant immuno-regulator cells in the human body. They are involved in different inflammatory disorders including arthritis, inflammatory bowel syndrome, respiratory disorders, allergic encephalitis, and ischemic cardiovascular diseases. Owing to the specific and unique features of mesenchymal stem cells regarding their role in immunomodulation in addition to their negligible immunogenicity, their surface membranes are thoroughly explored and studied for the synthesis of NG and in nanotherapeutics advanced applications (Barminko, Gray, Maguire, Schloss, & Yarmush, 2013; Chamberlain, Fox, Ashton, & Middleton, 2007; Maan et al., 2015; Nitzsche et al., 2017).

The Neutrophils are the most abundant blood-borne white blood cells and are considered to be an important and integral member of the cell group of innate immunity in the body of a healthy human. They have a critical rule and provide the first defense line of the immune system owing to its special granules. The bone marrow rate of production of neutrophils increases multiple folds in response to inflammatory reactions indicating strong relation and neutrophils' high affinity to inflammatory sites. It also demonstrated the contribution of neutrophils to the pathogenesis and the progress of some inflammatory disorders such as sickle cell, ischemic strokes, Behcet's disease, a chronic obstructive pulmonary disorder, inflammatory arthritis, and acute pancreatitis. This strong affinity between chronic and inflammatory diseases and neutrophils was employed for tailoring nanocarriers with powerful targeting capabilities which mainly take advantage of the surface molecules and phenotypic changes that take place after the neutrophils leave the bone marrow. Fresh neutrophils need to be activated or aged through adhesion molecules and a huge number of chemoattractant sensing receptors respond to different signals including pathogen-induced or derived molecules or distress signals. After activation, neutrophils accumulate in the inflamed or infected sites to execute their functions. The activation process involves different conformational or phenotypic changes in integrin adhesion receptors including $\alpha 4\beta 1$ integrin, $\alpha L\beta 2$ integrin, and $\alpha M\beta 2$ integrin (Chu, Dong, Shi, Zhang, & Wang, 2018; Dong et al., 2019; Kang et al., 2017; Wright, Moots, Bucknall, & Edwards, 2010; Zhang, Xu, Manwani, & Frenette, 2016).

Among the white blood cells, macrophages are considered to be important cellular modulators and effectors in the processes of damaged tissue repair, regeneration, and inflammation. They are multifunction, large versatile leukocytes that originate and differentiate from mononuclear cells circulating in peripheral blood. They have strong participation and an important role in immune responses whether innate or adaptive. Macrophages act as guardians and respond to inflammation or damage via several mechanisms. Their functions include clearing and destroying foreign materials, apoptotic cells and different microorganisms, phagocytosis, and processing and presentation of antigens. They display distinct and specific functional phenotypes from pro-inflammatory to anti-inflammatory with pronounced plasticity according to variable environmental signals and types of tissues. The importance of microphages also extends to the regulation of tumor

growth, different inflammatory diseases, and recognition of pathogens. They respond quickly to pathogens-associated cues due to the presence of chemokine receptors on the surface of their membrane leading to increased trafficking and enhanced migration to the inflamed sites. Macrophage cell membranes also express adhesion molecules which help in the cells' adhesion and recruitment. Macrophages also possess different families of receptors with various functions including scavengers for adhesion and phagocytosis, GPI-anchored receptors for recognizing apoptotic cells and signaling, Ig for inflammatory reaction regulation, antibody-based binding, killing, and uptake, and the Toll-like receptors for responding to peptidoglycan and lipopolysaccharides. Macrophages are involved in different inflammatory diseases such as Crohn's disease, atherosclerosis, multiple sclerosis, and rheumatoid arthritis. Owing to the plasticity and heterogeneity in the macrophage functions, they could provide increased probabilities for their engineering through dynamic environmental cues. Macrophages are the major contributors to engineered nanotherapeutics based on cell membranes through monitoring and tracking their polarization state and phenotype to control and modulate the pathway of inflammation resulting in inflammation successful resolution (Cheng, Wang, & Huang, 2017; Mosser & Edwards, 2008; Shi & Pamer, 2011; Svenson & Prud'homme, 2012; Taylor et al., 2005).

The lymphocytes including natural killer cells, T-cells, and B-cells are considered to be a critical subgroup of leukocytes. They nearly exhibit the same appearance but have different employabilities and functions. The T-cells and B-cells are counted to be significant effectors and modulators of the adaptive immune system owing to their recombined antigen surface receptors as well as their capabilities to produce antibodies, regulate immune responses, destroy tumor cells and kill infected cells. The lymphocytes are inherently mobile and in continuous motion in the form of big clusters circulating in-between the blood and lymph in high endothelial venules-like structures. In response to inflammation, the lymphocytes are derived to migration and the endothelial venules-like structures are observed. For the migration and homing processes to take place, the expression of G protein-coupled receptors which responds to chemokines, TCR, and other costimulatory molecules. These homing receptors combine or interact with chemokine receptors for providing a specific command code for the determination of the lymphocyte destinations where homing receptors are essential for migration and are involved in the engagement with corresponding specific tissue ligands. For example, some receptors are expressed responding to interferon γ-induced inflammation while others are expressed responding to interleukin-4, 5, or 13. In addition, home to specific tissues is also regulated by tissue-specific homing receptors, L-selectin is required for lymph node homing, $\alpha 4\beta 7$ integrin for gut and digestive system associated lymphoid tissue while cutaneous lymphocyte antigen for skin-homing. The lymphocytes are integral components in various inflammatory diseases including tuberculosis, asthma, blood cancer, and immune dysfunction diseases. The permanent availability or expression of the lymphocyte related ligands at the sites of endothelial venules-like structures lie behind the

selectivity of their homing process resulting in many successful opportunities for targeted delivery of nanoparticles and drugs upon using lymphocyte cell membranes in the synthesis of NG (LaRosa & Orange, 2008; Miyasaka & Tanaka, 2004; Venet et al., 2009; Ward & Marelli-Berg, 2009).

Therefore, it could be concluded that making use of the whole cell membrane as a coating or covering materials for different nanoparticles has the advantage of preserving most of the features and specifications displayed by the source cells offering tremendous effective therapeutic chances and opportunities. Different formulations based on camouflaging nanoparticles aby coating them with cell membranes and transforming them to nanoghost were designed for selective targeting inflamed tissues and taking complete charge of the inflammation microenvironments owing to their unique membrane surface proteins and homing properties. Nanoghost have different applications in inflammation reaction including immune modulation, detoxification, photoactivatable therapy, and imaging due to their accumulation in the required sites and prolonged circulation time (Dong et al., 2019; Zhao et al., 2019; Zhou, Cao, Tu, Zhang, & Deng, 2019).

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CHAPTER

Biomimetic nanosystems in theranostics 23

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23.1 Introduction

Nature has consistently propelled us, and the efficiently ordered biological frameworks have helped researchers in the fabrication of novel medications (Green & Elisseeff, 2016). Quintessential nanosystems ought to play out an application-arranged capacity with the delayed course by limiting vague focusing on and expanding explicit cooperations, much the same as different cellular frameworks in the human body (Chen, Zhang, Zhu, Xie, & Chen, 2017). The plasma membrane is among the most crucial cell segments known for performing different fundamental capacities needed for life subsistence. The boundary layer isolates the inside of a cell from the external environment and intervenes in signal transduction and transportation of molecules into and out of cells (Osaki & Takeuchi, 2017). It comprises 3 significant establishments: (1) hydrophobic obstruction for ionic/soluble substances by the bilayer structure formed by phospholipid chain, (2) transportation of substances and signals across the membrane employing conformational changes formulated by proteins embedded in the membrane, and (3) domination of cell-cell acknowledgment by carbohydrates located in the plasma membrane of eukaryotes. As the plasma membrane participates in pretty much every correspondence between a cell and its environmental factors, the biological membrane is of huge interest for idiosyncratic therapeutic applications (Xu, Hu, & Chen, 2016).

Recently, plasma membrane mimetic surface engineered materials have been broadly examined, while nanoparticles coated with biomimetic film copolymers were the delegate endeavor of the arena. As one of the primary segments of the cell film, the phospholipid bilayer is the primary segment to be imitated. The utilization of phospholipid-loved copolymers as a prevalent contender for the surface alteration of materials in the nanorange and drug conveyance with enriched biocompatibility and additional capacities have been acknowledged. Fig. 23.1 illustrates conventional nanotheranostics integrated with both diagnostic and therapeutic moieties. Principally, amphiphilic block copolymers of polyethylene glycol (PEG)-distearoyl phosphatidylethanolamine (DSPE) has been endorsed by the Food and Drug Administration (FDA) for clinical applications (Ishida et al., 2006).

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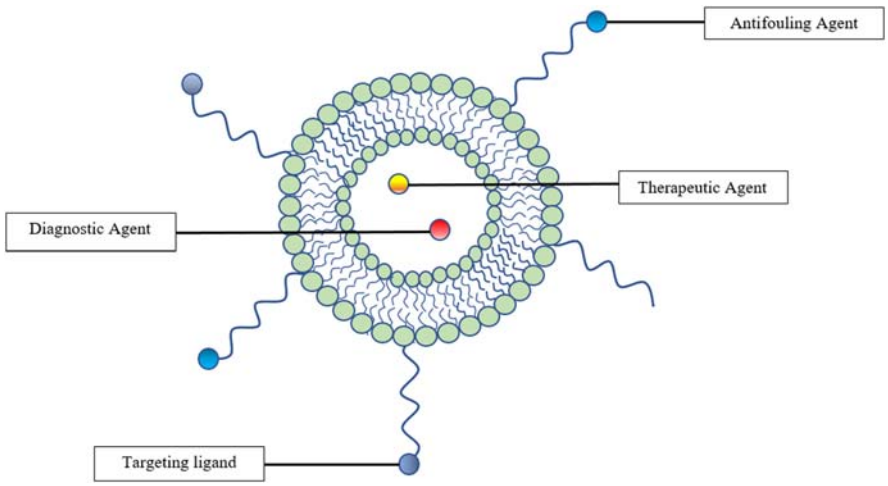

FIGURE 23.1

Conventional nanotheranostics integrated with both diagnostic and therapeutic moieties.

As of late, nanomaterials camouflaged with cell membrane innovation have arisen as a propitious procedure to outfit the nanoparticles with fantastic natural functionalities, which centers around modifying the nanoparticles with the characteristics procured from the source cells and proffer them a wide range of functionalities (Yang et al., 2019; Zhen, Cheng, & Pu, 2019). The nanomaterial camouflaged with appropriate membrane vesicles constitutes a nanoparticle core and a layer of characteristic cell film on a superficial level (Hu et al., 2011). In contrast to traditional nanoparticles, the cell layer camouflaged nanoparticles have delayed in vivo course time, lower immune system clearance, and higher adequacy for explicit focusing, showcasing an incredible potential for interpretation from the lab seat to the clinical setting. Until this point, different kinds of cell films, for example, red-platelet (RBC) layer, white-blood-cell layer, platelet layer, malignant growth cell layer, etc. have been utilized to build cell layer camouflaged nanoparticles. Every cell film has unmistakable attributes in the explicit regions of medication conveyance, imaging, and phototherapy, poison balance, insusceptible balance, etc. The chapter highlights recent advances in the development of biomimetic nanosystems for several diseases theranostics.

23.2 Fabrication of biomimetic nanosystems

In the vast majority of investigations involving the design of biomimetic core-shell structures [the center alludes to the stacked freight (for instance, inorganic nanoparticles, RNAi agents, drugs, etc.)] whereas, the shell alludes to the lipids

[for example Distearoyl-sn-glycero-3-phosphoethanolamine-Poly(ethylene glycol)], a single-step or a two-step methodology is employed. A two-step methodology necessitates the preparation of the core followed by a coating of lipid shell. Lately, the burgeoning of single-step methodology has proffered the analysts more noteworthy straightforwardness and is progressively favored (Krishnamurthy, Vaiyapuri, Zhang, & Chan, 2015).

The single-step method utilizes the mixing of polymer, lipids, and loaded cargo (particularly inorganic nanoparticles), utilizing self-assembly. The nanoprecipitation strategy or the emulsion technique is typically utilized in a single-step method. The emulsion technique alludes to the blending of water-immiscible organic stages and aqueous solutions to allow the coating of lipids over the cargo. For instance, Anticancer medication of curcumin-exemplified nanoparticles for the treatment of breast cancer was recently reported (Palange, Di Mascolo, Carallo, Gnasso, & Decuzzi, 2014). In the union obtain of which, an organic arrangement made out of dropwise addition of curcumin, poly lactic-co-glycolic acid, and dipalmitoylphosphatidylcholine to a 4% ethanol constituting DSPE-PEG, prompting nanoparticles (diameter = 170 nm) exhibiting therapeutic activity. The nanoprecipitation technique alludes to the process of eliminating the organic dissolvable in the emulsified combination of an organic arrangement also, a fluid arrangement that permits nanoparticle development. In this technique, several boundaries, for example, the proportion of lipid to polymer and the natural to fluid volume proportion, represent the last molecule width. Concerning inorganic nanoparticles, a straightforward single-step technique was generally employed utilizing mechanical mixing or bath sonication via Van der Waals and hydrophobic cooperations. Essentially, Wen et al. as of late introduced a DSPE-PEG2000 covered counterfeit catalyst, Cu2-xTe nanoenzymes, displaying peroxidase-emulating and glutathione oxidase-imitating exercises for reactant antitumor immunotherapy (Wen et al., 2019). The as-orchestrated Cu2-xTe nanosystems with oleic acid as surface ligands are hydrophobic and stable scattered in physiological conditions accomplished when scattered in DSPE-PEG2000 fluid arrangement with sonication in an ultrasonic bath. Bismuth/phthalocyanine manganese nanocomposites for trimodal imaging coordinated photodynamic therapy and photothermal therapy for malignancy (Wang et al., 2020). Dropwise addition of Tetrahydrofuran dissolved Bi/MnPcE4 nanodots into the water/ tetrahydrofuran (v/v, 5:1) arrangement constituting DSPE-PEG3000, inferring self-assembly of Bi/MnPcE4 nanocomposites and accompanied by purification by dialysis.

The nanocomplexation of stacked freight (particularly for medications and RNAi specialists) was nanocomplexed with biodegradable and biocompatible polymers as a center first, before being covered with the lipids to frame a shell utilizing electrostatic communications by the immediate hydration, sonication, or expulsion strategies. Implementation of different blending methodologies to meld the payload with lipid vesicles, for example, Li et al. planned a tumor hypoxia alleviation stage by typifying the hemoglobin, a proficient oxygen transporter, into the endoplasmic reticulum-directed liposomes with a help of photothermal

treatment/photodynamic treatment to upgrade the immunogenic disease cell passing (Li et al., 2019a). The endoplasmic reticulum focused on liposomes, made out of cholesterol, DSPE-PEG2000, and Trauma center focusing on pardaxin peptides (FAL)-altered DSPE-PEG2000, were dried into a flimsy before being hydrated within the sight of Hb arrangement (FAL-Hb-lipo). The FAL-Hb-lipo ended up being ready to amass in the endoplasmic reticulum and deliver adequate oxygen to a hypoxic tumor microenvironment for hypoxia to help upgraded reactive oxygen species generation.

23.3 Fabrication strategies using the real cell membrane

Cell lysis and purification of membrane ought to be worked tenderly to attain extreme maintenance of layer proteins. The absence and presence of nuclei in the source cells form the basis of different procedures that can be performed. In particular, the segregation of membranes from nucleated cells is more convoluted than that of nucleus-free cells. By and large, repeated freeze-thaw or hypotonic treatment is executed to eliminate intracellular constituents of the source cells. Cleansed vesicles are therefore expelled through polycarbonate layers to acquire cell vesicles for the combination (Parodi et al., 2013). In expansion, other idiosyncratic strategies for biomembrane seclusion have additionally been accounted for. In a new case, utilizing cytochalasin B, scientists have effectively invigorated macrophages to deliver numerous microvesicles for nanoparticle covering and affirm that the technique is more predominant than the conventional cell film extraction strategy (Li et al., 2019b). For prokaryotes, for instance, bacteria surrounded by peptidoglycan, plasma membrane disengagement is considerably more troublesome. In any case, the external film vesicles discharged by numerous microorganisms can be gathered utilizing ultrafiltration, and cell lysis isn't needed (Gao et al., 2015). Contingent upon the properties of conveyed freights, diverse center materials are chosen, for example, metals, nonmetals, polymers, etc. (Zhai et al., 2017). The assembling interaction of core nanoparticles changing in various types of materials is a similar path as the customary exposed nanoparticles.

There are innumerable techniques to combine plasma membrane vesicles with core nanoparticles. Film expulsion is the first and foremost used "top-down" approach, which includes the actual expulsion of plasma membrane vesicles and center nanoparticles through a permeable layer persistently utilizing a small-scale extruder (Hu et al., 2015). This technique is helpful and viable, nonetheless, it isn't reasonable for enormous scope creation. Along these lines, sonication is employed for the coating of cell layers with polymeric nanoparticles (Rao et al., 2017). Core nanoparticles are cobrooded with cell films and sonicated, prompting the fabrication of cell membrane camouflaged nanoparticles. Even though sonication is very helpful, in any case, the fundamental downside is that resultant particles shift essentially as far as size and consistency. As of late, a microfluidic

electroporation technique incorporating 4 successive parts including an inlet, merging channel, electroporation zone, and outlet was utilized for the amalgamation of magnetic nanoparticles and red blood cell membrane (Liu et al., 2019a). For complete inclusion, the proportion of biomembrane to nanoparticles ought to be exactly picked. Notwithstanding muddled, the microfluidic-based methodology, with high controllability and adaptability, is a more productive and dependable methodology for creating cell membrane camouflaged nanoparticles with complete film coatings and incredible steadiness. Additionally, there is a remarkable technique for in situ bundlings of nanoparticles from live cells. Incubation with various nanoparticles in explicit conditions causes cells to secrete vesicles of these nanoparticles (Silva et al., 2013). This in situ bundling strategy, without harming the cell layer, ought to be an ideal methodology for shaping cell membrane camouflaged nanoparticles, notwithstanding, that the uncontrollable nature may restrict its wide application.

23.4 Biomimetic nanosystems for the treatment of cancer

23.4.1 Chemotherapy

Regardless of the way that chemotherapy is quite possibly the most widely recognized disease restorative method, its after-effects are as yet the greatest clinical problems (Chabner & Roberts, 2005). The use of a common cell layer for vehicle surface functionalization displays the benefits of protecting medication viability, and immune evasion, effectively focusing on the tumor microenvironment just as decreasing undesirable after-effects to nearby solid tissues, which could be utilized for ameliorated malignancy chemotherapy (Hu et al., 2016; Zhang et al., 2018a; Zhang et al., 2019a). Stem cells are a sort of crude cells that can be separated into an assortment of explicit cell types, consequently advancing their improvement in biomedical applications (Blau & Daley, 2019; Stuckey & Shah, 2014). Umbilical rope inferred mesenchymal stem cells membrane doped polymeric nanoparticles could accomplish conveyance of chemotherapeutic medications, coming about in evident apoptosis inside tumor sores (Yang et al., 2018).

Melanoma cell film-coated polymeric nanoparticles first revealed the homologous targeting characteristic of cancer cell membrane camouflaged nanotechnology by presenting a lot higher cell grip to the first malignant growth cells contrasted with red blood cells-coated and uncoated nanoparticles. To ameliorate subcellular drug accumulation and tumor penetration capacity, yolk-shell structured malignancy cell layer coated nanoparticles were designed for malignancy chemotherapy (Nie et al., 2020). The cell layer coated nanoparticles with a yolk-shell structure was built by employing mesoporous silica nanoparticles as a coating to PEGylated liposome, additionally swaddled with malignancy cell layer abstracted from MCF-7 cell lines. Controlled rigidity proffered by the yolk-shell structure was advantageous for the change of shape into an ellipsoid form during

the invasion, bringing about encouraged dissemination limit in the extracellular network and improved tumor penetrance. Curiously, the cell intrusion route was compared to that of an enveloped virus. Additionally, cell membrane coated nanosystems could likewise be utilized for directed chemotherapy of homotypic metastatic tumors attributable to the homologous adhesion capacity. Different nanosystems for drug-release monitoring are depicted in Fig. 23.2.

23.4.2 Phototherapy

Phototherapy including photodynamic treatment and photothermal treatment has arisen as the localized noninvasive methodology for the methodical treatment of diseases. The utilized phototherapeutic specialists are nontoxic in obscurity yet become harmful in the presence of light illumination with a particular frequency by producing hyperthermia or reactive oxygen species. In expansion to little atom phototherapeutic specialists, an assortment of nanomaterials including metal-organic frameworks, metal or metal oxide nanostructures, semiconducting polymers, and 2D nanomaterials has been widely utilized as photosensitizers for photodynamic treatment and photo-absorbing specialists for photothermal treatment.

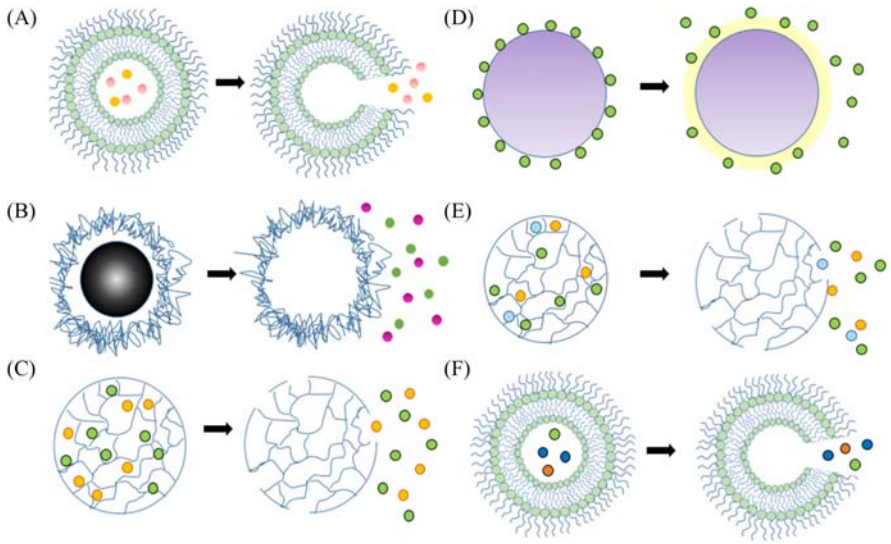

FIGURE 23.2

Biomimetic nanosystems for drug-release monitoring by various mechanisms. (A) release of MRI mediators with drug molecules; (B) drug molecules released by the decomposition of nanoparticle; (C) imaging probed coreleased with drug molecules from polymeric Michelle; (D) drug released from the surface resulting in imaging probe hyperintensity in MIR scan; (E) increase in imaging probe hyperintensity due to deshielding; (F) chemical shift agents and highly fluorinated compounds released together with drug molecules.

To amazingly limit phototoxicity and upgrade restorative explicitness, the phototherapeutic specialists ought to have the most noteworthy collection in the ideal tumor areas or metastatic injuries before their clearance from the body. A plasma membrane-based biomimetic system is the perfect contender to fold over nanomaterials for oncological phototherapy (Zhen et al., 2019).

Photodynamic treatment adequacy is enormously restricted by the tumor microenvironment because of its oxygen deficiency nature as oxygen assumes a significant part in reactive oxygen species generation. Thusly, biomimetic dioxygen self-improved nanobased platforms are exceptionally expected to beat tumor hypoxia furthermore, augment the accumulation of tumors. Intelligent O_2-advancing photodynamic treatment nanoplatform with homologous malignant growth cell focusing on capacity, acknowledged by the MnO_2 nanosheet-covered metal-organic framework core and malignant growth cell membrane shell was recently reported. The presented cell membrane structure managed the framework with ameliorated strength, and brilliant biocompatibility just as solid tumor focuses on capacity. Extraordinary synergist action for in situ endogenous generations of O_2 was demonstrated by the MnO_2 layer under an acidic tumor microenvironment, accordingly prompting improved cytotoxic O_2 creation inferable from the help of porphyrin-based metal-organic framework nanostructure. Photothermal treatment is essentially dependent on the laser illumination initiated hyperthermia to remove the tumor, in which the photothermal transformation execution and adequate aggregation of polyacrylic acid in tumor tissues are the keys. Lately, Bu et al. suggested multifunctional half-breed cell film covered attractive nanoparticles for augmented photothermal treatment of neck and head squamous cell carcinoma. The platelet−disease foundational microorganism half breed layer covered nanoparticles had the resistant sidestepping capacity determined from platelet layer and the homotypic focusing on capacity acquired from disease foundational microorganism film, which was predominant to single-cell layer covered nanoparticles. All the more critically, the mixture layer bunch displayed the most noteworthy temperature ascend in the tumor destinations and the most grounded antitumor action contrasted with different gatherings, particularly in an immunocompetent Tgfbr1/Pten 2cKO neck and head squamous cell carcinoma mouse model comparable to the human head and neck squamous cell carcinoma microenvironment. The heightened temperature during photothermal treatment interaction couldn't just remove the tumor yet, besides, annihilate the cell layer covering the nanoparticles to trigger medication discharge, accomplishing photothermal-synergized chemotherapy (Su et al., 2016).

23.4.3 Immunotherapy

Immunotherapy has accomplished extraordinary anticancer results in clinical preliminaries. Because of the variety, and heterogeneity of antigens articulation on tumors, Dark phosphorus quantum dots were coated onto tumor cell film and further stacked into a thermosensitive hydrogel constituting granulocyte-colony stimulating variable and lipopolysaccharide, along these lines creating a customized

tumor vaccine (Ye et al., 2019). The vaccine based on tumor cell membrane gave a bounty of autologous tumor antigens which could prompt solid invulnerable reaction. Meanwhile, the supported arrival of lipopolysaccharide and granulocyte-macrophage colony-stimulating factor from the hydrogel could likewise successfully enroll and enact dendritic cells, separately. At the point when joined with the safe designated spot barricade immune response PD-1, a durable and strong immunological reaction was initiated, furthermore forestalling tumor repeat and metastasis. Contemplating the spleen as the greatest fringe resistant organ with the capacity of disposing of senescent or harmed RBCs, sonication and physical extrusion were employed to acquire an intertwined malignant growth RBC layer (nano-Ag@erythrosome) (Han et al., 2019). Moreover, immune cell membranes have additionally pulled insignificant consideration in focused malignancy immunotherapy (Cheng et al., 2020; Li et al., 2020a; Liu et al., 2019b; Ochyl & Moon, 2019).

23.4.4 Other malignancy restorative modalities

Past the previously mentioned malignancy treatment strategies, many treatment methods, for instance, oncolytic virotherapy, so no dynamic therapy, and gas treatment SDT, as a noninvasive technique for disease treatment, quickly evolved as a ground-breaking option in contrast to customary photodynamic treatment because of its capacities to survive the lacks of deficient tissue entrance profundity and phototoxicity in photodynamic treatment. Like photodynamic treatment, the hypoxic tumor microenvironment has been the basic deterrent for enhancing the remedial impact. Thusly, tumor-focused O_2 conveyance frameworks [perfluorocarbon (Cheng et al., 2015), hemoglobin (Haney, Buehler, & Gulati, 2000), and so forth] or intratumor O_2 creation systems [MnO_2 (Zhu et al., 2016), catalase (Li et al., 2020b), and so forth] have been generally misused to adjust the oxygen-inadequate tumor microenvironment. In the arena of biomimetic sono dynamic therapy, Ag_2S quantum speck was wrapped in an RBC vesicle layer for increased sonodynamic therapy (Zhu et al., 2016). Ag_2S quantum speck was utilized as a sonosensitizer unexpectedly, in the interim, the decay of H_2O_2 in tumor cells was not just catalyzed by the red blood cell layer, yet additionally, long flow, great biocompatibility, and low immunogenicity of the nanosystem were guaranteed. Also, oncolytic virotherapy is another alluring technique for the treatment of disease utilizing replication-skilled infections to specifically imitate tumors and animates an antitumor insusceptible reaction to the fact that oncolytic virotherapy has presented extraordinary outcomes in both preclinical studies and clinical trials for cancer therapy, its curative effect is still restricted by the innate antiviral immune response and the nontargeted delivery of oncolytic adenoviruses. So far several endeavors have been successfully made for solving these problems. A robust antiviral immune shield with enhanced targeted delivery of oncolytic virus was attained by encapsulating oncolytic adenovirus with red blood cell membrane which was embedded with targeting ligands by genetic membrane engineering (Li et al., 2020b).

23.4.5 Cancer imaging and detection: diagnostic imaging

Avant-garde benefits in biomedical diagnosis and biological detection attributable to properties of the resolution, high sensitivity, multiple optical channels, and fast feedback have led to the development of novel fluorescent probes. Zhang et al. revealed malignancy cell layer pressed silver telluride quantum dot-based near-infrared II tests for improved in vivo tumor imaging with long blood flow time, splendid and stable fluorescence, and RBC layer covered imaging test for focused tumor imaging. (1) Arrangement of folic acid embedded RBC film covered upconversion nanoparticles. (2) Intravenous infusion with PBS or PBS constituting nanoparticles resulted in vivo upconversion radiance photographs of the tumor-bearing bare mice (Zhang et al., 2019b). The highest elevated fluorescence force with a high tumor-to-ordinary tissue proportion of 13.3 $\pm$ 0.7 was observed after intravenous infusion of the designed nanoprobes (Antaris et al., 2015). Also, near-infrared II fluorescent imaging is additionally a propitious imaging technique for cancer medical procedures route because of its high goal and sign clamor proportion (SNR), less tissue autofluorescence, and photograph dissipating (Zhu, Tian, Antaris, Chen, & Dai, 2019). PEG-coated rare-earth-doped nanoparticles and cancer cell film (CC-Nd@PEG) were created for the intraoperative tumor route in the near-infrared II window (Zhang et al., 2020). The authors reported the tumor-to-ordinary tissue proportion of the mice infused with CC-Nd@PEG as 4.73 $\pm$ 0.23 which when contrasted with infused Nd@PEG 2.20 $\pm$ 0.36 was impressively high. Also, the near-infrared II imaging-guided medical procedure was effectively directed with the help of CC-Nd@PEG. Likewise, the application of magnetic resonance imaging to clinical sickness findings is attributable to its nonintrusive, high difference in delicate tissues, high spatial goal, and staggered direct imaging (Jalandhara, Arora, & Batuman, 2011). To improve the pharmacokinetics and prevent conceivable nephrogenic fundamental fibrosis of Gd-based magnetic resonance contrast specialists, significant endeavors have been committed. RBC layer masked Gd-based polymeric nanoparticles presented longer flow time and, higher longitudinal relaxivity for ameliorated MRI representation (Nguyen et al., 2020). For tumor-focused bioimaging, a natural killer cell layer coated poly lactic-co-glycolic acid nanoparticle was fused with Gd-based magnetic resonance contrast specialists and near-infrared resonance fluorescent color (Pitchaimani et al., 2019). Pharmacokinetics and biodistribution examination indicated that natural killer cell film alteration enriched the biomimetic nanosystem with higher tumor aggregation (10% of the infused portion) and longer dissemination half-life ($\sim$ 9.5 h) in MCF-7 tumor-bearing mice. Besides, intravenous infusion of biomimetic nanosystem into the tumor-bearing mice directed ex vivo MRI. The improved magnetic resonance picture distinction was seen in tumor sites contrasted with that in encompassing delicate tissues, proposing the prevalence of biomimetic nanosystem in delicate tissue contrast by MRI.

23.4.6 Cancer imaging and detection: cancer recognition

Circulation of tumor cells in the peripheral blood circulatory system of cancer patients could proffer vital assessment data for early detection and identification of tumor cells, monitoring, and prognostics (Pantel & Alix-Panabières, 2007; Plaks, Koopman, & Werb, 2013). To date, innumerable innovations over the past decade to segregate circulatory tumor cells from the blood have been observed (Shen, Wu, & Chen, 2017). Among others, the immunomagnetic bead is the most ordinarily utilized technique utilizing a magnetic bead as a disengagement medium and antibodies on the outer surface of the magnetic bead for explicit binding to circulatory tumor cells. Albeit endorsed by FDA for clinical application, the proficiency of immunogenetic bead isn't exceptionally good because of the impedance from foundation cells, for example, white blood cells (Xiong et al., 2016). This difficulty has obliged analysts to set up new procedures to advance and refine circulatory tumor cells. Propelled by the new expositions about cell-cell cooperations and the improvement of cell membrane covering nanotechnology, the biomimetic immunogenetic bead has been employed to ameliorate the improvement proficiency of circulatory tumor cells. For instance, Electrostatic interactions mediated camouflaging of Fe_3O_4 nanoclusters with leukocyte layer which were additionally embellished with tumor-explicit neutralizer was reported (Hu et al., 2016). The vague leukocyte adsorption would be fundamentally smothered because of the way that homologous leukocytes don't shape bunches available for use. The subsequent biomimetic immunogenetic bead could catch $\sim$ 90% of uncommon tumor cells from the entire blood in 15 min with an imperceptible foundation from leukocytes. Furthermore, graphene nanosheets could likewise be coordinated with the high-polarization Fe_3O_4 nanoparticles utilizing the layer-by-layer get-together technique, which was additionally covered by CTC-focusing on immune response adjusted leukocyte films (Zhou et al., 2019). The high explicitness (catch proficiency: above 85.0%), upgraded hostile to leukocyte retention capacity (virtue: above 94.0%), and great affectability (as not many as three circulatory tumor cells in 1 mL of blood) were accomplished by the biomimetic immunogenetic bead, showing the leukocyte film cover could altogether decrease homologous leukocyte cooperation and acknowledge high-virtue circulatory tumor cell disconnection. More critically, the biomimetic immunogenetic bead was effectively applied to clinical disease patients' blood tests with great accuracy and repeatability (mean relative standard deviation 8.7% $\pm$ 5.6%). Notwithstanding single cell layer covering, half and half cell layer could be likewise utilized to cover MB. Rao et al. built up a bioinspired elite circulatory tumor cell segregation framework by combining platelet and leukocyte film, also, covering it onto magnetic bead just as adjusting circulating tumor cells focusing on antibodies on a superficial level (Rao et al., 2018). The subsequent hybrid membrane-coated immunomagnetic beads acquired the one-of-a-kind properties from source cells, for example, upgraded circulatory tumor cells restricting capacity through circulatory tumor cells-platelet cooperations, and improved circulatory

tumor cells' immaculateness by lessening homologous white blood cell-white blood cell collaborations. Contrasted with business immunogenetic beads, the circulatory tumor cell partition effectiveness of hybrid membrane-coated immunomagnetic beads was improved to 91.77% from 66.68%, also, the circulatory tumor cell virtue was improved to 96.98% from 66.53%. The leukocyte layer could likewise be combined with the tumor cell layer under a homologous tumor-focusing on capacity (Ding, Zhang, Cheng, & Xian, 2020). The biomimetic nanoplatform displayed proficient catch, simple seclusion, and delicate identification of circulatory tumor cells inferable from the improved restricting capacity toward circulatory tumor cells and decreased impedance from the foundation white blood cells. Additionally, this nanoplatform was effectively applied in the explicit acknowledgment and effective confinement of circulatory tumor cells in blood tests from people diagnosed with cancer.

23.4.7 Cancer imaging and detection: imaging-guided treatment

Imaging-guided malignant growth treatment has pulled into broad consideration lately by coordinating imaging tests and restorative specialists into one transporter, which accomplishes the exact area of tumors, constant checking of medication conveyance, furthermore, appraisal of remedial impact (Chen et al., 2017). Nanoparticles enveloped by cell layer can keep away from safe acknowledgment because of the "markers of self" on cell film, proffering "covertness" capacity to the incorporated nanoparticles. The exceptional biointerfacing capacities empower their utilization as focused vehicles for improved malignancy imaging-guided treatment. A biomimetic MR/NIR fluorescence double modular imaging-guided photodynamic treatment nanoplatform with superparamagnetic iron oxide nanoparticle as the MRI contrast specialist was designed, what's more, the stacked Ce6 produced NIR fluorescence for imaging and produced reactive oxygen species for photodynamic treatment (Li et al., 2018). The subsequent biomimetic nanoplatform displayed magnificent double modular imaging capacity, what's more, critical photograph prompted tumor cell executing impact with the assistance of malignancy cell film covering to upgrade the phone take-up of nanoparticles. Disease cell film shrouded PA/near-infrared resonance fluorescence double modular imaging-guided photothermal treatment nanosystem was additionally manufactured (Chen et al., 2016). Profited by the functionalization of disease cell film, the biomimetic nanosystem uncovered ongoing observation of in vivo powerful appropriation and tumor microstructure data with a high spatial goal and profound infiltration. Under near-infrared laser illumination, the biomimetic nanosystem displayed profoundly effective photothermal treatment execution to kill the xenografted tumor, demonstrating the incredible potential to be utilized for disease-focused on phototherapy and imaging. Careful resection is the principal line treatment technique for cancer patients, accordingly, cell-layer-based imaging-guided helpful nanoplatform assumed a significant part in a medical procedure to explore tumor treatment because of the following points of interest.

To begin with, the imaging tests enveloped by cell film can avoid the phagocytosis of the reticuloendothelial framework, delay blood flow time, and improve explicit aggregation to tumor locales. Secondly, tumor locales can be precisely found and tumor edges can be separated from encompassing ordinary tissues. Finally, the presentation of other treatment strategies can additionally dispense with lingering sores and metastases, in this manner decreasing the tumor repeat rate.

23.5 Bacterial diseases

Antibiotic resistance is considered perhaps the greatest danger of the 21st century by the World Health Organization and a potential calamity for human wellbeing and the worldwide economy as stated by the World Economic Forum. The growing resistance to antibiotics is alarming, subsequently the requirement for novel classes of antibacterial candidates. Microbes not just straightforwardly cause infections, for example, pneumonia, osteomyelitis, meningitis, sepsis, etc., yet additionally influence the restorative productivity of normal operations, particularly medical procedures and relocate medication. The poisons and metabolites delivered by pathogenic microbes assume hostile parts in causing tissue harm related to numerous irresistible infections. The antimicrobial treatment has for quite some time been the favored treatment for bacterial infections. In any case, the long haul and continuous utilization of antiinfection agents have caused increasingly more development of multidrug-resistant microscopic organisms. In this manner, creating elective therapeutics to annihilate bacterial contamination with high well-being furthermore, antibacterial action, in the interim, without bacterial obstruction, is critical to advance clinical interpretation. Alongside the extraordinary progression of cell layer-based nano, theranostics holds an extraordinary guarantee in antibacterial medicines

23.5.1 Targeted conveyance of antibacterial medications

Misusing focused on the conveyance of antibacterial specialists could fundamentally lessen the recurrence and dose of the drug, accordingly upgrading their bioavailability. Given the common pathogen-host connections, cell layer-based biomimetic nanosystems could achieve the point of augmenting antitoxin proficiency and diminishing medication opposition by focused conveyance of antitoxins into irresistible destinations. For instance, Angsantikul et al. determined layers from gastric epithelial cells and covered them with antitoxin stacked polymeric centers for focused antiinfection conveyance against *Helicobacter pylori* contamination (Angsantikul et al., 2018). The surface of the gastric epithelial cell layer acquired different receptors from source cells which had a solid restricting limit to *H. pylori* (Kaplan-Türköz et al., 2012; Parreira et al., 2014). The outcomes indicated a conspicuous burst delivering conduct of antitoxins in uncovered nanoparticles, while more delayed medication discharge from antibiotics from biomimetic nanoparticles,

which was credited to the reduced medication dissemination out of the polymeric network by film covering. Besides, in a mouse model of *H. pylori* disease, antibiotics from biomimetic nanoparticles exhibited more unrivaled remedial adequacy than free medication and were nonfocused on the drug conveyance framework. Acknowledging the on-request arrival of antibacterial medications in the sick destinations is critical to simultaneously limiting the fundamental poisonousness and upgrading the restorative impact of restorative specialists. Wang et al. covered bacterial pretreated macrophage layers onto the outside of gold-silver nanocages, which showed essentially drawn-out blood flow time and astounding biocompatibility (Wang et al., 2018a). Macrophages could perceive assorted unfamiliar organisms through the intricate receptors on their layers that are explicitly tied to microbe-related subatomic examples. The built bioinspired nanoplatform accomplished profoundly successful bacterial-focused on antimicrobials conveyance through the upregulated perceiving receptors on macrophage layers after pretreatment with explicit microbes. Moreover, the photothermal impact of macrophage layer covered gold-silver nanocages under NIR light couldn't just harm the pathogenic microorganisms, yet besides understanding an on-request arrival of antibacterial drugs, bringing about the unrivaled adequacy for treating limited bacterial disease.

23.5.2 Detoxification

Among the produced cytotoxic proteins, pore-forming toxins are the most widely recognized (Los, Randis, Aroian, & Ratner, 2013). These poisons annihilate cells by harming the uprightness of cell layers and to some extent disturbing the host resistant reaction (Edelson & Unanue, 2001). It has been shown that the hindrance of pore-forming toxins can diminish the destructiveness of pathogenic microbes (Hu, Fang, Copp, Luk, & Zhang, 2013; Wang et al., 2015). The subatomic constructions of toxins are different, consequently, it is exceptionally required to build up a comprehensively material detoxification stage that can kill poisons dependent on their capacity as opposed to their explicit atomic construction. Since the toxins principally interface with cell films, a biomimetic toxin nanosponge was intended to work as a wide toxin imitation (Chen et al., 2019). The nanosponge comprised of a polymeric nanoparticle covered by a red blood cell layer was equipped for engrossing and redirecting the layer harming the destructiveness of pore-forming toxins. In vitro studies affirmed the detoxification of a broad scope of pore-forming toxins regardless of their atomic structures, encompassing α-poison, melittin, and streptolysin-O. The nanosponge showed astounding detoxification viability against α-poison and improved endurance pace of poison-tested mice. The extraordinary layer poison proclivity demonstrated the biomimetic nanosponge would be widely investigated as an expansive range detoxification framework for bacterial disease. Despite its guarantee, the association between in vitro balance of toxins and in vivo helpful adequacy stays indistinct. The whole secreted proteins of methicillin-resistant *Staphylococcus aureus* were utilized to actuate lethality in mice and to examine the connection between

in vitro toxin balance and in vivo restorative adequacy (Wu et al., 2015). Right off the bat, they measured the capacity of the biomimetic poison nanosponge against the hemolytic movement of whole secreted proteins in vitro. At that point, they considered the concealment of whole secreted proteins-incited lethality with biomimetic toxin nanosponge utilizing a mouse model. The outcomes suggested that biomimetic toxin nanosponge presented essentially diminished whole secreted proteins-actuated lethality by killing the hemolytic movement. Besides, the decreased lung harm and brought down articulation of NF-kB in the spleen were exhibited in mice infused with whole secreted proteins preincubated with biomimetic toxin nanosponge, showing the high capability for detoxification application. Enlivened by the uncommon highlights and elements of miniature or nanorobots, a biomimetic engine wipe was designed by coating a layer of RBC film on the surface of a biocompatible gold nanowire engine. This engine wipe showed ultrasound-pushed development in undiluted entire blood. The designed wipe could adequately and quickly eliminate layer harming poisons from natural liquids because of the synergistic poison ingestion and an upgraded liquid vehicle. Other than a single RBC film covering, a half breed layer made out of platelet and RBC film was utilized to shroud acoustic gold nanowire-based nanorobot (Fig. 23.3) (De Ávila et al., 2018). The subsequent biomimetic sans fuel robot

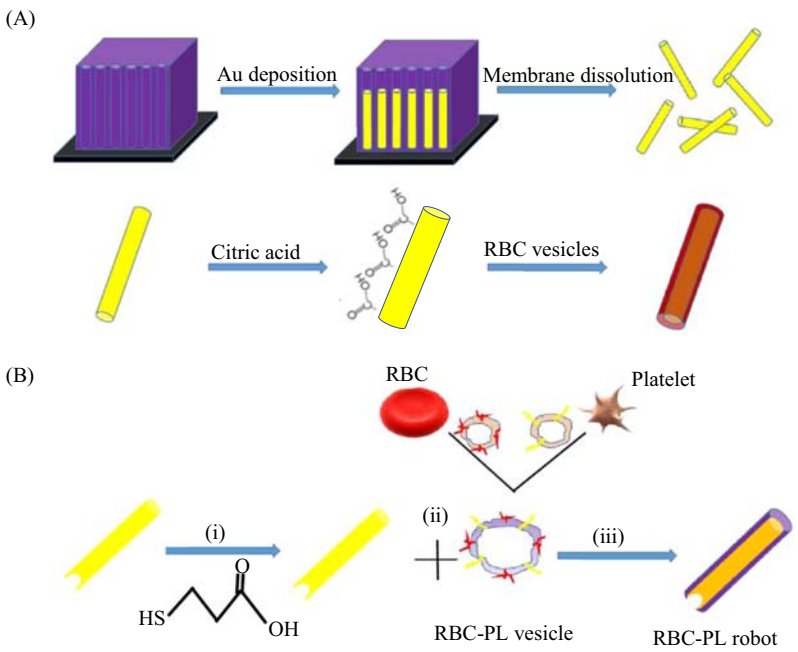

FIGURE 23.3

Acoustic gold nanowire-based nanorobot for detoxification.

had loads of organic capacities because of the different useful proteins acquired from source platelets and RBCs, for example, attachment and authoritative to platelet−following microbes (for example, *S. aureus*) and balance of pore-framing toxins. Also, the biomimetic nanorobot showed amazing impetus execution and against biofouling property, which permitted the robot to work in complex physiological liquids ceaselessly.

23.5.3 Antibacterial immunization

The advancement of antibacterial antibodies intends to decrease the reliance on antitoxins and hamper the outspread of drug-resistant bacterial strains (Atkins & Lipsitch, 2018). Until this point, various antibacterial immunizations have been authorized and demonstrated success in bringing down the horribleness and mortality related to bacterial microbes (Andre et al., 2008). The usually utilized antibacterial antibodies incorporate inactivated microorganisms, constricted microscopic organisms, or the subunit of microbes made of bacterial polysaccharides or proteins (Bundle, 2016; Cordeiro, Alonso, & de la Fuente, 2015; Micoli, Costantino, & Adamo, 2018; Pollard, Perrett, & Beverley, 2009). Even though these immunizations have indicated ground-breaking antimicrobial results, their inescapable use has far to go because the security of these nanoplatforms stays dubious. As of late, cell layer-based biomimetic nanomedicine has indicated an enormous guarantee in regulating antimicrobial resistance. The antimicrobial antibody was fabricated by gathering and coating the external film vesicles of *E. coli* onto the outside of gold nanoparticles (Gao et al., 2015). When subcutaneously infused into mice, the immunization made a trip to nearby depleting lymph hubs furthermore, quickly initiated the actuation and development of DC in lymph hubs. Besides, the antibody could evoke bacterium-specific T and B-cell reactions, guaranteeing a tough antibacterial insusceptible reaction. The outcomes demonstrated that bacterial film-based biomimetic nanoplatforms had the option to animate inborn invulnerability and create a solid antibacterial insusceptible reaction. To tackle the impediments concerning the solidness, versatility, and reproducibility of antibacterial antibodies, Wang et al. revealed nitrogen cavitation methods to produce film nanovesicle-based antibacterial immunizations. The nitrogen cavitation could rapidly produce nanovaccines if microorganisms (microbes) were distinguished, without warmth or substance harm to film proteins (Wang, Gao, Li, Wang, & Wang, 2018b). Also, the subsequent antibody involved twofold layered film vesicles and appeared successfully upgraded inborn and versatile invulnerability, consequently improving mouse endurance in *Staphylococcus aeruginosa*-instigated sepsis. The previously mentioned poisons can likewise be utilized for the improvement of pathogen antibodies (Angsantikul, Thamphiwatana, Gao, & Zhang, 2015; Hu & Zhang, 2014).

Conventional pathogen combination draws near include compound or warmth intervened detoxification measures of poisons, which may annihilate the protein structure, prompting traded-off immunogenicity and antigenicity. To dodge these impediments, biomimetic cell layer covering was utilized for immobilization and

killing of harmful antigens. A biomimetic self-moving micromotor for oral inoculation was designed by toxin embedded RDC layer and magnesium-based center, trailed by a layer of mucoadhesive chitosan and, enteric polymer responsive to pH alterations (Wei et al., 2019). The self-governing impetus qualities managed by the micromotor center added to the improved antigen conveyance, what's more, maintenance on the intestinal divider, in this manner coming about in advanced mucosal invulnerability. Later on, the cell membrane-based poison confinement technique might be stretched out to kill what's more, securely convey a broad scope of poisonous antigens.

23.6 Inflammatory sicknesses

23.6.1 Atherosclerosis

The trademark highlight of atherosclerosis is the arrangement of plaque, as a result of the aggregation of lipids, cells, and extracellular lattice under the inward mass of the vein (Falk, 2006; Lusis, 2000; Stehbens, 1975). The by and large asymptomatic nature at the beginning phase of the disease demands regular examination as complex plaques detected at the late phase of atherosclerosis are inclined to burst. The failure of present imaging modalities presents an extraordinary requirement for the development of imaging methodology for atherosclerotic plaques and injuries that can give organic data in the course of infection movement. In light of the normal cooperation of platelet with various parts during atherogenesis, a Platelet layer coated onto the PLGA center stacked with a far-red fluorescent color was recently reported (Wei et al., 2018). The designed nanosystem was proficient in exploring through flow and especially circulated in the atherosclerotic destinations. In vivo focusing on test additionally demonstrated binding of designed nanosystem exclusively to all around the created atherosclerotic plaque, yet additionally to preatherosclerotic locales with no noticeable infection sign. Contrasted with preadministration of MRI contrast specialist embedded biomimetic detailing, the atherosclerotic plaque at the aortic curve could be unmistakably distinguished after 1 h of organization, recommending its capacity of giving extra data to more readily survey the improvement of atherosclerosis. Present clinical settings generally administer oral medicine treatment to patients in the early phase. Notwithstanding, their remedial adequacy is undermined by the helpless pharmacokinetics and low circulatory half-life, accordingly requiring organization of a higher portion which brings genuine foundational results. The appearance of biomimetic nanotechnology proffers an unrivaled chance for tackling the situations looked at by customary medication conveyance frameworks. Rapamycin, a powerful enemy of atherosclerotic specialists, was stacked into PLGA, which was additionally shrouded with RBC film to get the center shell organized nanocomplex with an exceptionally functionalized biointerface (Wang et al., 2019). RBC film-covered nanoparticles effectively sidestep macrophage-interceded phagocytosis in the blood and aggregate in the set up atherosclerotic plaques, consequently figuring it out

supported medication delivering energy and compelling weakening of the atherosclerosis movement. Besides, the biomimetic nanocomplex showed a good security profile in the long-term organization, demonstrating its potential as a protected up-and-comer for persistent fiery sickness on the board.

23.6.2 Rheumatoid arthritis inflammation

Rheumatoid arthritis is a persistent immune system fiery confusion, portrayed by extreme synovial and fundamental irritation, bone disintegration, also, ligament harm (Firestein, 2003; Scott, Wolfe, & Huizinga, 2010; Smolen et al., 2018). The pathogenesis of rheumatoid arthritis is complex, including enactment and penetration of safe cells alongside the arrival of dangerous incendiary atoms into the synovium of ligament joints. The present clinical methodology for patients is for the most part dependent on the organization of sickness altering against rheumatic medications or immunosuppressive specialists. Regardless of much advancement has been made in treating RA, the remedial impact is as yet unsuitable due to helpless medication aggregation at the aroused site and the unpredictability of fiery organization. Late investigations found that cell layer-covered nanoparticles could extraordinarily lessen the resistant incitement capability of nanoparticles, which profited the treatment of immune system infections (Fontana et al., 2018). Ideal biomimetic nanocarriers were assessed against immune system infections, including the shape, steadiness, surface charge, and size of the biohybrid nanosystems, both in the cradle and in naturally important media (plasma and reproduced synovial liquid). Irritation and thickening of the synovial layers resulted in unrepairable harm to different components of the joint, at last, prompting agony and incapacity. The biomimetic nanomedicine-based calming technique has demonstrated success in lightening RA movement and seriousness. Neutrophils, a sort of polymorphonuclear leukocyte, have been viewed as the principal line of protection against irritation and tissue harm. Accordingly, neutrophil-like nanoparticles by intertwining neutrophil film with PLGA polymeric center were designed, and its utilization as an expansive range calming specialist for arthritis diagnosed patients were tested (Jin, Luo, Zhang, & Pang, 2018; Zhang et al., 2018b). Because of the acquired surface antigens from source cells, neutrophil-like nanoparticles had a receptor-mediated grip on cytokine-enacted chondrocytes. Remarkably, biomimetic nanoparticles could hinder the support of arthritogenic factors, infiltrate profoundly into the ligament framework, and give chondroprotection in aggravation incited ligament harm. In the mice model with incendiary joint inflammation, neutrophil layer covered nanoparticle demonstrated critical enemy of joint inflammation adequacy by killing diffusive arthritogenic factors furthermore, stifling by and large infection movement and seriousness.

Other than the previously mentioned superiorities, cell membrane camouflaged nanoparticles could likewise fundamentally improve the medication gathering in the aggravated joint districts of rheumatoid arthritis. Platelets assume a significant part in the immunological and, incendiary reactions, discovered to exist in plenitude in synovial fluid samples. To kill the provocative enhancement work of

platelets, a platelet layer has been utilized to cover the PLGA nanoparticle stacked with an immunosuppressive specialist for focused medication treatment of rheumatoid arthritis (Boilard et al., 2010; Shi et al., 2020). This investigation showed that platelet film covering permitted the nanoparticle to especially collect in the kindled synovial tissues through various instruments, like characteristic platelet. No undeniable paw growing, bone or ligament disintegration, nor any pannus tissue development in the medication stacked platelet mimetic gathering was observed, proposing that this methodology amazingly expanded the restorative viability against synovial aggravation, while decreasing the unfriendly results to nontarget organs. Contemplating the basic part of invulnerable cells in the provocative measure, it seems captivating to utilize safe cell membrane coated nanoparticles to be implemented in a wide scope of fiery illnesses.

23.6.3 Diabetes

Diabetes is a sort of persistent metabolic illness, described by hindered glucose resistance and hyperglycemia. Fiery measures appear to assume a significant part in the turn of events of diabetes and its late complexities, particularly in type II diabetes (He et al., 2018). Constant checking or fast recognition of blood glucose levels is urgent for the regulation of cancer. Enzymatic electrochemical detecting is normally utilized in blood glucose sensors because of its high affectability, quick reaction time, and ease of manufacture. Notwithstanding, the multitudinous segments in human blood, for example, proteins, cytokines, platelets, drug atoms, and particles, cause sensors to experience the ill effects of helpless selectivity when estimating entire blood or serum. Hence, it is profoundly expected to build up a glucose sensor with fundamentally improved selectivity just as without diminished affectability. It was accounted for that bountiful glucose carrier protein 1 (GLUT1) communicated on the RBC layer encouraged the dispersion of glucose over the cell film. Execution of permselective RBC film enhanced the selectivity of glucose sensors (Wheeler & Hinkle, 1985; Xie & Du, 2011). Contrasted with the uncoated sensor, RBC film covering could adequately forestall the entrance of meddling particles (for example, uric acid, ascorbic acid, galactose), while permitting the vehicle of glucose particles through GLUT1, exhibiting its boss selectivity and precision of glucose estimation. Furthermore, the RBC film-covered sensor indicated brilliant unwavering quality and long haul security at different glucose focus, even in human serum tests containing incalculable meddling particles. The methodology carries incredible guarantees in building up nonstop glucose observing framework for diabetic patients.

23.7 Conclusion

Biomimetic nanosystems have pronounced potentiality in enhancing the functionality of conventional nanoparticles. Emulation of attributes derived from source

cells, proffer capability of interaction with other cells by binding to sites of interest, overcoming the limitations and challenges involved in efficiency and compatibility of their delivery. The chapter summarizes and discusses the recent advances in the field of biomimetic nanosystems-based disease treatment. Howbeit, multiple challenges, and limitations need to be overcome including: (1) Preparation methodologies including microfluidic electroporation technique and physical extrusion being a sophisticated process that proffers low yield. (2) Scale-up manufacturing of nanosystems is relatively challenging and exploration of novel techniques high-yield, easy to implement attribute is important for the promotion of clinical impact and translation. (3) The biosafety of designed biomimetic nanotheranostics should be thoroughly assessed. (4) Problems with instability and penetration problem should be addressed.

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CHAPTER

Nanoformulations for cardiovascular therapy

24

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24.1 Introduction

Nanotechnology has also extended into the field of cardiovascular disease. Currently marketed nanoformulations of drugs are used in patients to help overcome challenges with drug solubility and absorption. Several delivery systems are under development to medicinally target pathways of vascular disease (Fig. 24.1).

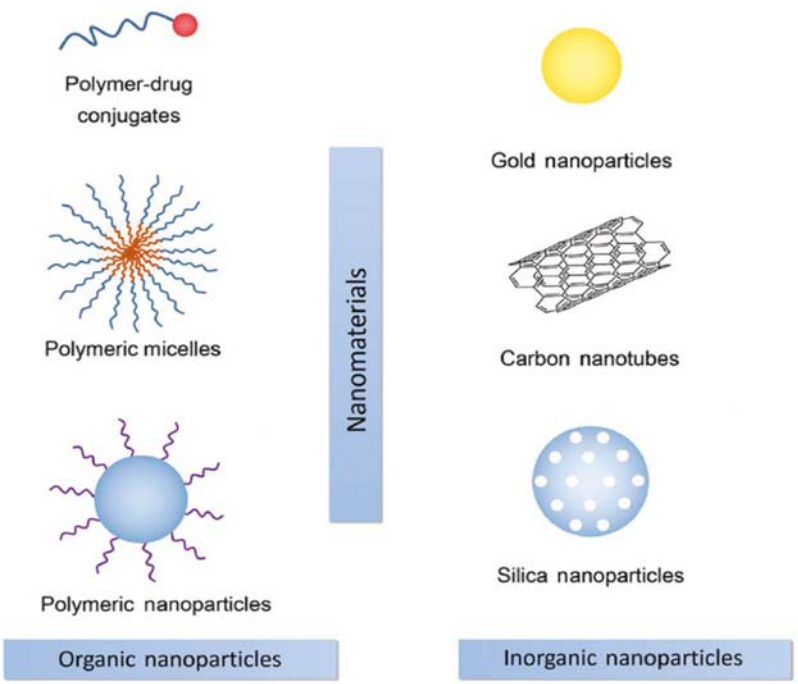

FIGURE 24.1

Nanomaterials representatives (organic/inorganic) applied for diseases therapy.

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Moreover, multifunctional theranostic nanoparticles are very promising for therapeutic delivery. These theranostic nanoparticles can serve to mix treatment with information from one or even multiple imaging modes to assess disease more comprehensively. Former work has highlighted the status of nanomaterials in cardiovascular imaging, including their potential to separately identify vulnerable signs at risk for rupture. This chapter will discuss advances in the nanoformulations application for vascular disease treatment.

24.1.1 Solving inflammation and defective efferocytosis

Atherosclerosis is an inflammatory disease characterized by the accumulation of lipids, diseased cells, and necrotic debris. Proinflammatory cytokines and leukocytes take action at different phases during atherosclerotic plaque formation (Libby, 2012).

Sharp inflammation is driven, in part, by the failure to clear apoptotic tissue from the diseased vessel wall due to a defect in efferocytosis (programmed cell removal), thus apoptotic cells accumulate, become secondarily necrotic, and discharge their proinflammatory intracellular fillings (Kojima et al., 2014; Kojima, Weissman, & Leeper, 2017). Importantly, this nonresolving inflammation leads to dangerous lesions that are at increased risk of rupture and thrombosis. Systemic antiinflammatory treatment is useful in stopping inflammation on cardiovascular disease in high-risk patients due to their ability to attain local delivery, atherosclerosis-targeted nanoparticles may be able to address these risks. Nanoparticles were able to overwhelm rapid elimination and short retention time of the free therapeutic agent in atherosclerotic plaques. Besides, their advantage on plaque progress and stability was importantly noted without side effects, indicated by normal clinical chemistry, hematology, and sustainability of mice following therapy (Wang et al., 2018).

Inflammation-targeting nanoparticles have also been created as theranostic nanoparticles. In a rabbit model of atherosclerosis, magnetic resonance imaging (MRI)−detectable liposomes were synthesized for prednisolone delivery to inflamed vessel wall (Lobatto, Fayad, & Silvera, 2010).

Liposomal encapsulation enhanced the pharmacokinetics of prednisolone and extended its circulating half-life, without general toxicity. After a single dose, rapid and continuous reduction in plaque inflammation was observed by MRI and correlated with 18F-FDG positron emission tomography/computed tomography—a certified method of tracing inflammation in atherosclerosis imaging (Rudd et al., 2008). Multimodal imaging showed that the nanoparticles stored in plaque macrophages without harmful effects, thus acting as a guide for imaging-based efficiency measure and showing the viability of aiming nanoparticles to human atherosclerotic areas.

Sager, Dutta, and Dahlman (2016) combined small interfering RNA (siRNA) targeting multiple cell adhesion molecules into a polymer-based nanoparticle. In apoE$^{-/-}$ mice that underwent coronary ligation, treatment with nanoparticles

encapsulating five siRNAs aiming leukocyte adhesion molecules meaningfully reduced vascular inflammation after myocardial infarction (MI) (Sager et al., 2016). The consequential decrease in leukocyte buildup led to a reduction in tissue damage.

24.1.2 Nanoparticles designed for cardiac regeneration

Nanomedicine can be defined as the application of nanotechnology to medicine for diagnosis and therapy (Pelaz et al., 2017). It aims to minimize the side effects of therapeutic drugs while increasing their selective accumulation, thus enhancing the ability of the treatment in clinics (Davis, Chen, & Shin, 2008). Traditional therapies are—in fact—often related to great side effects due to the natural toxicity of drugs, their broad spectrum of activity and the poor control over delivery (Jabir et al., 2012). Nanoparticles NPs have tunable properties that potentially allow any kind of application. Various types of NPs have been loaded with miRNAs and drugs to be used to transport therapeutic agents by different administration routes, offering several advantages compared to normal therapies (Fig. 24.2). Remarkably, a major drawback in the therapeutic use of miRNAs is their quick clearance and fast degradation in blood circulation and cellular cytoplasm mainly by ribonucleases, resulting in a short half-life (Sioud, 2005).

Furthermore, these molecules cannot enter the cell efficiently (Zhang, Zhao, Jiang, Wang, & Ma, 2007). Extracellular miRNAs are physiologically taken inside the cell by membrane-derived vesicles, lipoprotein and ribonucleoprotein complexes (Boon & Vickers, 2013). Among these systems, exosomes are the main effectors of miRNA carriage and exosome miRNA-loaded release has been

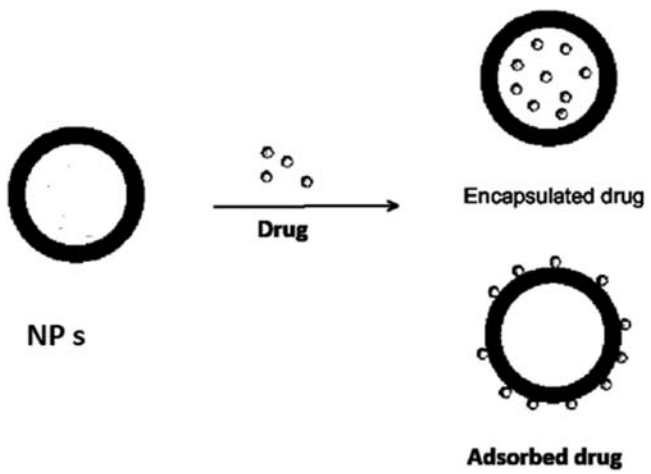

FIGURE 24.2

Schematic diagram of drug-loaded nanoparticles.

found to be participating in intercellular communications (Valadi et al., 2007). Therefore, the use of engineered miRNA nanocarriers represents a nature-inspired approach overcoming many limitations.

Along with miRNA delivery, the use of bioengineered nanocarriers can improve the circulation time, biodistribution and bioavailability of different drugs and proteins, as well as protecting them from degradation and inactivation (Patra, Das, Fraceto, Campos, & Rodriguez-Torres, 2018). Indeed, many currently available drugs are lipophilic and their systemic administration is faced by their limited aqueous solubility, with subsequent poor delivery and therapeutic effectiveness (Kalepu & Nekkanti, 2015). Consequently, the encapsulation of these molecules inside amphiphilic systems may enhance their efficacy and their long-lasting and sustained release at the desired site (Din et al., 2017).

24.1.3 Polymeric nanoparticles

Polymeric NPs have recently attracted interest for their great tunable properties, which make them extremely remarkable tools for controlled drug encapsulation/release (Fig. 24.3). Indeed, their physicochemical properties can be adjusted for accommodating nucleic acids, drugs, and proteins to improve their efficient release inside the cells (Patil & Panyam, 2009). This large group of NP based system include amphiphilic micelles, vesicles, dendrimers and polymersomes having unique structures and properties, which can be adjusted for hosting different kind of carriers (Chandarana, Curtis, & Hoskins, 2018). Most of the designed polymeric NPs offer new synthetic copolymers able to mix different functionalities such as targeting and selective carrier delivery systems (El-Say & El-Sawy, 2017). Moreover, given the developing use of miRNAs for cardiac regeneration many studies had developed polymeric NPs as miRNA carriers, alone or in combination with targeting therapeutic drugs.

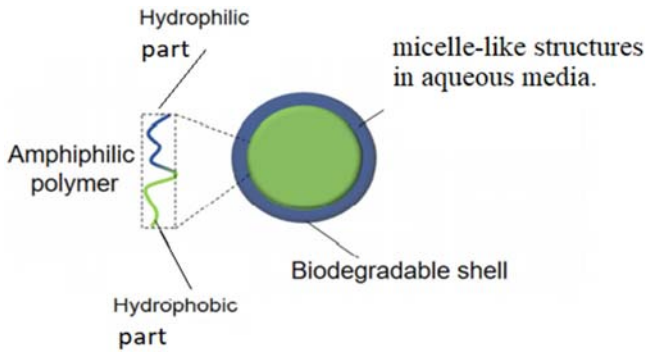

FIGURE 24.3

Schematic diagram of polymeric nanoparticles with overall composition and structure.

24.1.4 Targeting thrombosis

Platelet activation, the coagulation force, and fresh thrombus include unique factors that enable targeted delivery of therapeutic agents. Thrombus-targeted nanoparticles have been established for anticoagulants and thrombolytic agents delivery, which lead to vessel recanalization and reperfusion in most animals.

Nanoparticles were designed for controlled release of tPA (tissue-type plasminogen activator) using transthoracic ultrasound, where the application of ultrasound led to greater tPA offloading and thrombolytic activity at the affected artery. Also, it was found that antithrombin theranostic nanoparticles directly attenuated plaque coagulant activity within the injured arteries (Palekar, Jallouk, Myerson, Pan, & Wickline, 2016).

Investigated by magnetic resonance spectroscopy, nanoparticles were retained within the plaques and exerted rapid inactivation of any locally produced thrombin. These effects were observed without changing activated partial thromboplastin time or other general effects on coagulation.

Moreover, central inhibition of plaque thrombin reduced the expression of plaque inflammatory molecules and enhanced repair of the disrupted vascular endothelium, suggesting the antithrombin nanoparticles supported plaque stability. These studies proved the broad perspective that nanoparticles have for reperfusion therapy and anticoagulation with decreased bleeding consequences.

Biodegradable Polymers have found extensive applications in cardiology as scaffolds and coating matrices for drug-eluting stents (DES). Since polymers degrade once their function is fulfilled as they are required to fulfill special demands with their specific properties and biocompatibility.

In the past, synthetic polymers, like poly(ethylene) (PE), polyurethanes (PUR), poly(glycolide) (PGA), and polylactides (PLA), have been chosen for implants and other medical devices. While PURs are well established as scaffold materials for vascular grafts due to their excellent hemocompatibility (Han, Farah, Domb, & Lelkes, 2013; He, Hu, & Xu, 2013; Hu, Li, & Hu, 2012; Theron, Knoetze, & Sanderson, 2010), PGA is commonly used as a suture material for different surgical applications (Pillai & Sharma, 2010).

Further, PGA-containing scaffolds combined with poly($\in$-caprolactone) (PCL) (Diban, Haimi, & Bolhuis-Versteeg, 2013) are used for PGA-based drug delivery systems (Amjadi, Rabiee, Hosseini, & Mozafari, 2012; Yehia, Elshafeey, & Elsayed, 2012; Yi, Wu, & Jia, 2006). Overall, PLA has been strongly verified as temporary stent material in cardiology due to its long pathway records of in vivo biocompatibility (Bourantas, Papafaklis, & Kotsia, 2014; Onuma, Dudek, & Thuesen, 2013; van Alst, Eenink, Kruft, & Van Tuil, 2009).

Biopolymers coming from natural origin degrade physiologically by hydrolysis and are considered to be very biocompatible (Sisson, Schroeter, Lendlein, Lendlein, & Sisson, 2011). Typical examples of biodegradable polymers are polyhydroxy carboxylic acids, such as PGA, PLA, poly(3-hydroxybutyrate) (P(3HB)), poly(4-hydroxybutyrate) (P(4HB)), and PCL. P(4HB) is applicable for vascular

grafts and heart valves (Williams, Rizk, & Martin, 2013), while P(3HB) has not been approved for vascular applications due to its competency to activate extensive inflammatory responses in porcine models (van der Giessen, Lincoff, & Schwartz, 1996).

DES are specialized vascular stents which allow drugs local delivery in a controlled manner to reduce or prevent in-stent restenosis as a process of increased SMC proliferation (Grube, Gerckens, Muller, & ¨ ullesfeld, 2002; Kukreja, Onuma, Daemen, & Serruys, 2008). Biodegradable polymers, such as PLA and poly(lactide-co-glycolide) (PLGA), were extensively studied to optimize their properties and biocompatibility. Due to the degradation of the polymeric coatings, DES are expected to cause lower stent-thrombosis.

Fully biodegradable scaffold advantage is to provide a temporary mechanical support of narrowed blood vessel. In consequence, the vessel is allowed to heal and recover its physiological function before the implant loses its mechanical integrity (Iqbal, Gunn, & Serruys, 2013). Clinically approved scaffolds are mostly based on PLA (Iqbal et al., 2013). So far, the first clinically available scaffold is provided with a poly(L-lactide) (PLLA) frame and a poly(D,L-lactide) coating carrying Everolimus (BVS, Abbott, United States).

Biodegradable polymers can be produced from natural polymers and synthetic polymers for cardiac tissue engineering (Vroman & Tighzert, 2009). These kinds of polymers offer their advantages and disadvantages in cardiac tissue engineering. Thus, to gather the advantages of both natural and synthetic polymers, natural/synthetic composites have been proposed for some cardiac tissue applications (Hasan, Khattab, & Islam, 2015).

24.2 Natural polymers

Natural polymers are coming from natural sources, such as animals and plants, and since they are natural, they are composed of nanostructured proteins and are considered nanomaterials (Garbayo, Pascual-Gil, Prosper, & Blanco-Prieto, 2017). Due to their biodegradability, renewability, and abundant availability, they have been used in diverse applications in tissue engineering (Puoci, 2015). Some typical natural biodegradable polymers used for cardiac tissue engineering include fibrin gel, collagen, gelatin, chitosan, alginate, and Matrigel (Dhandayuthapani, Yoshida, & Maekawa, 2011).

24.2.1 Collagen

Collagen is one of the most common high-weight molecular natural polymers or proteins used for cardiac tissue engineering (Fig. 24.4). Collagen has advantages including thermal reversibility, biocompatibility, and strong cellular activities for cardiac tissue engineering efforts. Collagen has many different types (28)

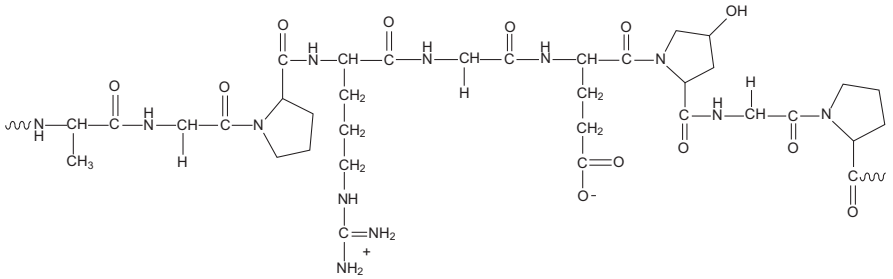

FIGURE 24.4

Structure of collagen.

discovered from various human tissues such as bone, skin, ligaments, tendons, and cartilage (Ricard-Blum, 2011).

However, collagen types I, II, III, and IV are commonly studied in tissue engineering (Nair & Laurencin, 2005), among these types of collagen, type I has been the most commonly used in tissue engineering due to its suitable biocompatibility (Ariganello, Labow, & Lee, 2010; Mirsadraee, Wilcox, & Korossis, 2006; Mirsadraee, Wilcox, & Watterson, 2007; Ravichandran, Islam, & Alarcon, 2015; Xu, Molnar, & Gregory, 2009; Yang, Motte, & Kaufman, 2010).

Collagen is one of most favorite biodegradable polymers in cardiac tissue engineering applications due to its high biodegradability, biocompatibility, low toxicity and hyposensitivity (Punnoose, Elamparithi, & Kuruvilla, 2015). Collagen scaffolds, especially nanofibrous scaffolds, have been examined for cardiac tissue engineering. Joanne et al. examined electrospun collagen scaffolds with biologically compatible solvents and cross-linking agents. This nanofibrous collagen scaffold was planted with human-induced pluripotent stem cell-derived cardiomyocytes and was epicardially delivered in a mouse model of dilated cardiomyopathy. In vivo and in vitro results indicated that a human induced pluripotent stem cell-derived cardiomyocyte seeded electrospun scaffold was a potent biomaterial for the stabilization of dilated cardiomyopathy and thus suitable for future clinical use (Joanne, Kitsara, & Boitard, 2016).

Collagen scaffolds have drawbacks that include low mechanical properties upon hydration. One way to improve the mechanical strength of collagen-based scaffolds is intermolecular cross-linking of collagen scaffolds via chemical and physical methods (Dong & Lv, 2016).

Lin et al. examined stiffness-controlled 3D collagen type I scaffolds for the differentiation and proliferation of mesenchymal stem cells into cardiac progenitor cells. To obtain the stiffness required in 3D collagen type I scaffolds, collagen was cross-linked with different ratios of hydroxyl succinimide (NHS) and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) cross-linkers. Results showed that the collagen scaffolds cross-linked with 50/50 EDC mM/NHS mM cross-linkers not only demonstrated a higher Young's modulus but also showed a better

interconnectivity. Another way to improve the mechanical strength of collagen scaffolds is by blending collagen with other materials, such as inorganic materials and natural synthetic polymers (Dong & Lv, 2016).

24.2.2 Chitosan

Chitosan is a linear polysaccharide (Fig. 24.5) derived from chitin by partial deacetylation (Dutta, 2016; Hudson & Smith, 1998). Chitosan is characterized by low toxicity and high biocompatibility due to its structural similarity to natural glycosaminoglycans. Chitosan biodegrades into nontoxic products through enzymatic hydrolysis during in vivo tissue applications. Chitosan, and its derivatives, increase cell recognition and cytocompatibility for tissue-engineering applications, and are often coated or grafted onto scaffold surfaces (Deng, Li, Griffith, Reul, & Suuronen, 2010).

Chitosan hydrogels have also been examined as scaffolds for cardiovascular applications. For example, Aussel et al. investigated chitosan-based hydrogels for developing a small-diameter vascular graft. Results from in vivo studies in different animals and in vitro studies of chitosan-based hydrogels proved the good hemocompatibility properties in vivo and in vitro biocompatibility of hydrogels (Aussel, Thébaud, & Bérard, 2017).

24.2.3 Alginate

Alginate is a natural linear polysaccharide (Fig. 24.6) which is obtained from the cell walls of brown algae (Slaughter, Khurshid, & Fisher, 2009). Alginate is likely a biodegradable polymer for cardiac tissue engineering due to its high biodegradability, nontoxicity, and biocompatibility, as well as its physical gelation process, and nonthrombogenic nature (Rosellini, Cristallini, & Barbani, 2009).

Several methods as crosslinking, immobilization of specific ligands, and the conjugation of other materials have been used for modification of mechanical properties of alginate (Sun & Tan, 2013). Sondermeijer et al. modified an alginate-based scaffold attached covalently with synthetic cyclic RGDfK (Arg-

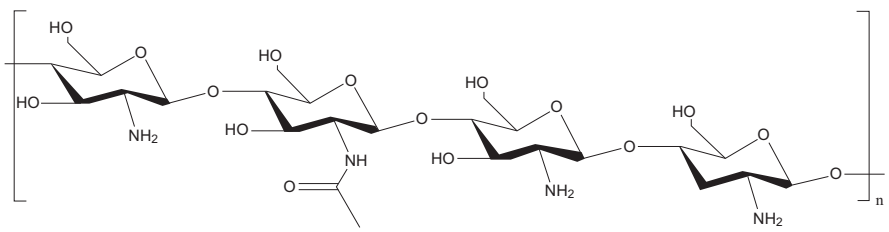

FIGURE 24.5

Structure of chitosan.

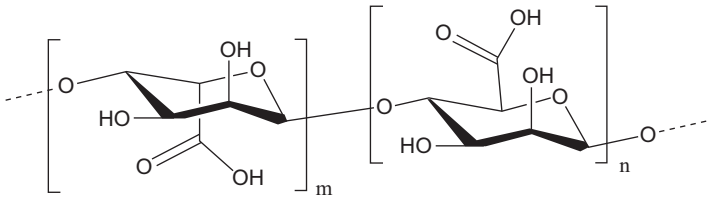

FIGURE 24.6

Structure of alginate.

Gly-Asp-D-Phe-Lys)-peptide to improve the survival of transplanted cells and angiogenesis in damaged myocardium tissue. The modified scaffolds were investigated in rats, it was observed that these scaffolds enhanced cell viability (Sondermeijer et al., 2017).

However, these natural polymers have some drawbacks such as rapid degradation, insufficient electrical conductivity, immunological reaction, and poor mechanical properties for cardiac tissue engineering (Ige, Umoru, & Aribo, 2012).

24.3 Synthetic polymers

Synthetic biodegradable polymers including PLA, PGA, PLGA, polyethylene glycol (PEG), PUR, PCL, and poly(*N*-isopropyl acrylamide) have all been used in cardiac tissue engineering applications. Synthetic biodegradable polymers are considered potential materials for cardiac tissue engineering due to their attractive physical and chemical properties such as strong mechanical properties, controlled structure, great processing flexibility, and no immunological fears (Guo & Ma, 2014).

24.3.1 Poly(ethylene glycol)

PEG, a common synthetic biodegradable polymer, was synthesized by ring-opening polymerization of ethylene oxide and has been used as a tissue engineering biomaterial (Zhu, 2010). PEG properties, like solubility in water and organic solvents, nontoxicity, low cell adhesion, and protein binding, made it a suitable applicant for tissue engineering (Alcantar, Aydil, & Israelachvili, 2000). However, the PEG polymer is bio-inert, thus, it is not an ideal environment for cell growth, adhesion, and survival (Rane, Chuang, & Shah, 2011). PEG-based gels are usually used in cardiac regeneration approaches because they have advantages over natural hydrogels like; easy control of chemical composition and scaffold architecture, and adjustable mechanical properties (Zhu, 2010).

Somekawa et al. examined the effect of thermoresponsive PLA−PEG gel injection in a rat MI Model compared with an alginate gel. Results proved that the PLLA-PEG/ PDLA-PEG and alginate gel well-maintained cardiac function (Somekawa, Mahara, & Masutani, 2017).

24.3.2 Polycaprolactone

PCL is a synthetic biodegradable polymer prepared by the ring opening polymerization of ε-caprolactone (Mclauchlin & Thomas, 2012). This material is one of the most widely used synthetic polymers in tissue engineering due to its excellent elasticity, mechanical properties, toughness, and biocompatibility. PCL is a suitable biodegradable polymer for applications where a long degradable time is needed because it has a long degradation time (about 2−3 years) (Mano, Sousa, & Boesel, 2004).

However, PCL exhibits a long degradation time which it is an obstacle for use in cardiac tissue engineering (Song, Ahmed, & Li, 2017). Two strategies have been used for the modulation and enhancement of mechanical and degradation properties of PCL: the incorporation of nanostructured filler material into the PCL material and the use of PCL as a copolymer or as one of the components of a blended material (Mondal, Griffith, & Venkatraman, 2016).

Ghaziof et al. synthesized PCL/multiwalled carbon nanotube (MWCNT) composite scaffolds, with different amounts of MWCNTs. Results showed that the addition of the MWCNTs in the nanocomposite scaffolds enhanced mechanical properties (Ghaziof & Mehdikhani-Nahrkhalaji, 2017).

24.3.3 Poly (lactic acid)

PLA, a hydrolyzable aliphatic semicrystalline polyester polymerized by lactic acid, is a biodegradable synthetic polymer that has a variety of applications in tissue engineering (Bertuoli, Ordoño, & Armelin, 2019). PLA has a low degradation rate and is more hydrophobic than PGA (Kannan, Salacinski, & Butler, 2005). PLA has been widely studied for cardiac tissue applications because of its biocompatibility, biodegradability, nontoxicity, and good mechanical integrity (Bertuoli et al., 2019). However, the long degradable time of PLA is the main drawback of its use in cardiac tissue applications (Lopes, Jardini, & Maciel Filho, 2012).

The porous nanofibrous PLA scaffolds for the construction of cardiac tissue with CPCs were developed. The CPCs derived from mouse embryonic stem cells were seeded into scaffolds to engineer cardiac constructs in vitro and were implanted subcutaneously in nude mice (Valente et al., 2016).

The combination of PLA/(polyaniline) PANI can be an important candidate for cardiac tissue engineering applications. Electrospun conductive nanofibrous PLA/PANI scaffolds (diameter of the nanofibers were about 500 nm) were

developed by Wang, Wu, and Hu (2017). This nanofibrous sheet acts as a promising potential in cardiomyocyte-based 3D bioactuators and cardiac tissue engineering.

Synthetic polymers, however, have some drawbacks such as a lack of cell attachment and less biocompatibility in cardiac tissue engineering (Do, Khorsand, & Geary, 2015).

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CHAPTER

Advanced nanoformulations for neurological therapeutics

25

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25.1 Introduction

Disorders and diseases of the central nervous system (CNS) have become more widespread due to the aging population (Kanwar, Sriramoju, & Kanwar, 2012; MedlinePlus, 2020). Several conditions affecting the CNS include neurodevelopmental disorders, tumors, stroke, neurodegenerative disorders, as well as traumatic injuries (Bellotti, Schilling, Little, & Decuzzi, 2021; Kanwar et al., 2012). These conditions affect millions of people with no age discrepancy leading to distressing consequences owing to advanced cell degeneration, cell death, and eventually, permanent disabilities and death (Ballios, Baumann, Cooke, & Shoichet, 2011; Bellotti et al., 2021). Moreover, the CNS has unique anatomical and physiological features that weaken the therapeutic efficacy of existing therapies (Bellotti et al., 2021). In numerous cases, many people don't even know they have these diseases, and therefore understanding their symptoms is very important to achieve a proper diagnosis and effective treatment (Zhou, 2019).

Regardless of the various technological advancements, the delivery of drugs such as nucleic acids, proteins, imaging agents, and other macromolecules to the CNS is still challenging because of the limitation of the drug molecules to access the CNS in therapeutic concentrations. The microvessels for the nervous system are a hindrance to the passage of molecules to the brain. This is nature's way of protecting toxic and infectious substances from reaching the nervous system. The main challenge has been attributed to the existence of factors such as the blood-brain barrier (BBB) and the blood-cerebrospinal fluid (BCSF) barrier (Barchet & Amiji, 2009; Pakulska, Ballios, & Shoichet, 2012).

Generally, the BBB, which is formed by the brain capillaries endothelial cells and their endocytic activity tight junctions, acts natural barrier that safeguards the brain and controls the homeostatic, nutritive, and immune environment of the CNS besides regulating the interchange of molecules between the CNS and the

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blood (Banks, 2009; Scherrmann, 2002). Though the BBB prevents the entrance of possibly toxic substances into the brain, it also restricts the passageway of traditional therapeutic molecules (Banks, 2009; Scherrmann, 2002). For the blood-cerebrospinal fluid barrier (BCSFB) on the other hand, the stroma having fenestrated blood vessels underneath the endothelial cells permits large molecules to diffuse from the capillaries via the fenestrae but the tight junctions of the epithelial cells block them (Barchet & Amiji, 2009; Scherrmann, 2002). The relative impermeability of the BBB and BCSFB is due to the tight junctions and adherents' junctions between capillary endothelial cells. Naturally, the transport system of the nervous system has limited transport pathways such as fenestrations, intercellular gaps, transendothelial channels, and pinocytotic vesicles. This selective transport system usually is for active efflux. The BBB and BCSFB are charged with maintaining the essential brain homeostasis however, it's a major challenge in the treatment of many nervous system diseases (Kasinathan, Jagani, Alex, Volety, & Rao, 2015). Therefore, there is a need to have a delicate balance between the selective uptake of the nanocarriers through the barriers, maintenance of the integrity of the BBB and BCSFB that can be affected by the vs membrane binding that can cause nanoporation leading to side adverse effects and passage of harmful substances to the nervous system.

Most of the systems for targeting the nervous systems are hypothesized that when they are administered, they will reach the site of action unchanged, bind to the needed site then elicit the intended action. Depending on the distribution this might not be the case. The delivery systems could undergo modification as they travel through different body organs such as the lung, stomach, blood, and different barriers (Hamidi, Azadi, & Rafiei, 2013). As the delivery system comes in contact with plasm proteins this could affect the surface charge, may also experience an increase in its particle size, and the surface functional groups could react with different substances or adsorb surfaces that could occlude them (Karmali & Simberg, 2011). Moreover, the residency time of the delivery system in the blood affects the stability. The longer they stay in circulation the more likely they degrade (Dou et al., 2020). Therefore, the challenge of formulating nervous system-directed nanocarriers is to design systems that can withstand degradation and metabolism until they reach the site of action.

Current strategies used to bypass these natural biological barriers including surgical methods or direct injection of the therapeutic molecules into the CNS (Hoang & Nimjee, 2019; Stockwell, Abdi, Lu, Maheshwari, & Taghibiglou, 2014) are invasive and can intensify the danger of damaging the neurons as well as causing local inflammatory reactions (Kasinathan et al., 2015). It is therefore very important to develop methods with minimal invasion which can precisely deliver the targeted drugs into the CNS for efficient and effective treatment of CNS disorders. A nanoscaled drug delivery system has been reported as a promising method to evade these challenges as well as the barriers (Chhabra, Tosi, & Grabrucker, 2015) and accessing the remote parts of the brain (Sriramoju, Kanwar, & Kanwar, 2014). The nanosized drug carriers have shown the incredible capability

of delivering therapeutic and diagnostic aids after systemic administration when compared to conventional methods due to their extraordinary drug loading capacity, directed action, minimal toxicity, and improved therapeutic effect (Chhabra et al., 2015; Sriramoju et al., 2014). This book chapter, therefore, focuses on nanosystems for drug delivery across the BBB and BCSFB into the CNS for the treatment of neurological disorders. Specifically, the chapter will provide the current status of therapeutic nanomaterials and nanoformulations for neurological therapeutics. Future directions such as improving their permeability and reducing their neurotoxicity will also be addressed.

25.2 Biomaterials for formulating nanosystems for drug delivery across the nervous system

The application of materials in the nanoscale range to serve as means of diagnostic tools or to deliver therapeutic agents to specifically targeted sites in a controlled manner as well as enhance the properties of conventional drugs is a rapidly growing technology (Patra et al., 2018). They have continued to gain research interest in the modern medical sector due to their innovative implications in drug delivery and gene therapy (Prasad et al., 2018) due to their small size, relatively close to that of proteins, nanomaterials can move freely in the human body as compared to bigger materials and easily cross the different barriers immunity (Al-Hatamleh et al., 2019; Patra et al., 2018). Due to their high surface-area-to-volume ratio, nanomaterials can change the basic properties and bioactivity of drugs.

Enhanced pharmacokinetics and biodistribution, reduced toxicities, enriched solubility and stability, controlled release, and site-specific distribution of therapeutic agents are some of the features that nanocarriers can integrate with drug transport systems (Din et al., 2017). Hence they have been used in nanoformulations of medicinal drugs since they can offer enhanced efficacy of drugs as well as allow therapeutic agents to be specifically directed on an organ, specific cells, and tissues thereby lessening the exposure of healthy tissues to drugs (Jeevanandam, Chan, & Danquah, 2016; Prasad et al., 2018).

Various therapeutic nanomaterials have been approved by the food and drug administration as nanocarriers and nanoformulations for the treatment of neurological diseases such as Parkinson's disease, dementia, and Alzheimer's disease (AD) among others (Bhaskar et al., 2010; Prasad et al., 2018) and hundreds of nanocarrier based products are presently accessible at several stages of the preclinical and clinical development (Webster, 2008). In the next section, we discuss various nanoformulations gaining eminence in the pharmaceutical industry for improved drug formulation. Specifically, we focus on polymeric nanoparticles (NPs), solid lipid NPs (SLNs), nanostructured lipids, and nanoemulsions used as nanocarriers and nanoformulations in therapeutics of neurological disorders.

25.2.1 Polymeric nanoparticles

Polymeric NPs are particles with a size ranging from 1 to 1000 nm that can be loaded with active compounds trapped within or surface-adsorbed onto the polymeric core (Zielińska et al., 2020). Advantages associated with the uses of polymeric NPs as drug carriers include their prospective use for controlled release, the capability to guard the drug and other molecules with biological activity against the environment, as well as the advancement of their bioavailability and therapeutic index. They have also shown a possibility to both encapsulate drugs and thereby guarding them against excretion and metabolism, and to transport active agents across the BBB without inflicting any damage to the barrier. Natural polymers (biopolymers) can be attained from a range of renewable resources and can be degraded into other molecules such as water, carbon dioxide, and small inorganic molecules hence denoted as environmentally friendly (Han et al., 2018; Tosi, Costantino, Ruozi, Forni, & Vandelli, 2008). Since some of these polymers already exist in nature, their production entails extraction followed by synthesis. This may include a combination of fermentation, filtration, compounding/granulation, hydrolysis, esterification, poly-condensation, oxidation, and dehydration. In the next section, we discuss the commonly used natural polymeric materials including chitosan, starch, alginate (ALG), cellulose, and dendrimers.

25.2.1.1 Chitosan

Chitosan, a nontoxic biopolymer, is gotten from several sources such as crustacea shells, insect cuticles, and some fungi cell walls by the deacetylation of chitin (Qi, Xu, Jiang, Hu, & Zou, 2004). It is a straight-chain amino polysaccharide with several features such as adsorbability, antimicrobial activity, permeability, and moisture retention making it promising for use in drug delivery (Abd El Hady, Mohamed, Soliman, & El-Sabbagh, 2019; Han et al., 2018). Chitosan derivatives such as glycol chitosan, thiolated chitosan, and arginine-chitosan among others have also been explored for drug delivery (Malik, Gupta, Gupta, Gogoi, & Bhatnagar, 2018; Tsai, Chen, Lin, Ho, & Mi, 2019). The processes through which these derivatives are obtained include acylation, alkylation, sulfation, hydroxylation, and carboxymethylation among others (Han et al., 2018).

Starch, one of the most abundant natural polymers, is one of the most commonly used polymers in drug delivery due to its hydrophilicity, biodegradability, and biocompatibility with tissue and cells. Starch NPs with particle sizes ranging between 10−1000 nm have been widely studied as controlled release nanocarriers. For example, curcumin-loaded starch NPs exhibited enhanced solubility in an aqueous solution as compared to free curcumin, and curcumin was observed to release out from starch NPs in a sustained way under physiological pH for 10 days (Chin, Mohd Yazid, & Pang, 2014). Starch NPs have also been shown to increase the effectiveness of drugs capability (Ismail & Gopinath, 2017).

25.2.1.2 Cellulose

Cellulose is a linear-chain homopolymer consisting of D-glucose monomers that are covalently linked via a β-1−4 glycosidic bonds with an intra- hydrogen bonding experienced between oxygen and hydrogen atoms of the neighboring glucose molecules linked by the glycosidic bond within the polymer (Wood, 2013). In addition, parallel stacking also known as cellulose elementary fibrils is promoted by interchain hydrogen bonds along with van der Waals forces.

Cellulose nanomaterials are of growing interest due to their attractive intrinsic properties such as biodegradability, extraordinary surface area, lightweight, chirality, and the capacity to form efficient hydrogen bonds through the cellulose chains or inside other polymeric matrices (Tayeb, Amini, Ghasemi, & Tajvidi, 2018). Nanocellulose in form of cellulose nanocrystals and cellulose nanofibrils (CNFs) obtained from animals, plants, fungi, and bacteria using several methods including hydrolysis, oxidation, and/or mechanical decomposition of biomass and cultivation of gram-negative bacteria suspended in water for hydrogel production have been reported (Kaja Kupnik, Vanja, & Maja, 2020). For example, CNF/quercetin (QT) nanoformulations have been prepared with the nanoformulation exhibiting enhanced dietary performance and antioxidant activity than QT (Li et al., 2018a). Moreover, sustained release of QT was demonstrated in vitro indicating that CNF is an ideal natural nanoscale dietary carrier and offers high encapsulation efficiency for healthcare. Nanocelluloses have also been used in wide medical applications such as wound dressing owed to their anti-infection features and their capability to increase tensile properties of the scaffold; bone, cartilage, and dental restoration as it facilitates phosphate and calcium deposition; and in drug delivery and cancer treatment due to their potential to improve drug release kinetics (Tayeb et al., 2018).

25.2.1.3 Alginate

Alginate (ALG), an anionic unbranched copolymer containing alternating D-mannuronate and L-guluronic acid connected by 1,4-glycosidic connections, has appealed to noteworthy interest in pharmaceutical and biomedical applications as a matrix material of nanocarriers owing to its intrinsic biological properties, such as noble biocompatibility, biodegradability, immunogenic, mucoadhesive, nontoxic, and water holding properties (Choukaife, Doolaanea, & Alfatama, 2020; Uyen, Hamid, Tram, & Ahmad, 2020).

ALG NPs can be developed using ionic gelation, covalent crosslinking, emulsion, self-assembly, and complexation methods, with ionic gelation and complexation being widely used for ALG NPs formulate (Choukaife et al., 2020). Calcium chloride and poly-l-Lysine have been used to develop ALG NPs with the size of 250−850 nm. The particle size depended on chloride and poly-l-lysine concentration, along with their order of addition (Rajaonarivony, Vauthier, Couarraze, Puisieux, & Couvreur, 1993). In the complexation method, chitosan has widely been used. In the study by Mukhopadhy et al., pH sensitivity chitosan-ALG (CS/ALG)

NPs were prepared by formation of an ionotropic pregelation of an ALG core entrapping insulin, followed by chitosan polyelectrolyte complexation for successful oral insulin administration (Mukhopadhyay, Chakraborty, Bhattacharya, Mishra, & Kundu, 2015). The NPs had an average particle size of 100−200 nm in dynamic light scattering, with an almost spherical or subspherical shape and ∼85% of insulin encapsulation.

25.2.1.4 Dendrimers

Dendrimers are emergent polymeric materials with biomolecule-like properties known for their distinct structures and adaptability in the delivery of drugs due to their high surface-to-volume ratio and their numerous surface groups (Madaan, Kumar, Poonia, Lather, & Pandita, 2014). They have shown their prospective capabilities in capturing and conjugating the high molecular weight hydrophilic/hydrophobic entities permitting a high drug load and multifunctionality (Menjoge, Kannan, & Tomalia, 2010; Xu, Zhang, & Wu, 2014). They are said to have the capacity to expedite the delivery of therapeutics across numerous cell membranes or biological obstacles through an endocytosis-mediated cellular internalization and hence they have been used in the evolution of new drug carriers associated with various therapeutic and biomedical applications (Madaan et al., 2014; Menjoge et al., 2010; Xu et al., 2014).

Their internalization mainly happens via clathrin- and caveolae-mediated energy-dependent endocytosis and comparatively via macropinocytosis making them function as a proton sponge enabling the escape from endosomes and lysosomes due to their large number of secondary and tertiary amines with a pKa at or below physiological pH (Ke et al., 2009). Fig. 25.1 below illustrates the mechanism of a dendrimer-mediated drug-delivery system.

Various compositionally differentiated dendrimers have been reported expansively for drug delivery and imaging as summarized in Table 25.1 below.

25.2.2 Lipid-based nanoparticles

Lipid-based NPs (LBNPs) have been reported as the most clinically progressive and promising colloidal nanocarriers for biomolecules (García-Pinel et al., 2019). Due to their extraordinary temporal, and thermal stability as well as loading capability, they can safely and effectively deliver bioactive organic molecules such as nucleic acids overcoming major barriers preventing the advancement and use of genetic medicines (García-Pinel et al., 2019). LBNPs constitute an extensive and diverse group of NPs which include liposomes, SLNs, and nanostructured lipid carriers (NLCs) which are described below.

25.2.2.1 Liposomes

Liposomes are spherical vesicles having an aqueous core enclosed by lipid bilayers (Din et al., 2017). They are the most studied drug carriers due to their biocompatibility and biodegradability (García-Pinel et al., 2019; Yingchoncharoen, Kalinowski, & Richardson, 2016). Besides, their structure which is analogous

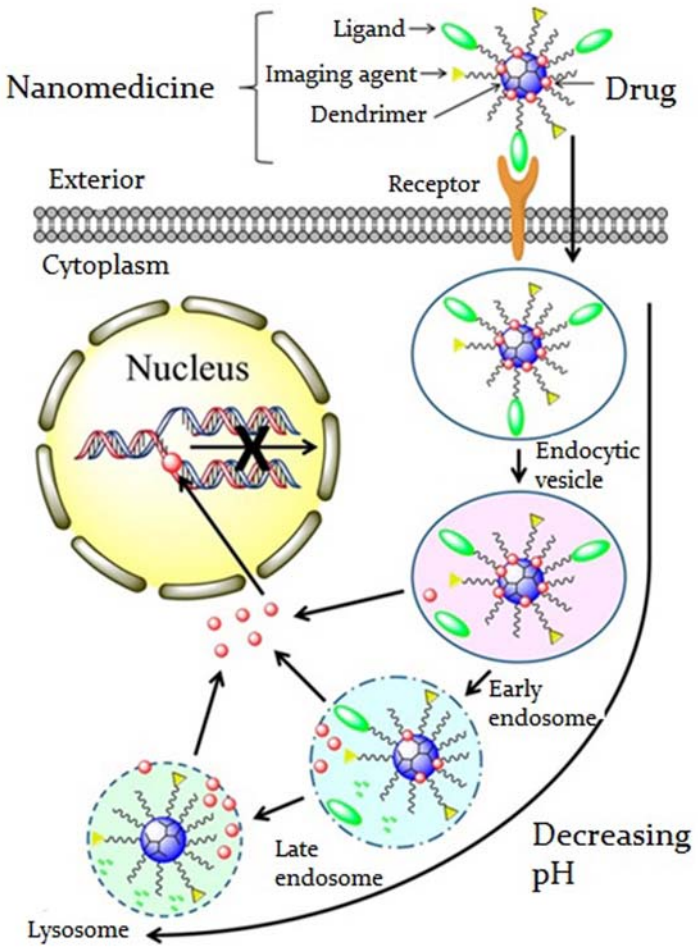

FIGURE 25.1

An illustration of the mechanism of dendrimer intracellular delivery of therapeutics.

Adapted with permission from Xu, L., Zhang, H., & Wu, Y. (2014). Dendrimer advances for the central nervous system delivery of therapeutics. ACS Chemical Neuroscience, 5(1),2–13. Copyright © 2014, American Chemical Society.

to that of the cell membrane, makes them accommodate both hydrophobic and hydrophilic drugs in their aqueous central part and lamellae, respectively (Din et al., 2017; Patra et al., 2018; Yingchoncharoen et al., 2016). Liposomes vary in composition, size, surface charge, and method of preparation. Properties associated with liposomes include enhanced delivery of the drug, protection of the active drug from environmental factors, improved performance features of the product, prevent early degradation of the encapsulated drug, biocompatibility, biodegradable,

Table 25.1 A list of selected dendrimers targeting the delivery of drugs into the Central Nervous system.

Polymeric nanomaterial	Drugs delivered	Ligands	Disease	Findings	Reference
PEGylated Poly (amidoamine) (PEG-PAAM) dendrimers	Doxorubicin	Transferrin (Tf) and wheat germ agglutinin (WGA)	Glioma	A minimized nonspecific acceptance by the normal cells was observed as well as a 2–threefold improvement of the transport across the BBB as paralleled to free drug and broke avascular C6 glioma spheroids in vitro.	He et al. (2011)
Poly(amidoamine) dendrimers	Doxorubicin	Conjugated with transferrin (Tf) and tamoxifen (TAM)	C6 glioma spheroids	A pH-triggered Doxorubicin release was observed indicating a relatively fast drug release at weak acidic conditions; improved transport across the BBB as well as reduced tumor volume of the avascular C6 glioma spheroids in vitro.	Li et al. (2012)
Polyethyleneglycol-Polyamidoamine dendrimers	pEGFP-N2 plasmid	Lactoferrin (Lf)	–	Concentration-dependent uptake in brain capillary endothelial cells (BCECs)	Huang, Ke, Liu, Jiang, and Pei (2008)
Polyamidoamine dendrimers	Borneol	Folic acid	Glioma	Reduced the cytotoxicity of PAMAM against both HBMEC and C6 cells and enhanced BBB permeability with the improvement of transportation	Xu et al. (2016)
Polyethyleneglycol-Polyamidoamine dendrimers	pEGFP-N2, pGL2	Transferrin		Increased transfection efficiency	Huang et al. (2007)
Poly(propyleneimine) dendrimers	Paclitaxel	Sialic acid	–	Enhanced delivery of anticancer drugs to the brain for higher therapeutic outcome	Patel, Gajbhiye, Kesharwani, and Jain (2016)
Poly(propyleneimine) dendrimers	Docetaxel	Polysorbate 80	Glioma	Significant reduction of the tumor volume by the docetaxel-loaded P80 conjugated dendrimers	Gajbhiye and Jain (2011)
Dendrigraft poly-L-lysine (DGL) dendrimers	Doxorubicin and gene agent pORF-hTRAIL	Peptide HAIYPRH (T7)	Glioma	Synergistic growth inhibition and less toxic antiglioma activity.	Liu et al. (2012)
Dendrigraft poly-L-lysine (DGL) dendrimers	Plasmid hGDNF	Angiopep-conjugated nanoparticles	Parkinson's disease	Higher cellular uptake and gene expression in brain cells compared to unmodified counterparts; best improved locomotor activity and apparent recovery of dopaminergic neurons.	Huang et al. (2013)

cost-effective formulations of expensive drugs, and efficient treatment with reduced systemic toxicity (Din et al., 2017; Patra et al., 2018; Teixeira, Carbone, & Souto, 2017).

25.2.2.2 Solid lipid nanoparticles

Solid lipid nanoparticles (SNLs) are composed of physiological lipids that stay in the solid state at room and body temperatures and can form a matrix material such as di- or triglycerides and fatty acids for drug encapsulation (García-Pinel et al., 2019; Yingchoncharoen et al., 2016). Owing to their expanded stability, they offer better security against the decay of medication, thus proving to be more beneficial than liposomes in the delivery of various drugs since their fabrication cost is also less when compared with liposomes (Yadav, Chauhan, Saraf, Singh, & Singh, 2020). However, SLNs have a moderate drug-loading capability and can lead to drug ejection because of the crystallization process under some storage settings which might hinder their usage (Rajabi & Mousa, 2016).

25.2.2.3 Nanostructured lipid carriers

Nanostructured lipid carriers (NLCs) include a combination of solid and liquid lipids representing the second generation of lipid-based nanocarriers developed from SLNs to overcome their limitations (García-Pinel et al., 2019). As a result, NLCs have an advanced loading capability and drug ejection during storage is minimized by evading lipid crystallization owed to the existence of the liquid

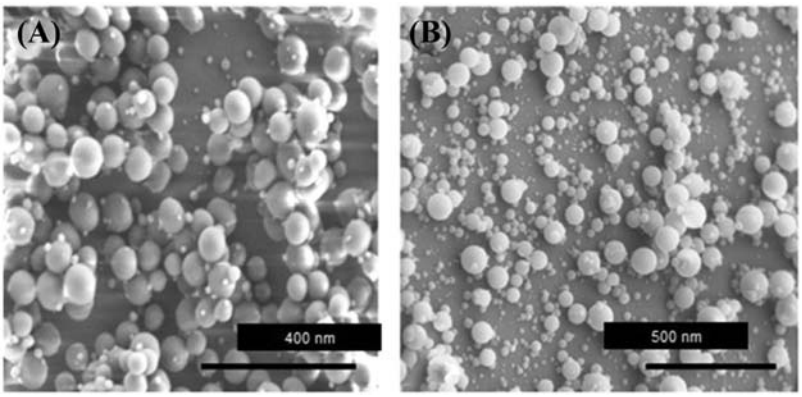

FIGURE 25.2

TEM images of nanostructured lipid nanocarriers with (A) triestearin and (B) tripalmitin, as the key lipid component.

Adapted from García-Pinel, B., Porras-Alcalá, C., Ortega-Rodríguez, A., Sarabia, F., Prados, J., Melguizo, C., et al. (2019). Lipid-based nanoparticles: Application and recent advances in cancer treatment. Nanomaterials (Basel), 9(4), 638, an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).

lipids in the formulation as illustrated in Fig. 25.2 (García-Pinel et al., 2019). These NPs can also be loaded with hydrophilic and hydrophobic drugs, their surface can undergo modification lending them site-specific to targets as well as offering controlled drug release and can display low in vivo toxicity (García-Pinel et al., 2019). However, drug ejection can occur when the lipid changes the nanocarriers matrix throughout the storage period (Obeid, Tate, Mullen, & Ferro, 2018).

Nanoformulations made from SLNs and NLCs have shown the potential to overcome the challenges of crossing the BBB since they can utilize physiological transport mechanisms (Pottoo et al., 2020; Teixeira et al., 2017). The NLCs and SLNs normally stick to the surface of enterocytes for the absorption of the loaded drugs in the interior of the enterocytes forming micelles followed by the formation of chylomicrons upon re-esterification through monoacylglycerol or phosphatidic acid pathway (Poovi & Damodharan, 2018). Successive stabilization by phospholipids follows exposure to the lymphatic transport system to get into the systemic circulation by lymphatic drainage at the thoracic duct (Bramini et al., 2014; Poovi & Damodharan, 2018). For example, drug-loaded SLN/NLCs can absorb apolipoproteins from the systemic circulation and then are taken up by endothelial cells via low-density lipoprotein (LDL)-receptor-mediated endocytosis and consequently selectively unloads the drugs at the targeted neuronal tissue hence reducing toxicity (Pottoo et al., 2020). Another way that has been reported to facilitate the transport of drugs across the BBB is conjugating ligands and monoclonal antibodies corresponding to the receptors on the BBB onto liposomes (Samad, Sultana, & Aqil, 2007). Besides, the liposomes can also trap both hydrophobic and hydrophilic groups, restricting the decay of the materials to allow for a controlled and precise release of the drugs at their targets (Samad et al., 2007).

For example, PEGylated-lipid NPs (PLNs) conjugated to Fas ligand antibody present in the brain ischemic region and used for therapeutic targeting have been reported (Lu et al., 2014). In this particular study, the results obtained suggested that the PLNs conjugated to an antibody specific to the Fas ligand established an ideal brain targeting drug delivery system for brain ischemia (Lu et al., 2014). Also, absorbing NFL-TBS.40−63(NFL) on lipid nanocapsules has been evaluated on their targeting efficiency on neural stem cells from the brain and the spinal cord, with the results indicating a preferential uptake by the neural stem cells from the brain, demonstrating that NFL-LNC represented a promising therapeutic strategy to selectively deliver bioactive molecules to brain neural stem cells (Carradori, Saulnier, Préat, des Rieux, & Eyer, 2016). More studies on lipid-based nanoformulations are summarized in Table 25.2 below.

25.2.3 Inorganic nanoparticles

Inorganic NPs such as silver NPs, Gold nanoparticles (AuNPs), and carbon nanotubes (CNTs) have been extensively studied as therapeutic agents in biomedical fields owing to their exceptional physical/chemical properties, ease of surface modification, and outstanding biocompatibility (Wang, Li, Cheng, & Yuan, 2016).

Table 25.2 Selected Lipid-based nanoformulations for drug delivery across the BBB for neurological disorders.

Liposomes	Drug delivered	Ligand	Disease	Findings	References
(T7&SHp-P-LPs/ ZL006)		T7 peptide and stroke homing peptide	Cerebral ischemia	Data demonstrated that T7&SHp-P-LPs could be used as a safe and effective dual-targeted nanocarrier for ischemic stroke treatment	Zhao et al. (2016)
Solid lipid nanoparticles (SLNs), and nanostructured lipid carriers (NLCs)	Temozolomide (TMZ)	–	Glioblastoma	The NLCs delivered the TMZ into U87MG cells more efficiently, with higher inhibition efficacy than polymeric and SLNs	Qu et al. (2016)
Catanionic solid lipid nanoparticles (CASLNs)	Carmustine	Anti-epithelial growth factor receptor	Human brain malignant glioblastomas	Effective delivery to U87MG cells and antiproliferative efficacy against the growth of malignant brain tumors.	Kuo and Liang (2011a); Kuo and Wang (2015)
Catanionic solid lipid nanoparticles	Doxorubicin (Dox)	Anti-epithelial growth factor receptor	Inhibition of growth of U87MG cells	The CASLNs captured the largest quantity of Dox; growth-inhibition induced.	Kuo and Liang (2011b)
Solid lipid nanoparticles (SLNs)	Anticancer etoposide	Melanotransferrin antibody	Glioblastoma multiforme	The MA on ETP-SLNs triggered the melanotransferrin-mediated transcytosis and promoted the growth-inhibitory efficacy of U87MG cells.	Kuo and Chao (2016)
Lipid nanocapsules	Nuclease-resistant locked nucleic acid (LNA)	Peptide	Human glioblastoma	Clear reduction of miR-21 expression and substantial enhancement of cell sensitivity to treatment	Griveau et al. (2013)
Proteoliposomes	Recombinant human FGF20 (rhFGF20)	Small ubiquitin-related modifier	Parkinson's disease	Extended the half-life of the drug; increased BBB penetration, slow drug release, high encapsulation rate, and biocompatibility.	Niu et al. (2018)

(*Continued*)

Table 25.2 Selected Lipid-based nanoformulations for drug delivery across the BBB for neurological disorders. *Continued*

Liposomes	Drug delivered	Ligand	Disease	Findings	References
RVG29 functionalized liposomes (RVG29-lip)	N-3,4-bis (pivaloyloxy)-dopamine (BPD)	Virus glycoprotein	Parkinson's disease	Selective distribution of the RVG29-lip to the brain, striatum, and substantia nigra; enhanced therapeutic efficacy of the BPD-loaded RVG29-lip (BPD-RVG29-lip);	Qu et al. (2018)
PEGylated liposomes	Neurotrophic factor (GDNF) + nuclear receptor-related factor 1 (Nurr1)-		Parkinson's disease	Ultrasound-guided BBB penetration capability, continued drug release, and prolonged drug half-life.	Yue, Gao, Wang, Ding, and Teng (2018)
Self-assembling nano-micellar system	Levodopa (L-DOPA)		Parkinson's disease	Considerably amplified percutaneous permeation and systemic absorption of L-DOPA.	Sintov, Levy, and Greenberg (2017)

They normally have advantages over polymeric and LBNPs in the delivery of drugs into the CNS due to their excellent stability and distinct materials and size-dependent physicochemical properties (Fan et al., 2018). Physical and chemical methods are popular for the synthesis of these NPs but the use of toxic chemicals greatly limits their biomedical applications, particularly in clinical fields. The use of biological routes such as the use of bacteria, fungi, and plants for the synthesis and of NPs may lead to the development of clean, nontoxic, and environmentally acceptable "green chemistry" procedures (Siddiqi, Husen, & Rao, 2018). Biomolecules such as carbohydrates, fats, proteins, nucleic acids, pigments, and several types of secondary metabolites present in plants and their parts act as reducing agents to produce NPs from metal salts without producing any toxic by-products. Similarly, biomolecules such as enzymes, proteins, and biosurfactants present in microorganisms also serve as reducing agents indicating the potential of using such systems for the green production of NPs (Siddiqi et al., 2018). Synthesis of silver NPs that have antiplorative, antimicrobials, and antioxidant properties by bacteria, fungi, and plants have been reported (Baltazar-Encarnación, Escárcega-González, Vasto-Anzaldo, Cantú-Cárdenas, & Morones-Ramírez, 2019; Hemlata, Meena, Singh, & Tejavath, 2020; Yuan, Bomma, & Xiao, 2019). The synthesis can occur extracellularly which involves the trapping of metal ions on the outer surface of the cells and reducing them in the presence of enzymes or biomolecules or intracellularly where synthesis occurs inside the microbial cells. Intracellular synthesis requires a step for cellular disruption to release the bio-synthesized polymers (Siddiqi et al., 2018).

Currently, various inorganic-based NPs with diverse structures have been extensively studied since it is easy to modify them with either polymer or specific ligands to enable the delivery of therapeutics and macromolecules across the BBB thus helping in providing an appropriate amount of the drugs to the brain (Ding et al., 2020; Yan et al., 2020; Zhang et al., 2016). Additionally, their circulation in the blood increases the binding ability of the drug towards the specific receptors existing on the surface of endothelial cells of the BBB, consequently aiding in crossing the BBB (Arvizo, Bhattacharya, & Mukherjee, 2010; Lockman, Mumper, Khan, & Allen, 2002; Singh & Lillard, 2009). In the next section, we discuss formulations from some of these inorganic NPs.

25.2.3.1 Gold nanoparticles

AuNPs have shown a massive potential to overcome the inability to cross the BBB (Rizvi et al., 2018). This is due to their unique features such as good stability, biocompatibility, their capability to be synthesized in various sizes, and their surface affinity towards numerous functional groups which makes them cross the BBB with ease (Rizvi et al., 2018). This makes the AuNPs an added advantage in comparison to other NPs when used as drug carriers for the brain (Ghosh, Han, De, Kim, & Rotello, 2008; Giljohann et al., 2010; Murphy et al., 2008), although the ability depends on the size of the NPs and the preferred size has been reported to be between 20−50 nm (Rizvi et al., 2018; Sonavane, Tomoda, & Makino, 2008).

Studies have demonstrated that functionalizing AuNPs with various molecules such as glucose and wheat germ agglutinin horse-radish enables them to efficiently cross the BBB (Gromnicova et al., 2016).

25.2.3.2 Silica nanoparticles

Silica NPs (Si NPs), have shown potential as promising candidates for brain drug delivery because they are relatively low cost and they have good biocompatibility, and manufacturing controllability (He et al., 2008; Liu et al., 2010). For example, an enhanced transport efficiency across the BBB of polyethylene glycol-modified SiNPs was demonstrated by attaching lactoferrin (Lf) to the surface of the SiNPs (Song et al., 2017). Mesoporous silica NPs (MSNPs), have also been used as drug carriers since they not only have outstanding biocompatibility but also have extensive specific surface area for drugs or ligands loading (Ding et al., 2018). As reported by Kuang et al., MSNPs loaded with doxorubicin demonstrated the ability to penetrate through the BBB into a tumor area, considerably enhancing the accumulation of drugs in orthotopic brain tumors with negligible side effects (Kuang et al., 2018).

25.2.3.3 Carbon nanotubes

Carbon nanotubes (CNTs) have shown remarkable potential as favorable candidates in the development of innovative entities for CNS pathologies owing to their outstanding physicochemical properties and capabilities to interface with neurons and neuronal circuits (Xiang, Zhang, Guo, & Liang, 2020). Their biomedical and therapeutic applications have been extended to numerous neuropathological disorder therapies both in vitro and in vivo as essential therapeutic drugs (Xiang et al., 2020). They have been reported to be used as drug carriers and have also shown prospects as therapeutic drugs as well. For example, Zhang et al. used single-walled CNT modified with acetylcholine to treat AD (Yang et al., 2010). Another study reported a drug delivery system using CNTs where they significantly improved the effects of CpG oligodeoxynucleotide immunotherapy in the management of glioma and averting tumors (Zhao et al., 2011). CNTs have also been reported to cross the BBB as reported by Kafa et al. (2015). In this study, the authors investigated the capability of amino-functionalized multiwalled CNTs (MWNTs-NH_3^+) to cross the BBB in vitro using a coculture BBB model with their results indicating the potential of using CNTs as nanocarriers for the delivery of drugs and biologics to the brain, after systemic administration (Kafa et al., 2015). Likewise, Costa and coworkers investigated the in vivo biodistribution and brain uptake of conjugated Pittsburgh Compound B (PiB) derivative gadolinium (Gd^{3+}) complexes—to multiwalled f-CNTs (f-MWNTs) (Costa et al., 2018). Their results indicated that complexes could be efficiently loaded onto different f-MWNTs, with substantial enhancement in brain accumulation of the conjugates when compared to the free metal complexes (Costa et al., 2018).

25.3 Nanoformulations for neurological therapeutics

Due to the advancement in technology in chemistry, drug delivery, and the medical field has seen progress in the field of nanoneuro medicine. This offers real prospects to exploit unique therapeutic approaches to address diseases of the nervous system where limited options exist. As depicted in Fig. 25.3, NPs from natural sources such as proteins (albumin), polysaccharides, and chitosan, have shown to have properties that enable them to penetrate BBB. Synthetic biomaterials such as poly(lactic-co-glycolic acid), poly(ethylenimine), polyesters (poly(lactic acid), and inorganic agents like gold, silica, or alumina can be fine-tuned into NPs. Nanosystems with a size range between 1−1000 nm have been reported to have the ability to cross the BBB. Nanosystems can be morphed into shapes (spherical, cubic, and rod-like), and also the surface charge can also be manipulated and the surface affects the delivery of drugs to the CNS. To achieve targeting Nps can be functionalized with different types of ligands such as those capable of mediating protein adsorption (e.g., poly(sorbate) 80 (P-80)); those that interact directly with the BBB (e.g., transferrin proteins, antibody or peptides); those that capable of increasing hydrophobicity (e.g., amphiphilic peptides); and those that can improve

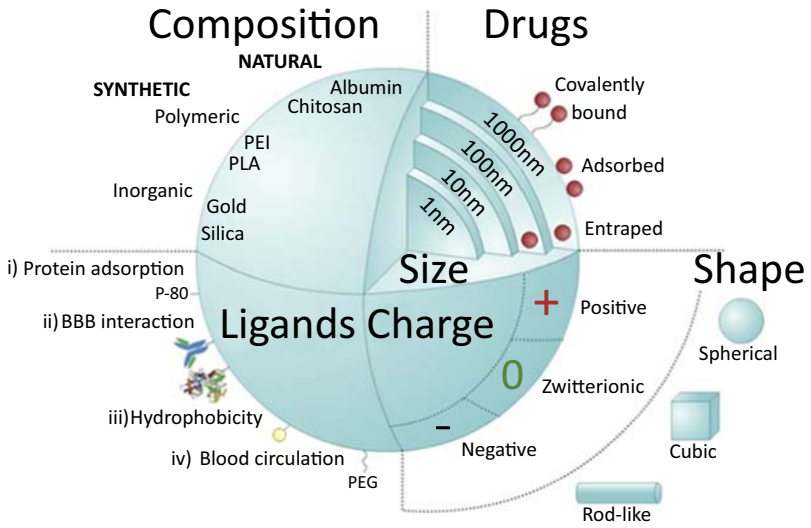

FIGURE 25.3

Properties and features that influence drug delivery to the nervous system and the ability to traverse the blood-brain barrier (Saraiva et al., 2016).

Reproduced with permission from Saraiva, C., Praça, C., Ferreira, R., Santos, T., Ferreira, L., & Bernardino, L. (2016). Nanoparticle-mediated brain drug delivery: Overcoming blood–brain barrier to treat neurodegenerative diseases. Journal of Controlled Release, 235, 34–47.

blood circulation [e.g., poly(ethylene glycol) (PEG)] increasing the possibility of the systems reaching the nervous circulations.

Drug delivery in the nervous system via nanomedicine offers potential in managing diseases that affect the system. In addition to improved therapies, newer, safer, and more sensitive and target-specific imaging modalities and advanced diagnostics for the detection of nervous system diseases are being developed. This section of the book chapter will cover, drug delivery to the nervous system and how nanoformulations are being employed to actively target the nervous system.

25.3.1 Passive targeting of the nervous system

The efficacy of an administered active pharmaceutical ingredient depends on its ability to cross the biological membranes and barriers from the site of administration to the site of action (Blanco, Shen, & Ferrari, 2015). Nervous drug delivery has several barriers that include the BBB, the BCSFB, and dilution effects due to the systemic (Begley, 2004). It is reported approximately 98% of small molecule drugs and 100% of proteins and nucleic acid therapies cannot be delivered to the CNS due to delivery barriers (Pardridge, 2006). Due to these delivery challenges transport of therapeutics, diagnostics, and imaging agents into the nervous system call for innovative strategies to over the limitations of the conventional dosage forms.

Passive targeting involves the delivery of a drug or drug-carrier system at the disease site because of Physicochemical, pharmacological, and disease factors. Under certain conditions such as inflammation state and hypoxia, etc., the integrity endothelium of blood vessels and barriers such as the BBB becomes more permeable than in a healthy state (Obrenovich, 2018). Therapies have been designed to take advantage of this anomaly to deliver drugs to the nervous system. These anomalies result in selectively enhanced permeation of drug molecules and nanosystems (Patching, 2017). For example, under normal conditions highly hydrophilic antibiotics such as aminoglycoside, penicillin, and cephalosporins cannot cross the BBB. However, in hyper inflammation states such as meningitis, the integrity of the BBB is compromised and hydrophilic antibiotics can be employed to treat bacterial meningitis (Schmidt & Täuber, 1993). In the section below, we discuss two features that affect passive targeting of the nervous system.

25.3.1.1 Particle size and shape

Particle size, shape, charge, and surface functionalization are important features for the penetration of the NPs into the brain and other organs where drug delivery is a challenge (Omolo et al., 2018). By manipulation of these traits, nanocarriers have been able to cross the nervous system barriers via passive targeting. Manipulation of size has resulted in NPs having the ability to cross the BBB irrespective of their nature. The use of the size of NPs to pass through the nervous system barriers has also been reported by Sokolova et al. who formulated 2 nm

inorganic AuNPs for uptake across the BBB. They studied the effect of the size of the NPs in traversing the neurovascular unit (NVU). The neurovascular unit (NVU) is a complex functional and anatomical structure that is made of endothelial cells that form the tight junctions in the BBB (Sokolova et al., 2020a). From the results of the study, the ultrasmall NPs could pass through the BBB. This represented a platform for drug delivery to the brain.

Apart from size, the shape has been used to passively target the delivery of drugs to the nervous system. Brown et al. reported that uptake of NPs into the brain was controlled by the size, molecular weight (MW), and shape. From the study, it was found that the uptake across the BBB was inversely proportional to the molecular weight. Particles with lower MW had higher uptake and vice versa. The study further determined that smaller particles (200 nm) had 17.sixfold more uptake than the 500 nm particles. Moreover, spherical particles had better uptake crossed the BBB than the road-shaped NPs (Brown, Habibi, Wu, Lahann, & Mitragotri, 2020).

Juthani et al. reported an advanced study where they employed ultrasmall core-shell Si NPs quantum dots (QD) for drug delivery to target malignant brain tumors. Dasatinib, fluorescence moieties, and tumor targeting peptides were loaded into the system for drug delivery and imaging. Findings via ex vivo autoradiography and fluorescence microscopy showed successful drug delivery and tumor inhibition (Juthani et al., 2020). Peptides have been reported to have difficulties in crossing biological membranes because of their high charge and hydrophilicity (Jena, McErlean, & McCarthy, 2020). However despite the QDs having surface functionalization with tumor-targeting peptides, still displayed a remarkable ability to accumulate in brain cells by traversing the BBB. Other similar studies have formulated several systems that have taken advantage of the small sizes of the NPs for nervous system drug delivery (Kenzaoui, Bernasconi, Hofmann, & Juillerat-Jeanneret, 2012; Lux et al., 2019; Sokolova et al., 2020b). Different NPs with differing sizes, shapes, and charges can be formulated. However, the caveat for their uptake is dependent on the intended biological effect which may vary from case to case.

25.3.1.2 Adsorptive-mediated transcytosis

The charge has also been employed for passive drug delivery. Via the adsorptive-mediated transcytosis concept, if cationic proteins are allowed to accumulate in the BBB, they can bind to the endothelial cell surface of the BBB. This binding results in the particles traversing the BBB (Hervé, Ghinea, & Scherrmann, 2008). The NPs with a positive charge can bind to the negatively charged luminal surface of BBB. Therefore, modification of the surface charge of the NPs can enhance the translocation to the brain. In their pioneer study, Kreuter et al. demonstrated the adsorptive-mediated transcytosis of NPs by formulating dalargin-loaded poly (butyl cyanoacrylate) NPs coated that were polysorbate 80 for brain delivery. dalargin is a drug with opioid activity. However, the conventional formulation via the intravenous route did not achieve the therapeutic effect of the drug. This was

attributed to the lack of adequate penetration of BBB resulting in low concentrations in the CNS to cause the opioid effect. In vivo studies demonstrated that dalargin-loaded PBCA particles had a hyperalgesic effect (Kreuter, Alyautdin, Kharkevich, & Ivanov, 1995). Further studies, on the same by Schroeder et al. where radiolabeled several NPs including poly(butyl cyanoacrylate) polysorbate 80 coated NPs, and examined the brain penetration. They determined in the absence of polysorbate 80, NPs that crossed the BBB decreased significantly (Schroeder, Schroeder, & Sabel, 2000). This technique has since been widely reported to enhance brain delivery (Alyautdin et al., 1997; Ambruosi et al., 2006; Jahansooz et al., 2020; Kreuter et al., 2003; Zhang et al., 2018). Investigation of the effect of polysorbate 80, has been found to act by appears to act by (1) decreasing nanoparticle clearance by the reticuloendothelial system (Ma et al., 2011), (2) interacting with BBB endothelial receptors like LDLs (Li et al., 2018b), and (3) possibly modulating tight junctions and efflux transporters (Rempe, Cramer, Qiao, & Galla, 2014). Moreover, the strategy has evolved by modifying the nanosystems with functionalized with positively charged or surface charge switching biomolecules, cell-penetrating peptides, TAT peptides, and aptamers, which by taking advantage of their physicochemical to enhance penetration and activity (Xie, Shen, Anraku, Kataoka, & Chen, 2019).

25.3.2 Active targeting of the nervous system

Active targeting is critical for the delivery of drugs, genes, and theranostics to the nervous system. Through active targeting delivery to the disease, sites are enhanced which leads to therapeutic efficiency and limits the side effects. Active targeting has been reported to concentrate the drugs to the disease sites when compared to free drugs or passively targeted nanosystems. This is achieved via the design of nanosystems to respond to physiological changes such as overexpressed receptors, Temperature, pH redox potential, and enzymes that occur due to diseases (Maji et al., 2019). This approach increases the affinities and destabilization of nanocarriers that result in the penetration and increased release of the targeted payloads. The active targeting systems in the nervous system are achieved via transporter-mediated transcytosis, receptor-mediated transcytosis/endocytosis (RMT), and the biomimetic approach. This section will discuss various methods of active delivery employed for targeting the nervous system.

25.3.2.1 Receptor-mediatedtranscytosis/endocytosis

The presence of certain receptors or overexpression of receptors has been employed for targeted drug delivery. In RMT systems with ligands that bind to specific receptors expressed such as nicotinic acetylcholine receptors, transferrin receptors (TRs), the insulin receptor, and the LDL receptor that are expressed on the brain endothelial cells are incorporated in the delivery systems to achieve receptor-mediated BBB transport. Moreover, certain diseases like the brain and metastasized cancer have overexpressed receptors which scientists can take

advantage of to achieve delivery of drugs, theranostic and diagnostic substances to the brain. For instance, glioblastoma multiforme (GBM) cancer has been elucidated and found to have overexpression of TRs. The presence of TRs has made it possible for targeted delivery in the brain to treat (GBM). Sheykhzadeh and Co-workers also reported a similar study by formulating transferrin-conjugated porous silicon for GBM. They evaluated the effect of inhibiting GBM migration via a microfluidic-based migration chip that mimicked the tight extracellular migration tracts in brain parenchyma. The results showed a successful inhibitory effect of the NPs on cell migration. Indicating a potential application of this platform for drug delivery (Sheykhzadeh et al., 2020). Kang et al. formulated NPs that contained iron-mimic CRT peptides targeting TRs. They conjugated CRT peptide, to poly(ethylene glycol)-poly(l-lactic-co-glycolic acid) NPs. The efficacy of the formulated NPs was evaluated using BALB/c nude mice with C6 glioma animal models. The NPs demonstrated better penetration and accumulation in the tumor sites when compared to the free drugs. The study highlighted the potential of CRT peptide as a targeting ligand for enhanced drug delivery in GBM cancer (Kang et al., 2015). There are other several studies have that taken advantage of receptors in the brain for targeted delivery (Cui, Xu, Chow, Wang, & Wang, 2013; Jhaveri, Deshpande, & Pattni, 2018; Luo et al., 2019). Receptor-mediated delivery to the nervous system shows promise, however, the effectiveness of targeting ligands is highly dependent on the receptor expression. The expression of the receptor is varied depending on different tumor types and the stage of the cancer disease. Moreover, some receptors are overly expressed on the surface of the cell which prevents penetration of the system into the disease site. Therefore, there is a need for a multireceptor and multiresponsive approach to have more effective systems.

25.3.2.2 Biomimetic approach

The mimicking of biochemical processes for targeting and delivery is an exciting approach to nervous system delivery. The biomimetic approach, exploits or mimics the biological structures and processes, to overcome the barriers to nervous system delivery. Such approaches include coating NPs with cell membranes, the use of lipoprotein-based delivery systems, exosome, viral, protein template, and peptide template-based delivery systems to bypass the nervous system barriers and deliver the payloads.

This approach has been reported to target diseases like Alzheimer's (AZ) that have been a challenge to treat. Through the biomimetic approach, there is a great potential for delivery and targeting of the disease. The formulation of nanosystems with multifunctionality for BBB permeability and targeting of the amyloid-beta (Aβ) and its aggregation is paramount in the management of AZ. Song et al. reported a biomimetic nanosystem from recombinant ApoE and synthetic lipids that could transverse the BBB and target the Aβ. They loaded α-Mangostin as the model drug for treating AZ. The results showed that the nanosystem had 54-fold better activity than the controls. The labeled system via visualization by confocal

microscopy showed an increase in penetration of the NPs across the BBB and amassing the NPs around the Aβ aggregates. The drug-loaded system further increased the binding affinity of the nanosystem to the Aβ. The drug-loaded system showed a 336% uptake and 29-fold degradation of Aβ when compared to the nontreated controls as shown in Fig. 25.4. In vivo studies further showed that for a period of 2 to 4 weeks, the drug-loaded nanosystem decreased amyloid deposition, attenuated microgliosis, and rescued the memory defect in SAMP8 mice, an AD mouse model (Song et al., 2016). Albumin has also been used to make biomimetic NPs for brain delivery by Lin et al. The formulated NPs used albumin-binding protein pathways for their action. The albumin proteins are usually overexpressed in tumors. Moreover, albumin provides amino acids and a much-needed energy source for fast replicating cancer cells. Therefore, albumin NPs are easily absorbed by the cancer cells thereby delivering the loaded payload to tumor cells. Moreover, albumin can also penetrate the BBB. From the study, the albumin NPs showed greater BBB penetration, intratumoral infiltration, and cellular uptake which translated to better treatment outcomes in both subcutaneous and intracranial glioma models, with reduced toxic side effects (Lin et al., 2016). Other reported biomimetic nanosystems for drug delivery to the brain have employed; High-density lipoproteins (Ma, Song, & Gao, 2018), Angiopep-2 peptide, and red blood cell membrane (Liu et al., 2020) to mention a few. The use of biostructures, materials, and processes to deliver and target the nervous system is

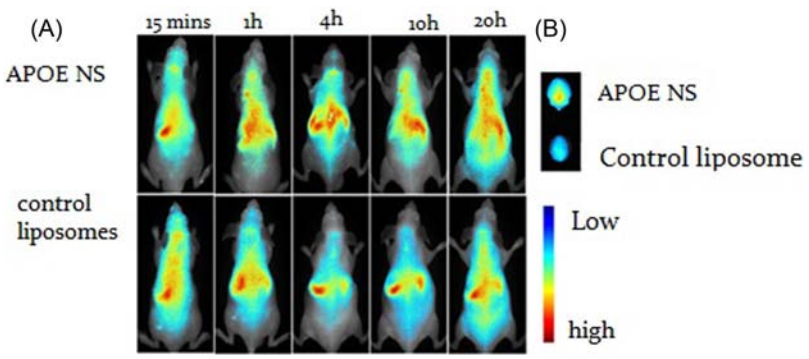

FIGURE 25.4

Distribution of the APOE biomimetic NS and the control liposomes after IV administration in mice. (A) After IV administration the In vivo distribution of the APOE nanosystem and the control liposomes at different times (15 min, 1, 4, 10, and 20 h) was imaged. (B) A fluorescent signal in the animal brain 4 h after administration via the tail. The imaging showed the APOE biomimetic had a high intensity when compared to the controls.

Reproduced with permission from Song, Q., Song, H., Xu, J., Huang, J., Hu, M., Gu, X., et al. (2016). Biomimetic ApoE-reconstituted high density lipoprotein nanocarrier for blood–brain barrier penetration and amyloid beta-targeting drug delivery. Molecular Pharmaceutics, 13(11), 3976–3987.

showing potential in overcoming the barriers to drug delivery and as means of avoiding neurotoxicity due to nanosystems.

25.3.2.3 Prodrugs approach

The advancement in chemistry and biology has resulted in the understanding of the BBB, the BCSFB, and different drug delivery strategies devised to assist in the delivery of drugs to the nervous system. Passage of drug molecules across the BBB by passive diffusion, the drug molecules should have a small molecular weight of less than 500 Da, be lipophilic, have a neutral physiological pH, and have minimum hydrogen bonding with water (Pardridge, 2007). Drugs that don't fit these physicochemical properties can be modified as prodrugs. Prodrugs are drugs attached to promote ties that undergo chemical or enzymatic biotransformation, before exerting their pharmacological activity (Rautio et al., 2008a). Prodrugs can be formulated to enhance CNS delivery by increasing lipophilicity of highly hydrophilic drugs, circulation time, brain penetration, and coupling with the targeting of drug moieties (Rautio, Laine, Gynther, & Savolainen, 2008b).

Highly hydrophilic drugs such as nucleosides, nucleotides, and their analogs find it difficult to penetrate the BBB. They are usually attached to hydrophobic promoieties, this assists in the penetration of the drug across the BBB. Conversely, highly hydrophobic drugs with solubility problems can be linked with hydrophilic promoieties. This technique has been reported to improve brain delivery and if there is a balance between hydrophilic and hydrophobic segments the system forms nanoassemblies. For example, Schiapparelli et al. synthesized camptothecin (CPT) self-assembling prodrug /hydrogel and evaluated it for the treatment of GBM. Via a reducible linker, CPT was conjugated to a peptide (Ac-Csy-Gly-Val-Val-Gln-Gln-His-Lys) as shown in Fig. 25.5. The prodrug could self-assemble into filaments, however when counterions were added the filaments formed hydrogels. This self-assembling prodrug hydrogel. The active drug was easily released from the gel when evaluated both in vitro and in vitro it showed better results than the naked drug (Schiapparelli et al., 2020). The advancement of the prodrug technique has resulted in systems that have stimuli-responsive, and with targeting ligands as promoieties. Such an advanced study has been reported by Zhao et al. who synthesized a redox potential responsive disulfide bond-conjugated polymer prodrug consisting of CPT, polyethylene glycol (PEG), and Tumor-Penetrating iRGD Peptide. The polymer prodrug of CPT-S-S-PEG-COOH self-assembled into micelles of nanosized 100 nm size and could disassemble in response to intracellular redox response. The micelles could cross the BBB and target glioma cells via $\alpha v \beta$ integrin and neuropilin-1-mediated ligand transportation both in vitro and in vivo. The system had a good antitumor effect both in vitro and in vivo and the antitumor effect was enhanced with laser light irritation (Lu et al., 2020). Similar advanced prodrug systems have been reported before in the literature (Mukerabigwi et al., 2019; Zhong et al., 2017). The prodrug technique has evolved from just trying to solve the physiochemical properties of drugs to more complex systems that include stimuli-responsive and ligands for active targeting. However, even the prodrugs

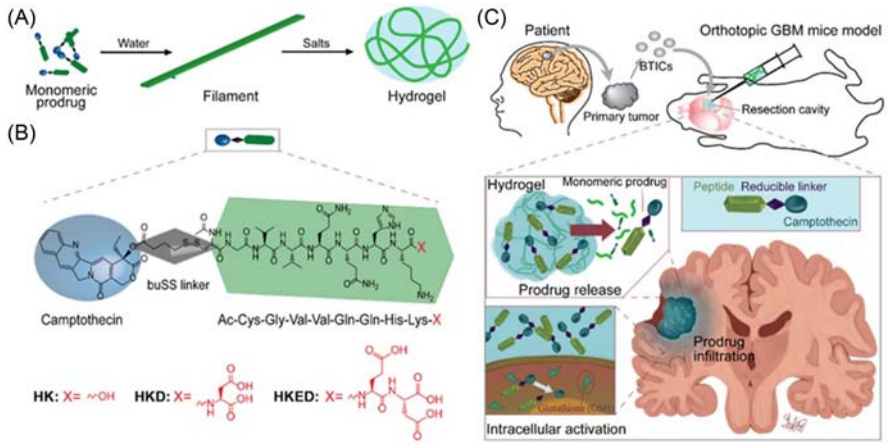

FIGURE 25.5

The self-assembling prodrug hydrogel and its application for the treatment of brain tumors. (A) Schematic representation of the self-assembly of the monomers of the prodrug into supramolecular filaments that forms a hydrogel when counterions are added. (B) Chemical structures of the drug, the reducible disulfide linker, and promoiety which is the peptide. (C) orthotopic GBM mice model initiated by human-derived brain tumor-initiating cells.

Reproduced with permission from Schiapparelli, P., Zhang, P., Lara-Velazquez, M., Guerrero-Cazares, H., Lin, R., Su, H., et al. (2020). Self-assembling and self-formulating prodrug hydrogelator extends survival in a glioblastoma resection and recurrence model. Journal of Controlled Release, 319, 311–321.

continue to evolve some of their classical limitations such as the slow onset of action due to slow cleavage of the bond between the drug and the promoiety, the inert molecules generated after cleavage of the prodrug may transform into toxic metabolites and even the prodrug itself could be metabolized into a toxic substance.

25.4 Neurotoxicity of nanosystem

Because of their size, NPs can cross the BBB via different mechanisms and pathways which can cause neurotoxicity, neuroinflammation, and neurodegeneration of the nervous system (Lin et al., 2020). It is paramount to note that the BBB represents a mechanism of defense of CNS against potentially neurotoxic molecules and structures, including NPs (Furtado et al., 2018). Data assessing the toxic effects of NPs on neural cells are scarce. Furthermore, it is challenging to conclude in vitro experimental models regarding the neurotoxicity of nanosystems. There have been reports on NPs that showed promising results in preliminary studies only to be abandoned for further studies after they demonstrated toxicity in vivo application (Elder et al., 2006). Systems such as SiO_2 and MnO NPs have been reported to have demonstrated neurotoxicity (Hu & Gao, 2010;

Wu, Wang, Sun, & Xue, 2011). As there is an increase in the application of the nanosystem to target the nervous system it should be noted as they induce their activity, they can modify the biological responses of the receptors and target sites which in turn could induce neurotoxicity. Furthermore, toxicity could be a result of the opening of tight junctions of the BBB by NPs. This affects its permeability as a result toxic particles that could not have passed a normal BBB could end up reaching the brain. The elimination and residency times of the NPs should also be determined. Most of the studies reported in the literature have not carried out neurotoxicity studies. This has affected the translation potential of the reported systems. Therefore, the risk and benefit ratio as a result of the application of NPs in the treatment of neurological diseases should be considered before their application.

25.5 Conclusions and future perspectives

Current approaches to improving delivery to the nervous system are focusing on circumventing the BBB and BCSFB via either direct injections or nasal drug applications. Most of the reported attempts for targeted delivery to the nervous system have been through passive targeting or active targeting. Passive targeting depends on the size, charge, and duration of circulation of the delivery system in the body. These techniques have drawbacks as they might fail in the translation of the reported research into safe and effective treatments for patients with nervous system diseases. For passive targeting to have an impact, the nanocarriers must be injected into the blood supply distribute in the body and then reach the BBB or BCSF in high concentration so that they can traverse the nervous system barriers. Moreover, the nanocarriers need to have a long residency time so that they can have enough contact time with the membranes for them to traverse. For the active targeted route for delivery to be effective there is a need for the design and synthesis of the biomaterials and ligands that can target the receptors and assist in traversing the barriers. The synthesis of these ligands and biomaterials might require multichemical steps and characterization including toxicity testing. The formulation of the nanocarriers requires in-depth, chemical, small-scale, and scaleup production, pharmacological, and pharmacokinetics testing especially crossings of the BBB and BCSF. Moreover, there is a need for in-depth testing for safety which includes toxicology, and the effect on the behavioral response of the administered nanocarriers. This is a major concern as most of the reported studies have not done such acute and long-term safety studies of the system in vivo. For the systems being reported appropriate routes of drug administration that provide a concentration of the administered nanocarriers to the disease sites and ease of administration for the management of chronic diseases are paramount for efficient delivery. For the reported system for delivery to the nervous to achieve its translational potential the raised concerns need to be addressed.

With the growth in synthetic and elucidations techniques, via nanotechnology, we can design and formulate intelligent drug delivery systems that match the complexity of the tasks required for nervous system delivery. Achieving this will require the multidisciplinary scientific community to work together in designing and formulating these systems.

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CHAPTER

Advanced nanoformulations for targeting, imaging, and therapy

26

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26.1 Introduction

In recent years, the emergence of nanotechnology has led to phenomenal advancements in the diagnosis and treatment of various diseases. Advanced nanomaterials have provided great possibilities for the development of smart pharmaceuticals. They are utilized for the development of multifunctional nanomedicines that can cross biological membranes and barriers and specifically target diseased cells. As one of the fast-growing fields, the use of multifunctional nanomedicines and nanosystems (NSs) is envisioned to improve both pharmacokinetics (PK) and pharmacodynamics (PD) properties with maximal therapeutic impacts and minimal side effects (Spencer, Puranik, & Peppas, 2015). Nanotechnology-based drug delivery systems (DDSs) efficiently supply several advantages, including small particle size, large surface area to volume ratio, functionalization possibility with high reactivity and more active sites, and desirable adsorption capacity. All these features can result in improved drug absorption and bioavailability, enhanced targeting, prolonged circulation half-life, and reduced side effects on normal cells/tissue (Su et al., 2019; Yin, Yuan, Gao, & Yang, 2020). Of note, recent investigations in the field of cancer nanotechnology have led to tremendous advancement in the design of tailored nanoparticles (NPs) in various forms conjugated with targeting ligands and other entities. As multifunctional nanomedicines, they can successfully be loaded with anticancer drugs and specifically/selectively target the chemotherapy-resistant cancer cells. Benefiting both passive and active targeting mechanisms, these NPs can accumulate within the tumor microenvironment (TME) (Omidi & Barar, 2014). The passive targeting is based on the enhanced permeability and retention (EPR) effect due to the fenestrated and leaky nature of irregular tumor microvasculature with pores and gaps (120−1200 nm) (Asgharzadeh et al., 2017; Barar & Omidi, 2013; Omidi & Barar, 2014), which defines the ability of NPs (100−300 nm) in accumulating within the TME. In the

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active targeting, however, NPs benefit from both the EPR effect and actively direct interaction with the overexpressed specific receptors on the surface of cancer cells. NSs can be designed in a way to release the cargo drug molecules in response to different exogenous and/or endogenous stimuli. In this line, they can be designed to respond to changes in the temperature/pH change, enzymes or antigens, or respond to physical stimuli (light, electromagnetic field, or heat), which have been the basis of considerable research interest (Barar, Aghanejad, Fathi, & Omidi, 2016; Fathi et al., 2017; Fathi et al., 2018a; Fathi, Abdolahinia, Barar, & Omidi, 2020a; Shakoori et al., 2017). Of note, nanoscaled treatment modalities, as a potential tool, can be exploited for the simultaneous imaging and treatment NSs that are known as nanotheranostics (Fig. 26.1) (Benko et al., 2021; Miao, Guo, Lin, Liu, & Huang, 2017). A nanoscaled theranostic system can be

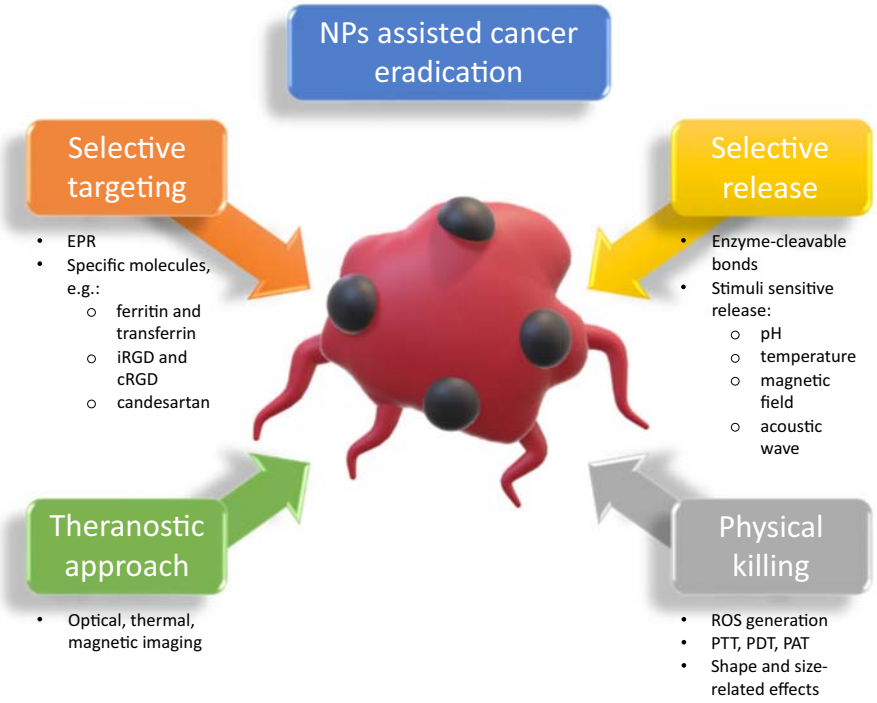

FIGURE 26.1

The schematic representation of advanced NPs-assisted cancer-eradiation via different approaches. *EPR*, enhanced permeability and retention, *iRGD*, internalizing RGD oligopeptide, *ROS*, reactive oxygen species, *PTT*, photothermal therapy, *PDT*, photodynamic therapy, *PAT*, photothermal ablation therapy.

Adapted with permission from Benko, A., Medina-Cruz, D., Vernet-Crua, A., O'Connell, C. P., Święt ek, M., Barabadi, H. et al. (2021). *Nanocarrier drug resistant tumor interactions: Novel approaches to fight drug resistance in cancer. Cancer Drug Resistance, 4(2), 264–297.*

defined as a single NS that can offer combined therapeutic and diagnostic impacts simultaneously. The high capacity of nanoplatforms to load imaging and therapeutic functions has a critical impact. Such NSs are capable of instantaneous targeted delivery of diagnosis and drug as well as the monitoring of the therapeutic response. As a result, they are projected to play a substantial role in the beginning era of personalized medicine, even though much research endeavors are needed to address issues related to such objectives (Xie, Lee, & Chen, 2010).

For clinical purposes, several noninvasive imaging modalities are in the practice, including (1) computed tomography (CT), (2) magnetic resonance imaging (MRI), (3) optical imaging (OI), (4) ultrasound, (5) gamma camera scintigraphy, positron emission tomography (PET), and single-photon emission computed tomography. The use of such imaging modalities may associate with some advantages/disadvantages, in large part because of the dissimilarities in spatial and the accuracy of temporal resolution, differences in the sensitivity of the probe detection approaches, and the distinctions in terms of the penetration depth of signals (Baetke, Lammers, & Kiessling, 2015). While the selection of the desired imaging modality depends on the type of required information, the useful way is to combine OI with CT as well as PET and MRI using nanoscaled agents that might result in an improved diagnosis (Arranja, Pathak, Lammers, & Shi, 2017). Recently, the use of ultrasmall metal nanoclusters with unique physicochemical properties (as theranostic agents) was shown to be beneficial in biomedicine applications. Many other NPs with the capability of delivering multiple drugs have previously been well evaluated. As a result, various NPs have been employed as theranostics, including gold nanoparticles (AuNPs), iron oxide nanoparticles, copper sulfide nanoparticles (CuS NPs), and mesoporous silica nanoparticles (MSNs) (Yao, Yuan, Chen, Leong, & Xie, 2018; Zheng & Xie, 2020). The main focus of the current chapter is to provide important insights into the development and application of multifunctional nanomedicines and nanotheranostics in terms of targeted imaging and therapy of diseases such as solid tumors.

26.2 Theranostic nanoformulation

The critical point of clinical success in the treatment of diseases is the diagnosis at the early stages of the initiation/progression and specific targeting of the diseased cells/tissue in the target site (Omidi, 2011). The term "theranostics" was first coined by John Funkhouser in 1998, which is the combined use of "therapeutics" and "diagnostics." It is used to define multifunctional nanomedicines, combining instantaneous diagnosis and treatment potentials in a single NS. Such nanoscaled medications could serve imaging and therapy simultaneously. To date, theranostics modalities have been considered one of the most effective all-in-one modalities for the detection and treatment of a wide range of diseases such as cardiovascular, and neurodegenerative disease, as well as various types of solid

tumors (Funkhouser, 2002). As a result, various multifunctional nanotheranostics have been developed and examined through different imaging techniques such as MRI/PET, MRI/CT, and MRI/PET/UT (Talegaonkar & Rai, 2020). Further, such NSs can be optimized to improve the PK and/or PD properties of cargo molecules, including drug bioavailability by facilitating drug absorption/diffusion across the biologic membranes and barriers while protecting them from degradation (Begines et al., 2020). It should be noted that the capability of the different NSs such as polymeric NPs for targeting can be influenced by their size distribution, surface charge, protection, hydrophobicity, and hydrophilicity of drugs (George, Shah, & Shrivastav, 2019).

Nanoformulation can carry therapeutic agents with distinct physicochemical characteristics and pharmacological behaviors to induce desired multifunctional impacts simultaneously by a single NS. Especially, hydrophobic and hydrophilic drugs have been loaded with adjustable drug loading ratios (Miao, Guo, Zhang, Kim, & Huang, 2014). Polymeric biodegradable nanocarriers are developed using synthetic polymers such as poly(amino acids), poly(alkyl-cyanoacrylates), poly (esters), poly(orthoesters), poly(urethanes), and poly(acrylamides), or natural polymers such as albumin, gelatin, chitosan, and heparin (Fernandez-Fernandez, Manchanda, & McGoron, 2011).

26.3 Inorganic nanoparticles for theranostics

Inorganic nanocarriers are metallic nanocarriers (e.g., iron, gold, copper sulfide, magnetic, etc.) or silicon and carbon nanostructures. Owing to their unique properties, metallic NPs such as gold, silver, magnetic, copper, and platinum have been shown to offer useful platforms for the catalysis (Jamkhande, Ghule, Bamer, & Kalaskar, 2019), development of polymeric composites (Moura et al., 2017), theranostic applications (Banerjee et al., 2017), sensor fabrications (Shaikh, Mane, Min, Hwang, & Joo, 2016), and labeling aims (Gracias, Tien, Breen, Hsu, & Whitesides, 2000).

26.3.1 Magnetic nanoparticles

Magnetic nanoparticles (MNPs) have been commonly utilized for biomedical applications, such as targeted drug delivery, bioimaging, gene therapy, biosensors, and magnetic imaging (Aghanejad, Babamiri, Adibkia, Barar, & Omidi, 2018; Azhdarzadeh et al., 2016; Barar et al., 2015; Fathi et al., 2017; Fathi, Rashidi, & Omidi, 2019; Fathi, Barar, Erfan-Niya, & Omidi, 2020b; Heidari Majd et al., 2013a; Heidari Majd et al., 2013b, 2013c; Kang et al., 2017a; Khosroushahi, Naderi-Manesh, Yeganeh, Barar, & Omidi, 2012; Mooguee, Omidi, & Davaran, 2010; Samadi Pakchin, Fathi, Ghanbari, Saber, & Omidi, 2020; Same, Aghanejad, Akbari Nakhjavani, Barar, & Omidi, 2016; Shakoori et al., 2017;

Siminzar, Omidi, Golchin, Aghanejad, & Barar, 2020; Zamanlu et al., 2018). MNPs have significant properties (e.g., small size, high magnetic saturation, zero coercivity, biocompatibility, biodegradability, and nontoxicity), which make them unique NPs with great potential to serve in different pharmaceuticals and biomedical applications. The benefits of using MNPs in targeted drug delivery are the specific identification of biological molecular targets and the use of the reduced amount of drug molecules, resulting in high therapeutic impacts with trivial side effects. Drug-loaded MNPs are controlled by the external magnetic field and lead the drug to the particular target tissue (McNamara & Tofail, 2015). Besides, MRI approach is one of the best way of biomedical imagings through different types MNPs such as iron oxide MNPs, superparamagnetic iron oxide MNPs, core-shell MNPs, and magnetic gel NPs. All these MNPs can be used as contrast agents in MRI.

For example, the use of MNPs has widely been considered in the treatment of breast cancer (Aghanejad et al., 2018; Barar et al., 2015; Heidari Majd et al., 2013b; Heidari Majd et al., 2013c; Siminzar et al., 2020; Singh, Singh, Lillard, & Singh, 2017). In this line, Nosrati et al. developed methotrexate-conjugated L-lysine modified MNPs to examine their anticancer effects on MCF-7 cells. The drug release was done via the peptide bond cleavage in the presence of a broad-spectrum serine protease (proteinase K) at low pH and the drug delivery of designed MNPs was monitored through MRI (Nosrati, Sefidi, Sharafi, Danafar, & Manjili, 2018). In another work, Huang et al. synthesized the folate-modified polymeric SPIONs for MCF-7 breast cancer treatment as well as drug delivery monitoring through MRI. Doxorubicin (DOX) was encapsulated in NPs via electrostatic interaction and hydrogen bonding, which yielded the pH-responsive NPs (Huang, Mao, Zhang, & Zhao, 2017). Omidi and coworkers developed mesoporous silica MNPs (SPION@SiO_2) for the targeted delivery of chemotherapy agent DOX to breast cancer MCF-7 cells (Siminzar et al., 2020).

26.3.2 Gold nanoparticles

AuNPs are considered paramount important NPs, in large part due to some significant advantages such as high x-ray absorption coefficient, localized surface plasmon resonance, responsiveness to the photoacoustic and electric stimuli, simple self-assembly through thiolation, and the possibility of surface modification (Ahn, Jung, & Lee, 2013; Akbarzadeh Khiavi et al., 2020; Bai et al., 2020). To improve the surface properties of AuNPs for application in drug delivery, they have been modified with a variety of cationic polymer or functional groups (e.g., carboxyl, amine, and thiol groups) that resulted in stable and effective drug immobilization and protection from enzymatic degradation. Cell-specific targeting molecules can also be conjugated to AuNPs complexes and deliver the desired drug to the specific target cells (Jeong, Jung, Am Hong, & Lee, 2014). Photothermal therapy (PTT) is a recently developed strategy in cancer treatment that utilizes NPs inside the tumor to generate heat in response to the exogenously used near-infrared

(NIR) laser ray. In this regard, AuNPs offer some useful characteristics such as their preferred biocompatibility, simple bioconjugation, the small size of NPs for tumor penetration, high conversion yield of light to heat, and capability of NIR absorbing. As a result, they have been used to target solid tumors. Once internalized and penetrated deep into the core of the tumor, they can be activated for PTT or sensitize the cancer cells to the other treatment modalities (Riley & Day, 2017). It should be also stated that, due to the longer wavelengths, the NIR rays can penetrate deeper into target tissue compared to other light sources (Wang et al., 2020a), in which AuNPs can play a key role in PTT/photodynamic therapy (PDT). Besides, AuNPs can also enhance the required dose in cancer radiotherapy leading to increased cancer cell abolition without damaging the tumor surrounding healthy cells/tissue (Siddique & Chow, 2020).

26.3.3 Copper sulfide nanoparticles

Copper sulfide (CuS) NPs, due to their excellent optical and electrical behaviors, have been considered for charge transport, light emission, thermal diffusion, and photocatalysis (Zhou, Tian, & Li, 2016). In comparison with other NIR absorbable materials (gold, silver, and carbon), CuS NPs were shown to be adjustable for the NIR absorption peaks in a wide region of (700 − 1100 nm) that can be converted to 900 nm via changing the stoichiometry (Sun, Li, Liang, & Yang, 2017). Copper ions in the form of small CuS NPs can be used as potential contrast agents for the T1-weighted MRI because of their unpaired electrons similar to MNPs. Moreover, the radionuclide [^{64}Cu] integrating into CuS NPs during the synthesis process without incorporating a radioactive metal chelating agent can be applied for PET imaging (Liang, Jin, Qin, & Xing, 2017). It is worth mentioning that the other significant advantage of CuS NPs is their small size which makes it easy to enter the deep parts of tumors and results in improved diagnosis along with the PTT effect (Liu, Ji, & Wang, 2019). The main challenges of CuS NPs application are their long-term toxicity potential and a relatively low photothermal ratio, which demands the use of higher laser powers or longer laser irradiation times, and hence a certain degree of damage might occur to normal healthy tissues. Such shortcomings have limited the in vivo applications of CuS NPs. The surface modification with certain ligands was shown to critically improve its usefulness as the PTT agent, most likely by changing their properties, including size, morphology, and elimination (Liu et al., 2019). Briefly, considering the properties of CuS NPs, they can be further modified with different entities such as imaging labels (e.g., radioisotopes, fluorescent dyes), targeting ligands (e.g., peptides, small molecules, aptamers, and antibodies), therapeutic agents (e.g., drugs, genes), as well as various polymers (e.g., chitosan, PLGA, PEG). Such modification can enhance the water solubility and biocompatibility of CuS NPs. As a result, they have been examined as nanotheranostics, which were shown to offer good potential for the combination of different targeting moieties, imaging labels, and therapeutic drugs. Collectively, such features make them a promising candidate that

can provide an effective and controllable delivery system for therapeutic agents and real-time monitoring of the progress of the treatment (Goel, Chen, & Cai, 2014).

26.3.4 Silicon nanoparticles

Silicon-based materials, especially with porous structures, have been established to serve as flexible platforms such as theranostics (Karaman, Kaasalainen, Kettiger, & Rosenholm, 2021). MSNs and porous silicon (PSi) are fabricated in two different ways. The MSNs are engineered via the bottomup approaches, while the PSi systems can be manufactured through the top-down processes. The pores of MSNs made by molecular templating strategy lead to high drug-loading efficacy. The pores of PSi are created via electrochemical etching under controlled conditions (Shen, Wu, Liu, & Wu, 2017). The silicon-based materials are biodegradable and can be degraded in the presence of residual water or humid air. This class of metal-free nanomaterials exhibits unique photoluminescence properties (e.g., size/surface-tunable fluorescence, high photoluminescence quantum yields, excellent antiphotobleaching capacity), which make them suitable for further surface modifications (Chen, Zhang, & Wu, 2020).

Several methods have been applied to fabricate the functional MSNs to serve as theranostics. For instance, MSNs-coated gold nanostars have been prepared and used for combined PTT and chemotherapy serving as effective nanotheranostics in the imaging and treatment of solid tumors, as reported by Miao et al. (Miao, Yu, Chen, Liu, & Su, 2020). Being excited by the NIR rays, the developed NSs displayed excellent photothermal effects. The proposed combination therapy consequently exhibited greater anticancer efficacy in HeLa and cervical cancer cells as compared to chemotherapy or photothermal treatment alone (Miao et al., 2020).

The surface of MSNs can be easily modified with antibody (Ab), aptamer (Ap), peptides, or other specific receptors to grant tumor-targeting NSs to maximize their tumor-target-guided imaging performance. Depending on the type of silica NPs (SiNPs)/MSNs, their features might differ from each other. For example, MSNs are preferred due to higher surface area, larger pore volume, and tunable pore size, whilst the choice of SiNPs might be contingent on the intended goal(s) (Zheng, Zeng, Hu, Wu, & Hou, 2018). The applications of silica NPs in bimodal imaging are CT-fluorescence bimodal imaging, MR and fluorescence bimodal imaging, fluorescence trimodal imaging, and PET-based multimodal imaging. All of them are useful in both types of silica NPs (Zheng et al., 2018).

26.3.5 Carbon-based nanoparticles

As safe and effective nanomaterials with wide nanomedicine applications, much attention has been paid to carbon-based nanomaterials (CNMs), including graphene oxide (GO), fullerenes, carbon nanotubes (CNTs), and carbon quantum

dots (CQD) (Saleem, Wang, & Chen, 2018). High delocalized π bonds, especially in the case of GO and CQD, appear to bond with hydrophobic drugs via π stacking and also their unique optical properties such as NIR absorption and emission. As a result, they can serve as promising NSs with potential for imaging and PTT applications (Li, Fan, Shen, Bányai, & Shi, 2019; Li et al., 2020). Therefore, the multifunctional DDSs can be designed with the possibility of loading more than one drug onto a single CNMs to function as NS with simultaneously real-time OI and PTT/PDT (Patel, Singh, & Kim, 2019). In cancer DDSs, the applied CNMs are most typically loaded with hydrophobic drugs, where the therapeutic impact can be promoted through combined PTT and chemotherapy. In vitro and in vivo evaluations indicated that the morphology of CNMs affects their performance. As a result, it has been demonstrated that the spherical, rough, and hollow NPs are premier for the combined gene therapy/PTT/chemotherapy resulting in the eradication of over 90% of diseased cells including metastatic and chemoresistant stage IV of breast cancer cells (Zhao et al., 2020a). Carbon dots (CDs) made by small graphene planes are extremely small (around a few nm) and can be prepared using chemical or physical techniques. CDs exhibit similar characteristics, including graphene-NIR fluorescence, PTT effect, and easy internalization. Perhaps, the most important feature of these nanomaterials is their removal from the body through kidney clearance (Benko et al., 2021).

Altogether, CNMs have been considered the most suitable carrier for treating cancer in the last decade as they can be employed with the PTT, PDT, and other complementary techniques for effective elimination of cancerous cells both in vitro and in vivo. It is noteworthy that they can provide targeted NSs which can impose their impacts on cancer cells with no/trivial damage to the healthy cells/tissues. Nonetheless, the physicochemical properties of CNMs might influence healthy cells, which can result in some inevitable restrictions in terms of the biomedical usage of CNMs.

26.4 Polymeric nanoformulations

The DDSs have been utilized to enhance the efficacy of the traditional administration routes through (1) a better-controlled/sustained drug release, (2) maintaining required therapeutic efficacy, and (3) the active targeting of diseased cells at the specific site. In this regard, polymeric nanoformulations are of particular interest for the fabrication of effective nanoformulations, in large part due to their great features such as biocompatibility, biodegradability, and nonimmunogenicity (Pillay, Essa, Kondiah, & Choonara, 2020). A polymer is a macromolecular structure that is engineered by topdown or bottomup methods of polymerization of one or several monomers to form linear or branched chains. Based on a variety of monomers with extensive structures and functional groups, there is great synthetic resourcefulness for the preparation of polymeric nanoplatforms (Begines, de-Paz,

Alcudia, & Galbis, 2016; Begines et al., 2019; Liu et al., 2018; Pla & Gomez, 2016). By the obtained progress in engineering therapeutic polymeric NPs, many polymeric nanotheranostics have been developed for clinical applications such as instantaneous imaging and treatment NSs, which offer desired features. Such NSs generally entail a polymer backbone with desired biocompatibility and stability, therapeutic moiety such as drug/gene molecules, and imaging agents like MRI contrast agent, photosensitizer, and fluorophore (Luk & Zhang, 2014). Either synthetic or natural-based sources have been utilized for the engineering of the biodegradable polymeric nanoformulations as demonstrated in Fig. 26.2. Some of these nanostructures will be discussed in the following sections.

26.4.1 Synthetic polymer-based nanotheranostics

Since synthetic polymers are the most stable and cost-effective polymeric entities, they are formulated to load high content of drug molecules and offer a good potential to be conjugated with different moieties such as Abs/Aps. They are designed to have desired stability in the blood and safely deliver drug molecules to the target sites. Some of the polymeric entities can even function as antidotes for the sequestration of toxins and neutralizing toxic peptides (Weisman, Chou, O'Brien, & Shea, 2015). In this line, some synthetic polymers such as linear polymers (Wada et al., 2015; You et al., 2007), dendrimers (Imaoka, Kawana, Kurokawa, & Yamamoto, 2013; You et al., 2007), and polymeric NPs (Weisman, Chen, Hoshino, Zhang, & Shea, 2014) have successfully been used.

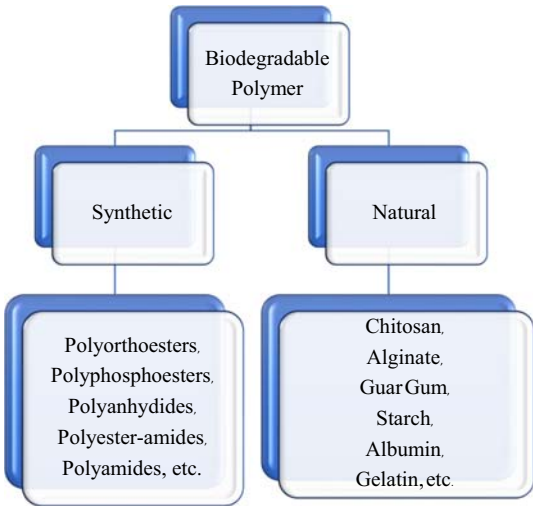

FIGURE 26.2

Classification of biodegradable polymers based on synthetic and natural routes.

Of various polymeric materials, some synthetic biocompatible polymers have been approved for clinical applications, including poly(ethylene glycol) (PEG), poly(D, L-lactic acid) (PLA), poly(lactide-*co*-glycolic acid) (PLGA), and poly (ε-caprolactone) (PCL). In synthetic polymeric NPs, the use of surfactants appears to be often necessary. As amphiphilic organic molecules, surfactants can self-assemble in solution mainly because of possessing a hydrocarbon chain bound to an anionic or cationic functional group. They are classified into cationic, anionic, and nonionic. Examples of cationic surfactants are benzalkonium chloride and tetramethylammonium hydroxide, and the anionic surfactants are docusate or sodium laurate. The nonionic surfactants contain the unions of hydrophobic and hydrophilic sections (Sakamoto, Lochhead, Maibach, & Yamashita, 2017). Usually, surfactants are commonly involved in the formulation of nanocarrier as stabilizing agents. They are often crucial to obtaining a well-structured NS by stabilizing the dispersion during the nanoemulsion process resulting in the decrease of NPs' surface tension and increase of affinity with lipidic structures (Belletti et al., 2016). Zhang and coworkers used a polyphosphoester-based nanocarrier for the codelivery of IR-780 radiosensitizer as a hydrophobic photothermal agent and curcumin ($NP_{IR/Cur}$). The engineered NPs exhibited sufficient drug loading, enhanced passive tumor homing, a prolonged blood half-life, and promoted curcumin bioavailability. The NS was reported to combine the therapeutic functions via the conversion of the NIR light to the heat as well as increased radiation, resulting in an increased local temperature and hence enhanced abolition of tumor cells. Therefore, as shown in Fig. 26.3, the developed NPs were proposed as an effective nanosystem for the radio-PTT of breast cancer with imaging potential to serve as the nano theranostics system (Zhang, Xu, Sun, & Yu, 2019). As a biodegradable and FDA-approved polymer, PLGA has a great capacity for encapsulating hydrophobic drugs with high efficiency via hydrophobic interactions, which can be formulated as nanoscale DDS. Pure PLGA polymeric NPs have a low loading capacity for hydrophilic drugs and are prone to immune clearance by the reticuloendothelial system. However, the amphiphilic copolymers (e.g., PLGA-PEG) can self-assemble into nanoscale micelles, composed of the hydrophobic core and hydrophilic shell, which offer improved solubility and stability. As reported previously by some researchers, the amphiphilic copolymers present a higher loading capacity for the encapsulation of drug molecules (Miao et al., 2017).

Vu-Quang et al. developed a nanoscale theranostic system based on folate-decorated PLGA-PEG NPs containing DOX as well as perfluorooctyl bromide (PFOB) and indocyanine green (ICG) respectively for ^{19}F MRI and NIR imaging. Upon some analyses using flow cytometry, confocal laser scanning microscope imaging, and ^{19}F MRI, it was found that the NPs could be significantly taken up by the human nasopharyngeal epidermal carcinoma KB cells, most likely via interaction with the overexpressed folate receptors (Fig. 26.4A, B), and a greater cytotoxicity effect for cells killing. In comparison with nontargeted NPs, the in vivo assay using folate-decorated NPs demonstrated higher MRI and

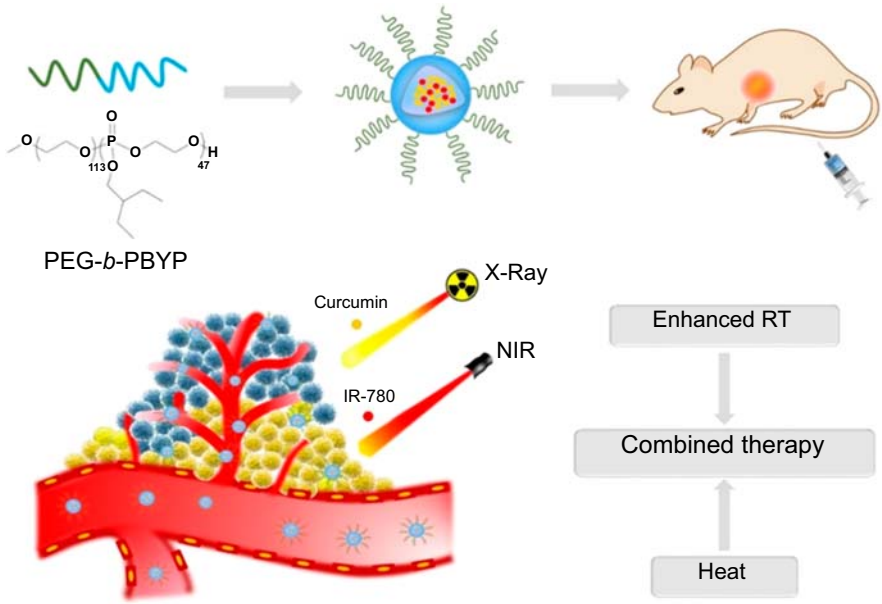

FIGURE 26.3

A schematic illustration of polyphosphoester-based nanocarrier containing curcumin (NPIR/Cur) for combination therapy.

Data were adapted with permission from Zhang, B., Xu, C., Sun, C., & Yu, C. (2019). Polyphosphoester-based nanocarrier for combined radio-photothermal therapy of breast cancer. ACS Biomaterials Science & Engineering, 5(4), 1868–1877.

fluorescence signals from tumors with ^{19}F MRI and NIR, respectively (Fig. 26.4C–E). The obtained results confirmed the use of PLGA-PEG-folate PFOB/ICG/DOX NPs as efficient nanotheranostics for concurrent targeted chemotherapy and traceable imaging (Vu-Quang et al., 2016).

26.4.2 Natural polymer-based nanotheranostics

Natural polymers are considered suitable choices for the development of advanced DDSs mainly due to their nontoxic potential, abundant existence in nature, biodegradability, and biocompatibility. They can be metabolized into safe byproducts in the body. As a result, they are considered nontoxic polymers at moderate concentrations. Natural polymers include peptide-/protein-based macromolecules such as albumin, gelatin, starch, collagen, and polysaccharides like chitosan, agarose, dextran, hyaluronic acid, alginate, carrageenan, guar gum, zein-pectin, pullulan, lignin, and cyclodextrin (Ding, Zheng, Zhang, & Kan, 2016; Myint et al., 2016). Monosaccharides, which are associated with O-glycosidic linkages, can be obtained from different sources such as plants (e.g., pectin,

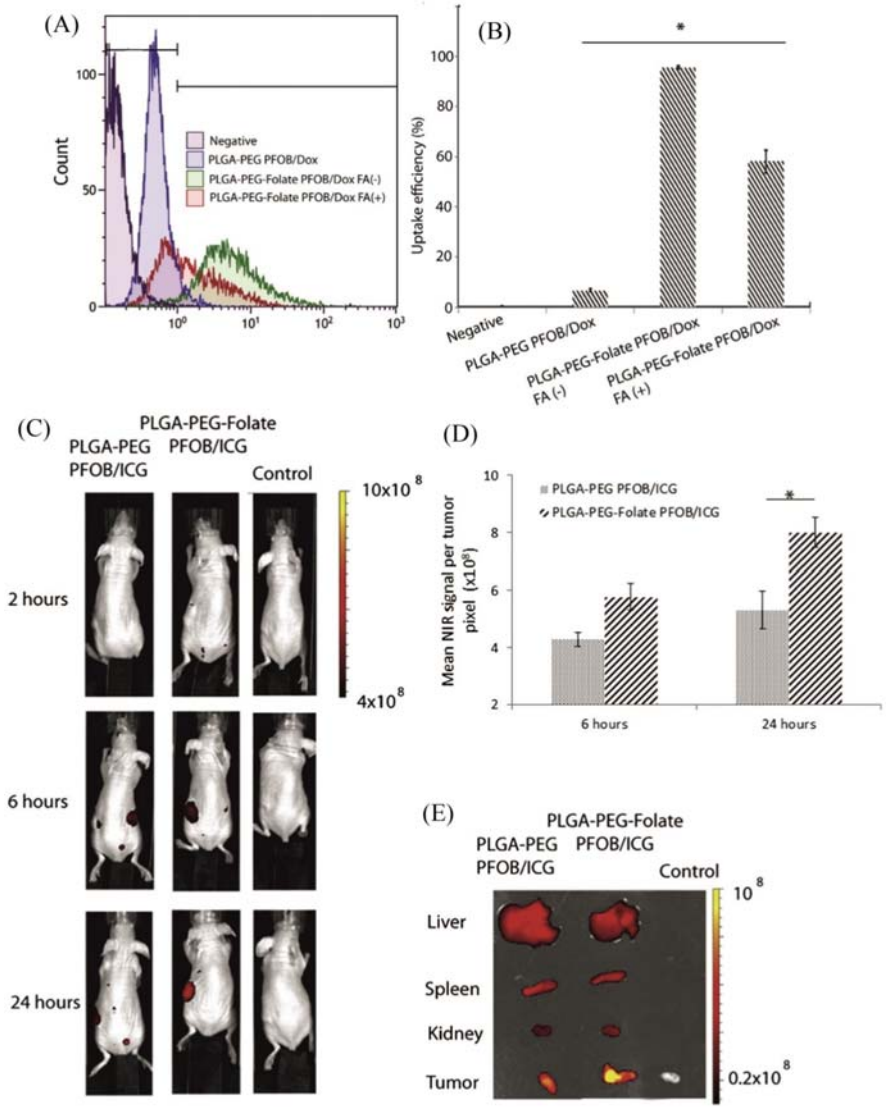

FIGURE 26.4

(A, B) Efficiency of folate-targeting in KB cells treated with PLGA-PEG-PFOB/DOX or PLGA-PEG-folate-PFOB/DOX in the presence of folic acid (FA) (+) and absence FA (–) after 3 h incubation. (A) Fluorescent histogram provided by FACs analysis indicating the uptake of DOX labeled NPS. (B) Diagram of mean uptake efficiency. (C) In vivo NIR imaging of mice treated with PLGA-PEG-PFOB/ICG, PLGA-PEG-folate-PFOB/ICG, or

(*Continued*)

cellulose, starch), animals (e.g., chitosan, chitin, glycosaminoglycan), microbial (e.g., dextran, pullulan, xanthan gum, gellan gum), and algal (e.g., agar, alginate, and carrageenan) sources. These macromolecules are biopolymers that are often found as complex polysaccharides (Kang, Opatz, Landfester, & Wurm, 2015; Nicolas, Mura, Brambilla, Mackiewicz, & Couvreur, 2013). The main issue of natural polymers is their broad and mixed molecular weights and variable chemistry that make some issues in the design and development of DDSs. Besides, many polysaccharides lack solubility in organic solvents and may associate with some limitations in terms of chemical modifications (Wen & Oh, 2014). The plenteous and accessible functional groups of natural polymers (e.g., hydroxyl, carboxyl, and amine groups) make them macromolecules of choice with great potential for facile modification towards more desirable biomaterials for intended biomedical applications. To modify the natural polymers and attain composite entities with desired features, the natural polymers can be combined with semisynthetic or synthetic polymers through cross-linking reactions, chemical/physical modifications, or surface mineralization.

26.4.2.1 Chitosan-based nanotheranostics

Chitosan (CS) is one of the most commonly used natural polymers in biomedical applications (Fathi et al., 2017; Fathi et al., 2018a, 2018b; Fathi et al., 2020b). It can easily be combined with other polymers, and offer great potential for surface modification. As a nontoxic and noncarcinogenic polymer with antibacterial properties, CS imposes low immunogenic behavior and displays significant compatibility with cells and tissues (Rosenblum, Joshi, Tao, Karp, & Peer, 2018). The abundant amino and hydroxyl functional groups allow the desired functionalization of CS. Such modification provides a great possibility for the engineering of nanocarriers and nanotherapeutics with simultaneous incorporation of therapeutic agents, molecular targeting moieties, and diagnostic/imaging modalities. Altogether, CS is a promising natural polymer for the engineering of advanced DDSs (Fathi et al., 2018b; Shukla, Mishra, Arotiba, & Mamba, 2013; Sun & Wan, 2007; Takeshita & Yoda, 2017). It should be noted that at pH below 6.5 (pKa of CS), the protonation of amine groups of CS can supply the cationic and mucoadhesive properties for CS, which might facilitate its interactions with anionic structures such as cell surface macromolecules and nucleic acid and fast absorption by cells (David, Jaidev, Sethuraman, & Krishnan, 2015; George et al., 2019; Jean et al., 2012; Nagarajan et al., 2015). The biological and physiological

◀ control (PBS) after 2, 6, and 24 h. (D) Quantification of NIR signal from (C). (E) Ex-vivo NIR imaging of organs from treated mice.

Adapted with permission from Vu-Quang, H., Vinding, M. S., Nielsen, T., Ullisch, M. G., Nielsen, N. C., & Kjems, J. (2016). Theranostic tumor targeted nanoparticles combining drug delivery with dual near infrared and 19F magnetic resonance imaging modalities. Nanomedicine: Nanotechnology, Biology, and Medicine, 12 *(7), 1873–1884.*

properties of CS can be improved by an amphiphilic modification, which can make CS a suitable biopolymer for various purposes such as the delivery of insulin (Wang et al., 2017), the formation of cisplatin-mediated polyampholytic CS-based NPs (Kang, Kwon, Kim, & Lee, 2017b), PEGylated and fluorinated CS for targeted delivery (Antoniraj et al., 2018; Belabassi et al., 2017), and modification of CS by means of folate and heptamethine cyanine particularly in terms of tumor-specific imaging and PDT (Fathi et al., 2018b; Zhang et al., 2017). The CS-grafted graphene oxide (CS-g-GO) carrier containing DOX and MNPs has been evaluated as a hybrid pH-sensitive nanotheranostics. The decreased release rate of DOX molecules with the increasing pH demonstrated the pH-dependent behavior of the carrier mainly due to the hydrogen bond formation of CS chains. The biocompatibility of the nanohybrid system was confirmed by cytotoxicity assays of healthy L929 cell lines, while the DOX-loaded nanohybrid system indicated an efficient anticancer effect against breast cancer MCF-7 cells. Magnetic behavior study revealed that the GO lessened the contrast potential of the MNPs, while grafting of MNP/GO with CS promoted its contrast efficacy which is very beneficial for MRI applications (Baktash, Zarrabi, Avazverdi, & Reis, 2021).

26.4.2.2 Alginate-based nanotheranostics systems

Alginate (Alg), an anionic linear biopolymer, is identified as the most derived polymer from the marine source. It is the second most amply available biopolymer. It has widely been used in the food industries, bioengineering applications, and the development of various DDSs. Owing to advantages of Alg (e.g., biodegradability, biocompatibility, flexibility, functionality, facile modification, and muco-adhesiveness), this polymer has received tremendous attention and is employed for a variety of applications in particular for the development of nanotheranostics (Hasnain, Nayak, Kurakula, & Hoda, 2020; Lee & Mooney, 2012; Nayak, Malakar, Pal, Hasnain, & Beg, 2018; Ruman, Fakurazi, Masarudin, & Hussein, 2020; Zhao et al., 2020b). Methods used to formulate alginate include spray drying, polyelectrolyte complexation, covalent crosslinking, ionic gelation, emulsification, and self-assembly techniques (Ciofani et al., 2008; Hasnain & Nayak, 2019; Singh & Singh, 2019).

26.4.2.3 Albumin-based nanotheranostics

Albumin is an FDA-approved biomacromolecule that has widely been used for therapeutics applications because of some inherent properties such as nontoxicity, nonantigenicity, biodegradability, biocompatibility, stability, and the ability for binding to positively- and/or negatively-charged molecules via multiple binding sites, as well as high drug loading capacity (Desai, 2007). There are several albumin-based NPs used for the delivery of various drugs, including albumin-bound paclitaxel (Abraxane) (Villano, Mehta, & Radhakrishnan, 2006), curcumin (Kim et al., 2011), DOX (Choi et al., 2015), tacrolimus (Seo et al., 2016), and rifampicin (Sung et al., 2009). Typically, there exist two different sources of albumin, including bovine serum albumin (BSA) (Maghsoudi, Shojaosadati, &

Farahani, 2008) and human serum albumin (HAS) (Dreis et al., 2007), which can be used for different purposes. Further, albumin from egg (i.e., ovalbumin) is a suitable candidate for the development of nanocarriers, even though its antigenicity is the main limiting issue (Chang, Stadmiller, Staskevicius, & Champion, 2017). Recently, Wang et al. reported on a theranostic system caged in HAS as a therapy NS against breast cancer. A prodrug of a drug/dye conjugate (DDC) was efficiently attached to HAS via a disulfide-exchange reaction to having a high drug-loading rate. The drug-release kinetics was monitored by a noninvasive fluorescence signal in real-time which revealed the usefulness of the NS. The designed DDC@HSA particles indicated tumor-targeting ability as glutathione (GSH)-responsive NS with the improved antitumor effect both in vitro and in vivo. Based on such findings, the NS has proposed a promising strategy as the HAS-based theranostic system for precision diagnosis and therapy purposes (Wang et al., 2020b).

26.4.2.4 Starch-based nanotheranostics

Starch is one of the most abundant carbohydrates in the human diet. As granular structures with different shapes and sizes, they can be found in most higher plants in leaves, stems, fruits, roots, and sprouts. Starch-based NSs (e.g., NPs, nanocrystals, nanofibers) with unique physicochemical properties have been considered potent biocompatible nanocarriers for encapsulation and delivery of various drugs (Acevedo-Guevara, Nieto-Suaza, Sanchez, Pinzon, & Villa, 2018; Pérez, Baldwin, & Gallant, 2009). So far, several starch-based multifunctional nanotheranostics systems have been developed and evaluated (Li et al., 2014; Verger et al., 2016). In a study carried out by Li et al., for example, a multifunctional nanoscale theranostic system composed of poly(methacrylic acid)−polysorbate 80-grafted-starch was developed. Then, the ability of the NS in crossing the brain microvessels of healthy mice was shown by both in vivo MRI and ex vivo confocal microscopy analyses. The obtained results confirmed the efficacy of DOX-loaded NS in the treatment of micrometastases compared to free-DOX (Li et al., 2014)

26.4.2.5 Hyaluronic acid-based nanotheranostics

Hyaluronic acid (HA) is a natural polymer, which is a nonsulfated glycosaminoglycan with some unique properties such as mucoadhesion, nonallergenic, biocompatibility, and facile chemical modification. HA has widely been employed for the development of different types of DDSs, theranostics, tissue regeneration, antiaging, and antiinflammatory medications (Vasvani, Kulkarni, & Rawtani, 2020). HA and its derivatives have been utilized as a tumor marker, for cancer treatment and also for monitoring its progression because of its mucoadhesive property. Additionally, HA-based NSs can specifically bind to some existing cell surface receptors of the liver, kidney, body fluid, vessels, and most tumor tissues. It can be used as a potential carrier of proteins, peptides, nucleic acids, and anticancer agents for targeted drug delivery (Kumar, Singhal, Narang, Mishra, &

Kumari, 2020; Zhang et al., 2020). More recently, Lin et al. reported a smart HA-based theranostic nanogel containing gold clusters (GC) for the NIR fluorescence-guided cancer treatment. The synthesis steps of methacrylate HA (mHA) nanogel are shown in Fig. 26.5A. The multifunctional mHA-GC hybrid nanogels were used to encapsulate DOX with high efficiency and excellent colloidal stability.

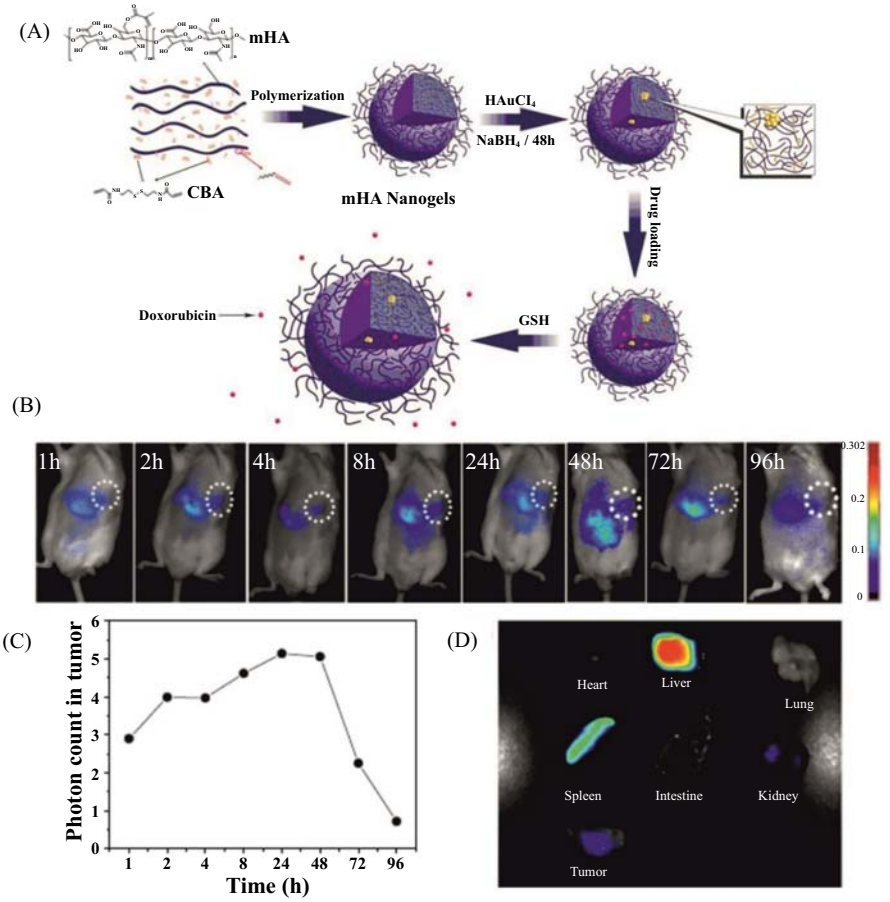

FIGURE 26.5

(A) Schematic presentation of methacrylate hyaluronic acid-gold cluster (mHA-GC) hybrid nanogel. (B) NIR fluorescence images of H22 tumor-bearing mouse after intravenous injection of mHA-GC hybrid nanogels. Tumor areas are indicated with the dashed circle. (C) The time-dependent fluorescent intensity of the tumor region. (D) The NIR fluorescence images of the major organs and tumor at 96 h postinjection.

Data were reused with permission from Lin, Y., Li, C., Liu, A., Zhen, X., Gao, J., Wu, W., et al. (2021). Responsive hyaluronic acid-gold cluster hybrid nanogel theranostic systems. Biomaterials Science, 9, 1363–1373.

The reducing TME containing GSH promoted the fluorescence signal of GC in the hybrid nanogels. The fluorescence tracking of nanogel indicated the selective cancer cell uptake and high intratumoral accumulation. The nanogel responsive to GSH could lead to the disassembly of the hybrid nanogels structure and enhanced drug release in the presence of GSH in the TME and cancer cells. Both, in vivo and in vitro tumor suppression evaluation demonstrated the significant cell inhibition capability of DOX-loaded hybrid nanogels compared to free DOX (Fig. 26.5B, C, D) (Lin et al., 2021).

26.5 Stimuli-responsive nanotheranostics clinical usage

The stimuli-responsive behavior of nanocarriers has improved the clinical translation of several nanotheranostics. Some nanocarriers that respond to magnetic, temperature, pH, and secretory phospholipase A2 (sPLA2) have been under clinical translation investigations (Mi, 2020). Two magnetic-sensitive vehicles including iron oxide magnetite and DOX-loaded iron and carbon (MTC-DOX) are under clinical trial for cancer therapy. The first one is based on the iron oxide magnetite, which is in phase I clinical trial for the treatment of prostate cancer. The second one is based on the DOX-loaded iron and carbon (MTC-DOX), which have been utilized in three clinical stages, including the unresectable hepatocellular carcinoma phase II and III safety, tolerance, and efficacy (survival time).

Additionally, the thermo-sensitive DOX encapsulated liposomes (ThermoDox) have been utilized in some clinical studies (ClinicalTrials.gov Identifier: NCT02181075, NCT02536183, NCT00441376, NCT00617981), including phase I and II studying to indicate the maximum tolerated dose, safety, PK, and hyperthermia effects in patients with recurrent regional breast cancer. Phase I investigation of DOX release from liposome by focused ultrasound in liver tumors with MRI and high intensity focused ultrasound combined study was used to determine DOX release in pediatric refractory solid tumor. In a phase 3 trial, ThermoDox was used for the radiofrequency ablation in the treatment of hepatocellular carcinoma (NCT00617981). Moreover, pH-responsive polymeric micellar carriers are used for solid tumors, soft tissue sarcoma, metastatic sarcoma, and sarcoma. The NS established improved therapeutic effect in phases I and II as a promising modality for further clinical evaluation. Besides, in the secretory phospholipase A2 (sPLA2)-sensitive nanoplatform, cisplatin incorporated liposomes (LiPlaCis) have entered phases I and II to study the safety, tolerability, and sensitivity of patients with advanced and metastatic breast cancer (ClinicalTrials.gov Identifier: NCT01861496).

As far as is known, the successful progress in the clinical translation of stimuli-responsive nanocarriers can face some challenges, including (1) the difference between the animal tumors model and human tumors due to the complexity of human tumors, (2) the toxicity, biosafety, and biodegradability of nanocarriers

that are essential factors, (3) the stimuli-responsive function in vivo with good stability and outcome, (4) the tumor accumulation and therapeutic efficacy of stimuli-sensitive nanocarriers that should be approved in a clinical trial, (v) the factors that might influence the stimuli-responsive features in vivo that should be validated, and (6) the right dose and administration routes (intravenous, or intraperitoneal injection) that should be determined (Mi, 2020).

26.6 Conclusion and final remarks

According to available data, the desired simultaneous targeting, treatment, and imaging/monitoring potentials combined in a single NS has opened a new horizon for the development of smart multifunctional nanomedicines and nanoscale theranostics platforms. These multimodal NSs can be further advanced to function as stimuli-responsive NS with dual-/multitargeting possibilities. Such multifunctional modalities can be engineered as multistage NSs employing microfluidics techniques. Notably, the rapid development of these NSs has extended their diagnostic and therapeutic usage beyond cancer therapy into other fields such as cardiology and tissue engineering. As a result, it is envisioned that such multifunctional NSs pave the way for the development of remotely controlled sophisticated nanotheranostics. Contrary to the obtained advances, to be safe than sorry, more basic studies seem to be necessary to get a deeper understanding of their performance and long-term impacts in biological settings in particular on healthy cells/tissues. The concerns of toxicity, immunogenicity, and slow elimination kinetics from the body should be thoroughly evaluated before the clinical test in humans. Collectively, these new nanoplatforms seem to provide great clinical potential with great hopes for the targeted diagnosis and treatment of different detrimental diseases such as various malignancies.

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CHAPTER

27 Cell-penetrating peptide(s): Design, synthesis, and site-specific nanotheranostic applications in cancer therapy

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27.1 Introduction

The role of theranostic is widely used as a treatment strategy for cancer treatment. In the present scenario, the diagnosis of cancer is the major limitation of science. Because the early diagnosis is treated with radiology and surgery if it is a solid tumor that can be localized and easily removed by operation (Gautam et al., 2019). The worldwide report said that the maximum number of cancer patients are of different stages which cannot be easily diagnosed in the early stages. The report of the American Cancer Society has collected the data from the National center for health statistics 98,160 new cancer patients and 608,570 deaths were recorded in the United States. They also said that the early detection, diagnosis, and change in habits (smoking, other food habits) reduce the 31% of cancer patients in comparison to 1990, also including the clinical trials data (Lee et al., 2021). As per the WHO, 11.6%lung cancer is followed by breast cancer 11.6%, and 10.2% colorectal cancer (Shrivastava et al., 2021). The death recorded is 18.4% of all deaths by lung cancer (Gautam et al., 2020), 9.2% in colorectal, and 8.2% by stomach cancer.

Theranostics is a portmanteau of therapeutics and diagnosis (Sharma et al., 2017). It is a kind of therapy that is initially diagnosed and then treated for the disease according to the prescribed medicines. This can be highlighted by labeling the marker over the nanodelivery systems and targeted. Their imaging and treatment strategies can be understandable which include site-specific targeting/delivery, time-dependent release, pH-dependent release, controlled release systems, liposomes, vesosomes (Gautam, 2022a), naoparticles etc., (Gautam, 2022b).

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The synthesis of the first CPP was Tat dodecapeptide (GRKKRRQRRRPQ) for human immunodeficiency virus 1in 1988 and the mechanism was cell internalization and nucleus translocation. After that in 1991, Penetratin peptide (RQIKIWFQNRRMKKK) was encoded having the ability to cross cell membrane in an energy-independent manner. The CPP can be a 5−30 amino acid long chain ability to deliver the DNA, RNA, protein, APIs to the effective site. The current chemotherapeutic agents for the treatment of cancer caused many side effects. The role of CPPs plays an important role in the targeted drug delivery along with the theranostic formulations.

27.1.1 Role of enzyme in tumor metastasis

Invasion by neoplastic cells is a key impediment to effective cancer treatments. Enzymes like hyaluronidase (Witzel et al., 2017), matrix metalloproteinases (Gonzalez-Avila et al., 2019), plasminogen activator (PA) (Andreasen et al., 1997), plasmin (Reuning et al., 1998), sialyltransferase (Drinnan et al., 2003), urokinase-type, and others, play a crucial role in the catabolism of extracellular matrix macromolecules. However, inhibitors of these enzymes can thwart this process. For effective metastasis, the cancerous cells must filtrate the matrix, especially the basement membrane. Invasion is the term for the process of tumor cells attaching to matrix components, proteolysis of matrix components, and tumor cell movement via the flaws in the matrix. Cancer is a challenging illness to cure because malignant cells have the potential to penetrate and spread. Malignant cells in the initial neoplasm must infiltrate localized host tissue, enter and survive in the circulation, extravasate into the tumor site, and establish a secondary colony to generate metastatic growth. Cells are arranged in the lattice of collagen fibers, glycoproteins (such as fibronectin), proteoglycans, and hyaluronic acid in the interstitial connective tissue. Type I, II, and III, or interstitial collagen, are the three primary kinds of collagen present in interstitial connective tissue. The release of a variety of proteases from the invading tumor is thought to cause deterioration of these natural barriers by infiltrating cancer cells. The PAs, particularly the urokinase-type, cathepsins, particularly B and D, and different metalloproteases are among the most studied proteases. Trypsin-like enzymes, chymotrypsin-like enzymes, thrombin, and heparinases are a few of the hydrolases linked to metastasis (Duffy, 1992; Fouani et al., 2017).

27.1.2 Proteases involved in metastasis

The proteases of the plasminogen-PA systems play a significant role in cancer spreading. Protease-mediated breakdown of ECM 3 is a critical rate-limiting step in metastasis, allowing tumor cells to locally penetrate and infiltrate blood arteries, escape the vasculature, and embed themselves in nearby tissues or organs (Meyer & Hart, 1998; Woodhouse et al., 1997). Furthermore, endothelial cells must synthesize these proteases in order for angiogenesis to occur (Pepper, 1997).

The serine proteases of the plasminogen-PA-plasmin system are among the proteases involved in the degradation of ECM during cancer development. The serine proteases also degrade the glycoproteins (like fibronectin, laminin, and type IV collagen). Plasmin is produced by plasminogen using two activators: (1) tPA (a protease found mostly in plasma and participates in thrombolysis) and (2) uPA (a protease found in a variety of body tissues and cells (Sugiura et al., 1999).

27.1.3 Plasminogen activator

The PA system, commonly known as the fibrinolytic system, regulates intravascular fibrin accumulation as well as a range of physiologic and pathologic events. Through their effects on angiogenesis and cell migration, the elements of the process have a role in tumor development, invasion, and metastasis in cancer. Most cancers include these components, and their presence indicates not just their function but also a prognostic value. Their expression is generally modulated by growth factors and cytokines, and most of them are up-regulated in cancer. Two types of PAs are present in humans, tissue-type (tPA) and urokinase-type uPA. Both the tPA and uPA are expressed in cancerous cells (Leurer & Rabbani, 2015). tPA is a glycoprotein of 70-kDa, it is synthesized by the endothelial cells and is assists to uphold vascular patency in response to intravascular fibrin formation. The key role of t-PA is assumed to be the dissolution of blood clots. tPA with its receptor annexin II which was generally found in macrophages, endothelial surface, and certain tumor cells controls fibrin deposition intravascularly. The high expression of annexin II in acute promyelocytic leukemia and other malignant conditions renders a bleeding risk in these disorders. uPA is another naturally occurring PA. The single-chained uPA has a stronger affinity for fibrin. Both the single-chained and double-chained versions of cell-associated uPA bind to the uPA receptor (uPAR). The interaction boosts uPA's ability to activate plasminogen. uPA with its receptor (uPAR) is generally engaged in various cellular functions, uPAR can also bind inactivated uPA from PAI-1, creating a uPAR-uPA-PAI complex that is immediately endocytosed (Nykjær et al., 1997). Numerous components of the low-density lipoprotein receptor family facilitate this process. It is also quickly removed from the circulation by the liver with a plasma half-life of around 5 min (Caiolfa et al., 2007; Gliemann, 1998; Nykjær et al., 1997). Many cells, including most tumor cells, have been identified to have uPA and its receptor. It can directly activate procollagenase in addition to functioning as a plasmin activator. It has a regulating influence on physiologic cell migration while also tumor development and metastasis via both of these routes.

Multiple inhibitors control the proteolytic activity of fibrinolytic enzymes. Plasmin is inhibited by the first group. The most essential component of the PAS in the control of both physiologic processes and the pathogenesis of numerous illnesses, including cancer, is plasminogen activator inhibitor type 1 (PAI-1) (McMahon & Kwaan, 2007). PAI-1 is a prominent participant in the pathogenesis of numerous vascular disorders, as well as cancer, among the fibrinolysis

inhibitors. Endothelial cells, liver, adipose tissues, vascular smooth muscle cells, and a substantial number of tumor cells all produce this 52-kDa glycoprotein that is PAI-I. It belongs to the SERPIN (serine protease inhibitor) family. Both uPA and tPA are inhibited by it (Durand et al., 2004; Stefansson et al., 2003; Vaughan et al., 2007). PAI-1 is found in a variety of tumor cell types, as well as stromal fibroblasts and tumor-associated endothelial cells in the tumor microenvironment. It has the ability to control tumor development, invasion, and angiogenesis due to its presence (Angleton et al., 1989; Muller et al., 1989). PAI-1 suppresses apoptosis including both normal vascular smooth muscle cells and tumor cells when added to cell cultures, thereby contributing to tumor growth and angiogenesis (Chen et al., 2004; Kwaan et al., 2000).

Active cathepsin B (CB) is a thiol-stimulated protease normally found in lysosomes. Depending on the source. At neutral and slightly alkaline pH, CB from tumor tissue appeared to be more active (Aoki & Harpel, 1984). Tumor CB's capacity to catalyze peptide bond hydrolysis at neutral pH may allow it to play a role in cancer dissemination. The cancerous cell shows a higher proportion of CB in the cell membrane than in normal cells. similar to CB, Cathepsin D (CD), is also a lysosomal protease with an acidic optimum pH. CD, as formed by breast cancer cells in vitro, can exist in multiple molecular weight forms. The processing of CD appears to be slower in cancer cells than in normal cells (Duffy, 1992).

27.2 Peptide synthesis

Currently, "peptide synthesis" includes a large range of techniques and procedures that enable the preparation of materials ranging from small peptides to large proteins. A peptide having a library of amino acids can be screened and optimized. After that synthesis process starts (Fig. 27.1). Chemically synthesized peptides serve very diverse purposes, including biopharmaceutical drugs, epitope mapping, peptide microarrays, and vaccine development (Petrou & Sarigiannis, 2018). Peptide synthesis necessitates the establishment of exact amide bonds between certain amino acids. Although each amino acid is both an amine and a carboxylic acid, the four dipeptides X-X, Y-Y, X-Y, and Y-A may be formed via direct interaction between two amino acids X and Y. To get quality output, such as Y-X, the amino moiety of Y and the carboxylic acid moiety of X must be protected. Only the carboxylic acid moiety of Y and the amino group of X is capable of reacting in this fashion. Higher peptides like Z-Y-X are made by expelling the protective group of Y from the dipeptide Y-A and interacting it with the amino acid Z, which is protected at the amino group. The free carboxylic acid group of Z, which is shielded by the carboxylic acid group of A, then can react with the free amino group of Y-A. An amino acid carboxyl group is protected by creating a benzyl ester. By creating a Boc derivative, the amino group is protected. DCCI is used to form an amide link between these two protected amino acids. The Boc

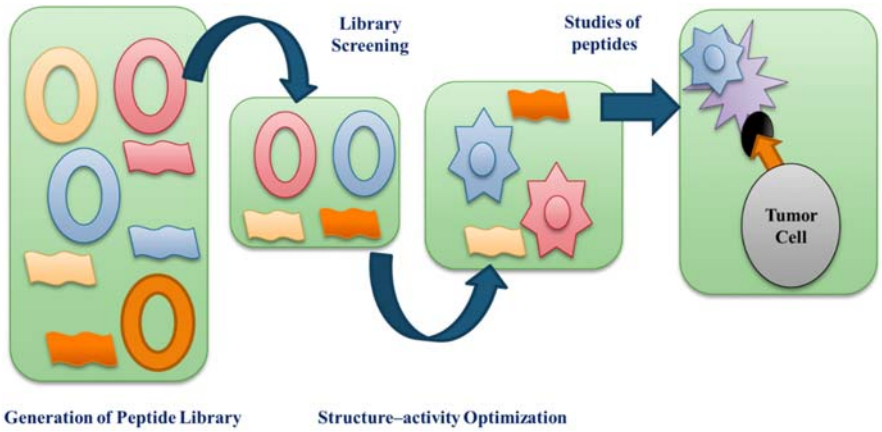

FIGURE 27.1

Schematic representation for the synthesis of the novel peptide having a high binding affinity towards cancer receptors.

group is hydrolyzed using trifluoroacetic acid to allow the peptide to continue to extend. The amino acid group is unattached.

Synthesis of peptides in solution was utilized to synthesize smaller peptides made up of just a few residual amino acids. Its key benefit is that, after each stage of synthesis, intermediate products may be separated, purified, and recombined to generate longer sequence peptides. In terms of chemical compositions and the combination of peptide blocks, this approach is exceedingly adaptable. Novel Techniques have been developed for solutions synthesis ranging from the design of functional groups for side chains, condensation, and usage of novel coupling chemicals of fragments for the synthesis of big molecules (Hiebl et al., 1999).

27.2.1 Solid-phase peptide synthesis

Peptide synthesis is far more sophisticated than just combining the necessary amino acids in a test tube to produce amide bonds. With 20 natural amino acids with a handful of synthetic ones, the combinations that may be created with this process are endless. The synthesis of peptides is both exciting and hard because of their intricacy. When two amino acids in solutions are combined, four distinct dipeptides are generated. SSPS comprises extending a peptide chain to a solid matrix with consecutive amino acid add-ons attached by the formation of a binding amide (peptide) between the incoming amino acid carboxy and the amino acid group previously constrained to the matrix till the desired sequence and length of peptide has not been synthesized (Amblard et al., 2006; Nilsson et al., 2005) (e.g., for a mixture of glycine and alanine the four dipeptides would be glygly, glyala, alagly, alaala. In this representation of peptides, the free amino

group or N-terminus is on the left-hand amino acid, and the free carboxylic acid group, the C-terminus is at the right-hand end).

To guarantee that just the required dipeptide is generated, both the basic group and acidic groups of distinct amino acids must be rendered incapable of reacting. The shielding of reactive groups refers to this "deactivation," and a group that is unable to respond is referred to as a protected group. The acids are shielded, and allowed to react, and thereafter deprotected in the classical method of synthesis, after which one terminus of the dipeptide is protected and reacted with a new protected acid, and so forth. In solid-phase peptide synthesis (SPPS), the amino acid is bonded at the one end of the peptide to a water-insoluble polymer and stays protected all through the peptide's development, requiring fewer protection/deprotection phases and allowing the reagents to be washed away without wasting any of the peptides. The synthesis in solution was well documented, and the purification of peptides in the novel suggested approach could only be carried out following cleavage, wherein most of the by-products ligate together during synthesis. Despite the disadvantages, the SSPSs have numerous benefits over the conventional method: the reaction may indeed be automated. There is no more concern with the solubilization of the peptides, as it stays attached to the solid matrix.

27.2.2 Methodologies of synthesis

Sequential synthesis, convergent synthesis, and chemical binding are the basic SPPS techniques. Sequential synthesis includes the gradual addition of amino acids to the desired sequence.

27.2.3 Sequential synthesis

Sequential synthesis was already depicted in Fig. 27.2. This approach is for the synthesis of peptides (small to medium) with up to 50 residues. Nevertheless, bigger polypeptides may be created utilizing the process of cysteine polymerization, dendrimer assembly employing lysine matrices, or construction of the Template Assembled Synthetic Protein construction (Banfi et al., 2004; Tuschscherer & Mutter, 1996). Cysteine polymerization takes place at amino and Carboxy terminals of the peptide by identifying cysteine residue. Cysteine possesses an oxidation-friendly sulfide group that forms disulfide bonds to polymerize the peptide at both the amino and carboxy tails to produce high molecular weight polypeptides. This approach generates a wide array of peptide oxidized species from cyclic monomer and dimer to higher-molecular polypeptides (Fig. 27.3). Cysteine chain polymer peptides are frequently the best peptide vaccine in terms of immunogenicity. The difficulty is to control the extent of polymerization to obtain a peptide species to produce different species whose circulation from batch to batch is not inevitably repeatable (Patarroyo et al., 2002). The t-Boc/Bzl is utilized to synthesize higher molecular weight peptide (Miranda & Alewood, 1999; Taylor et al., 2005), which was not possible with Fmoc/tBu due to the stearic hindrance

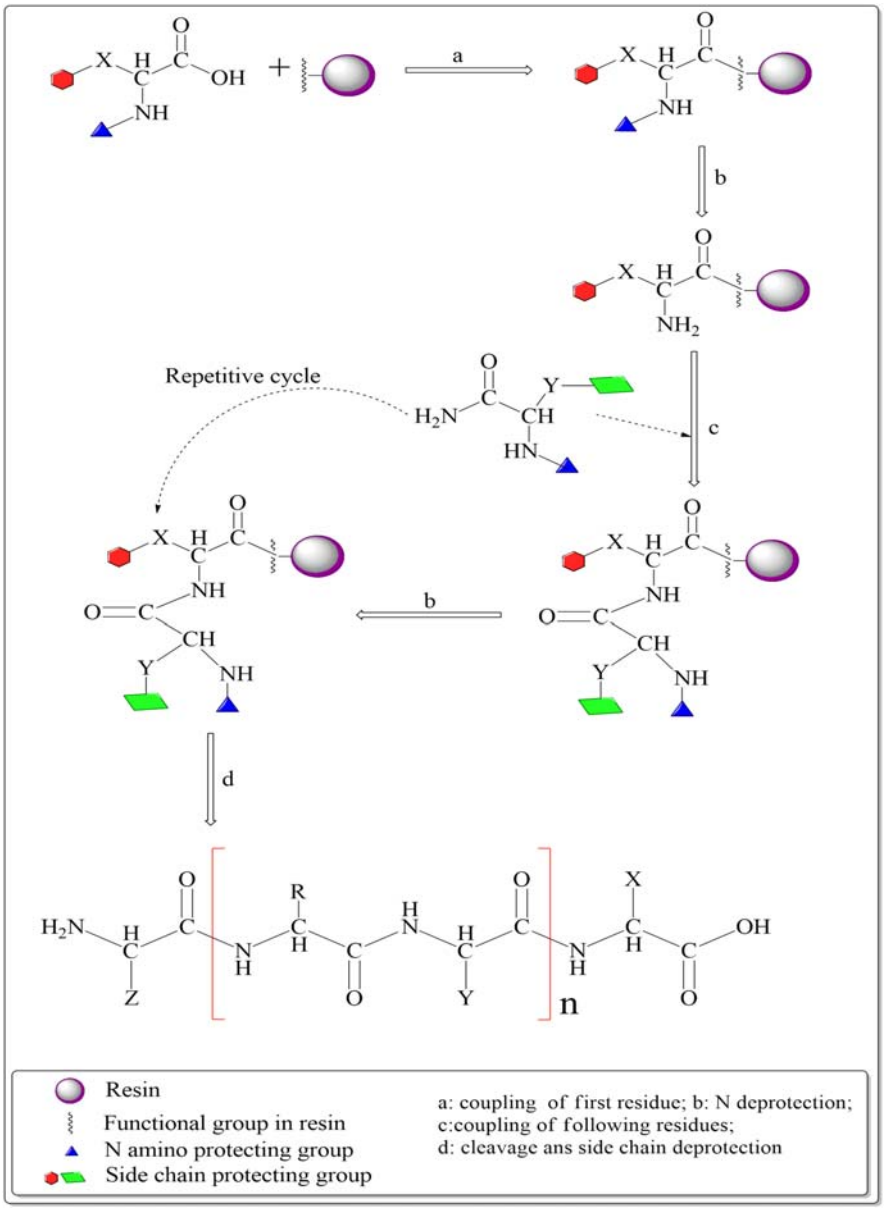

FIGURE 27.2

Steps involved in the synthesis of solid-phase peptide synthesis.

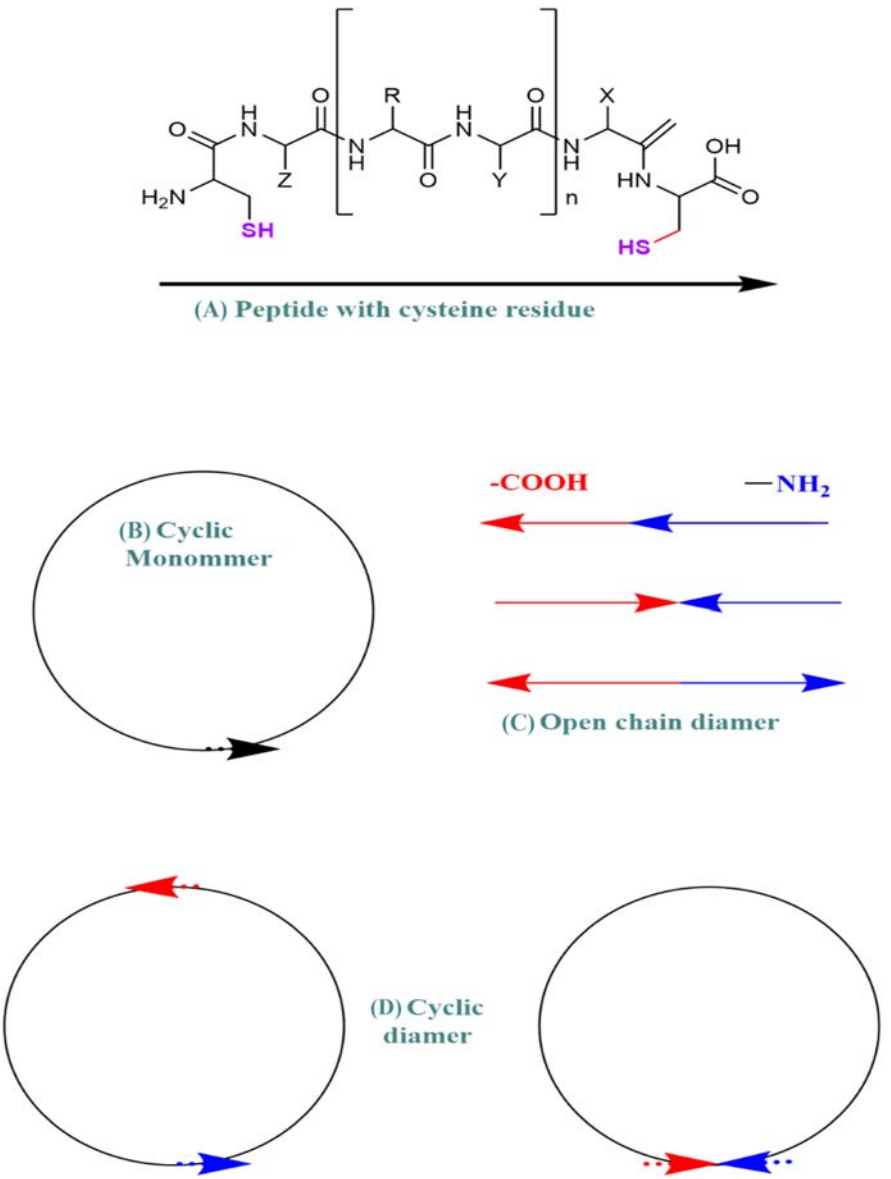

FIGURE 27.3

Role of cysteine in oxidative polymerization.

in the deprotection phase of the Fmoc group at the time when the peptide chain grows (Tickler et al., 2001). Dendrimers can be constructed to obtain high molecular weight peptides containing a core lysine matrix residue to which the peptide

sequences are linked with the amino group. By developing the dendrimers, it will be possible to synthesize the desired copies of the peptide sequence.

27.2.4 Convergent synthesis

Convergent synthesis, however, involves the synthesis and coupling of protected peptide segments. Convergent solid-phase peptide synthesis comprises the following steps: (1) solid-phase synthesis of protected peptides (these must retain the protecting groups of the N-amino and the side-chain functions after cleavage from the resin), (2) purification and characterization of the protected peptides, and (3) their solid-phase coupling. The final steps of the detachment of the free peptide from the solid support and formation of disulfide bridges, when necessary, are common to those carried out in a linear strategy and are discussed elsewhere in this volume. Some factors are crucial while synthesizing the peptide by convergent method, including the residues at the C- and N-terminal positions, the protecting group strategy Boc/benzyl Bzl or Fmoc/tert-butyl tBu], and the provision used for coupling of the solid-phase segment and the size of the protected peptides. Both the Boc/Bzl and Fmoc/tBu strategies have been utilized equally well enough for the fabrication of protected peptides, however, the milder circumstances utilized for the latter have contributed to its becoming gradually more preferred. Both protecting groups of Fmoc/tBu are flexible with the use of linkers (C-terminal carboxylic protecting groups) labile to extremely dilute acid. The modifications associated with the cleavage of shielded peptides from these linkers are synthetically uncomplicated as compared to the use of ultraviolet light or the usage of transition metal complexes that are occasionally necessary for the Boc/Bzl method. From the perspective of solubility, no significant differences have been noted between both forms of protected peptides (Gao et al., 2021; Isoda et al., 2021; Zhu & Van Der Donk, 2001).

27.2.5 Chemical ligation

For the chemical synthesis of big peptides and proteins, chemical ligation is a particularly promising technique (Baca et al., 1995; Yan & Dawson, 2001). It is based on the chemical bonding of short, unprotected peptides, which are simple to handle due to their high solubility in the synthesis solvents. The unprotected side chains of such peptides are complexed with units that react chemoselectively with just one group of the acceptor peptide. The chemical ligation method is the method of interest for the synthesis of various peptides and proteins by using ligands with the formation of thioester, oxime, disulfide, thiazolidine, and peptide bonds. Initially, the main problem. The distinctive instance of this tactic is the synthesis of phospholipase A (124 residues with 14 cysteine residues and 7 disulfide bridges), the synthesis of the microprotein S (Hackeng et al., 2000).

27.2.6 Experimental events of solid-phase peptide synthesis

SPPS can indeed be done in a variety of methods. For the modest and big-scale synthesis of only peptides or numerous peptides at a time, there are manual and automated processes available. In Fig. 27.4 the basic steps involved In the synthesis were incorporated.

Step 1- Attaching an amino acid to the polymer

The N-terminus and the C-terminus3 are the two extremities of a peptide chain, and the end which is coupled to the polymer depends mostly on the nature of the polymer. The amino acid is attached by reacting these with a bridging agent, then reacting the polymer with the opposite end of the linkage agent. This indicates that a peptidepolyamide connection can be generated that will not be hydrolyzed during further peptide formation processes. Di- and trisubstituted benzenes, like those illustrated below, are common linking agents (Fig. 27.5). These then join the C-terminus amino acid and resin together as follows:

Step 2—Protection

The next amino acid also needs to have its amino group protected to prevent the acids from reacting with each other. This is done by protecting it with FMOC (9-fluorenylmethoxycarbonyl). In addition, any amino acid side

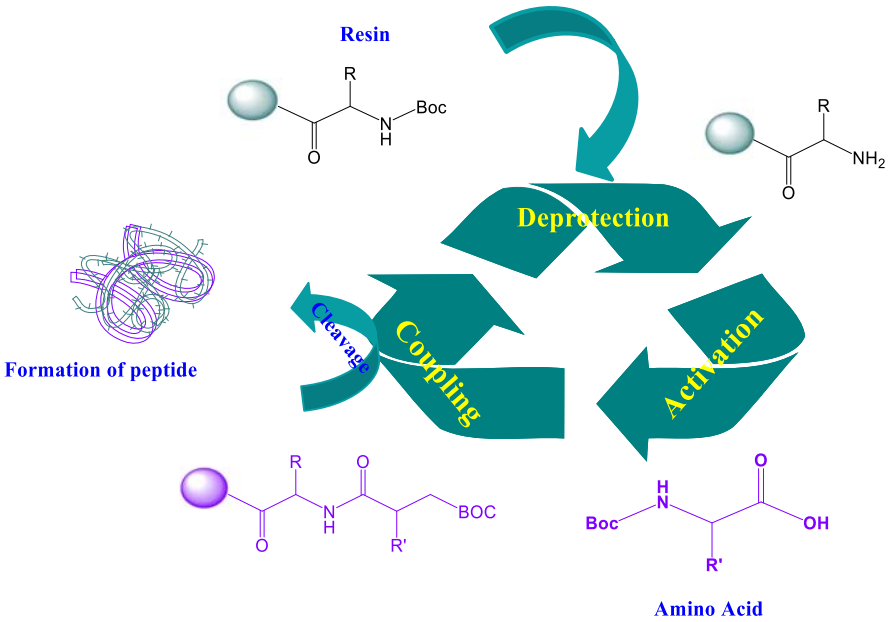

FIGURE 27.4

Schematic representation of steps involved in the synthesis of the peptide.

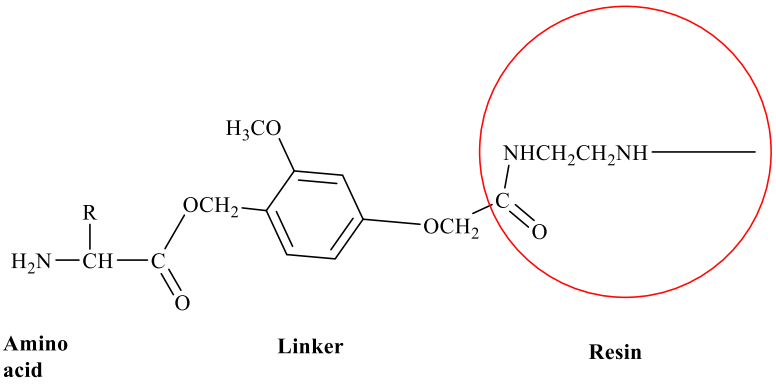

FIGURE 27.5

In the process of SPPS the intermediate steps involved in the reaction.

chains that are aromatic, acid, basic, or highly polar are likely to be reactive (see Table 27.1). These must also be protected to prevent unwanted branched chains from forming. There are four main groups used in this way: tBu (a tertiary butyl group), Trt (a triphenylmethyl group), tBOC (a tertiary butyloxycarbonyl group) and PMC (a 2,2,5,7,8-pentamethylchroman-6-sufonyl group). Examples of a carboxyl group protected with FMOC and examples of the different types of side-chain protection are given below.

To prevent the acids from interacting with one other, the amino group of the following amino acid must likewise be shielded. This is accomplished by using FMOC to protect it. Furthermore, aromatic, acidic, basic, or extremely polar amino acid side chains are prone to being reactive. These must therefore be protected against the formation of inappropriate branching chains. tBu, Trt, tBOC, and PMC are the four primary groups employed in this method (Fig. 27.6). Examples of carboxyl groups that have been preserved by FMOC, as well as examples of the many types of side effects.

Step 3—Coupling

The last amino acid attached to the polyamide is reacted with the amino acid protected with FMOC. DCC catalyzes the process, which is then reduced to DCU. Fig. 27.7 depicts the coupling reactions where RCOOH signifies the FMOC protected acid and H_2NR signifies the reactive end of the increasing peptide chain.

Step 4—Deprotection

Residual DCC is rinsed away with water from the insoluble polymer, and the FMOC group is eliminated using piperidine "a cyclic secondary amine." This is what is known as a trans amidification reaction.

Table 27.1 List of CPP-derived molecules selected with the theranostic formulations for effective cancer therapy.

Peptide	Peptide sequence	Type of delivery platform	Drug molecules	Model/ target	Outcomes	Reference
Matrix metalloprotein-triggered peptide	GPLGIAGQr9	Star-shaped nanoparticles	Curcumin	L929 (mouse embryonic fibroblasts) and A549 (human lung carcinoma) cells	Higher fluorescent intensity, 77% tumor growth inhibition	Guo et al. (2020)
LK peptide	α-helical amphipathic peptide rich in leucine (L) and lysine (K)	Triangular nanoprisms	Au	MDA-MB-231	Effective photothermal cancer therapy,	Ha et al. (2020)
HER2 targeting peptide and TAT	YDLKEPEH and GRKKRRQRRRPPQ	Liposomal probe	Doxorubicin	SKBR3 and 293 T cells	Higher tumor accumulation, negative regulation PTGES, ADORA2B, and CEBPA	Jia et al. (2020)
AngioPep-2	TFFYGGSRGKRN NFKTEEYC	Gold alloy nanoparticles	Fe-Au	C-6 glioma cells and L929	Cross Blood-brain barrier, 1.fivefold higher activity in C6 as compared to L929, decreased Ki67 level in rat	Hsu et al. (2020)

CRGDK and polyarginine (R9)	CRGDK & R9	–	BF2-oxasmaragdyrins	MDA-MB-231	Excellent photo thermal effects	Laxman et al. (2020)
Tumor-homing and penetrating peptide-F3	F3—Cys peptide (KDEPQRRSARLSAK PAPPKPEPKPKKAPAKKC)	Nanoparticles	methylene blue and Gadodiamide	MDA-MB-231	Apoptosis triggered by ultrasound, sonodynamic/ HIFU irradiation therapy	Li et al. (2019)
TAT & cRGD	Arg-Gly-Asp	Near-infrared triggered targeted nanoparticles	Doxorubicin	MDA-MB-231 and A549	Higher cytotoxicity, Effective chemo-photo thermal-photo dynamic therapy	Wu et al. (2019)
TAT	CGGYGRKKRRQRRR	Nanoparticles	Palladium	MCF-7 cells	Enhanced nuclear entry in metastatic cells resultant nuclear stiffness	Gao et al. (2019)
TAT	CGYGRKKRRQRRRGC	Phase-transformation Lipid Nanoparticles	10-hydroxycamptothecin	SMMC-7721 cells	Higher penetration rate, Early theragnostic	Zhao et al. (2018)
PEN, pVEC, and R9	RQIKIWFQNRRMKWKK (Penetratin), pVEC (Cadherin originated with an amino acid sequence of LLIILRRRIRKQAHAHSK), and R9 (RRRRRRRRR)	ZnS nanoparticles	Paclitaxel	SKOV-3 and HeLa cells	Increased drug solubility, better tumor targeting	Rejinold et al. (2018)

(*Continued*)

Table 27.1 List of CPP-derived molecules selected with the theranostic formulations for effective cancer therapy. *Continued*

Peptide	Peptide sequence	Type of delivery platform	Drug molecules	Model/ target	Outcomes	Reference
R9	(RRRRRRRRR)	Nanovehicles	Cabazitaxel	4T1 cells	Reduce metastasis level up to 96%	Hu et al. (2018)
REG	RGERPPR	Inorganic nanoparticles	Doxorubicin	U87MG cells	Enhanced tumor penetration, Mr imaging	Gao et al. (2018)
gH625	C-terminus (AcHGLASTLTRWAHY NALIRAFC-CONH2)	Magnetic nanoparticles	Iron	MDA-MB-231	Higher cellular uptake, enhanced endocytosis	Perillo et al. (2017)
TAT	GRKKRRQRRRPQ	Gold nanoparticles	Doxorubicin	MDA-MB-231-Br and CN34-Br	Enhanced cytotoxicity, Increased CNS Uptake	Morshed et al. (2016)
R8-dGR, R8-RGD, and R8-EGR peptides	Cys-RRRRRRRRdGR, Cys-RRRRRRRRRGD and Cys-RRRRRRRREGR	Liposome	Paclitaxel	C6 cells	Anti-glioma effect, CD34, CD133 studies, the expression level of integrin $\alpha v\beta 3$ and neuropilin-1	Liu et al. (2016)
Biotin	NH_2-RRRRRRRRK-biotin	Silica-coated 4-mercaptobenzoic acid-modified gold nanorods	Au	HeLa cells	Higher drug loading efficiency up to 55%, Uptake efficiency 70.7%	Li et al. (2016)

Peptide	CPP-FITC (RRRRRRRRRK [β-Ala-FITC]-amide) and CPP (GGRRRRRRRRR-amide)	Liposome	Stearic	MCF-7	Accelerated blood clearance, higher target efficiency	Ding et al. (2015)
	R8 (RRRRRRRR)	Nanoparticles	Docetaxel	A549	Increased penetration, Cell selecting affinity	Gao et al. (2015)
iRGD	CRGDKGPDC	Silicon nanoparticles	Sorafenib	PC3-MM2 cells	Good biocompatibility, sustain releases	Wang et al. (2015)
TAT	CRRRQRRKKR	Mesoporous silica quantum dots	Doxorubicin	A549, NIH-3T3, A2780 cells	Enzymatic activity, cellular penetration, intracellular drug delivery	Li et al. (2014)
α-helical cell-penetrating peptide	TH (AGYLLGHINLHHLAHL (Aib)HHIL-NH2) and TK (AGYLLGKINLKKLAKL(Aib) LLIL-NH2),	Liposome	Paclitaxel	C26 and HepG2 cells	Higher tumor spheroidal uptake, clathrin-mediated endocytosis	Zhang et al. (2013)

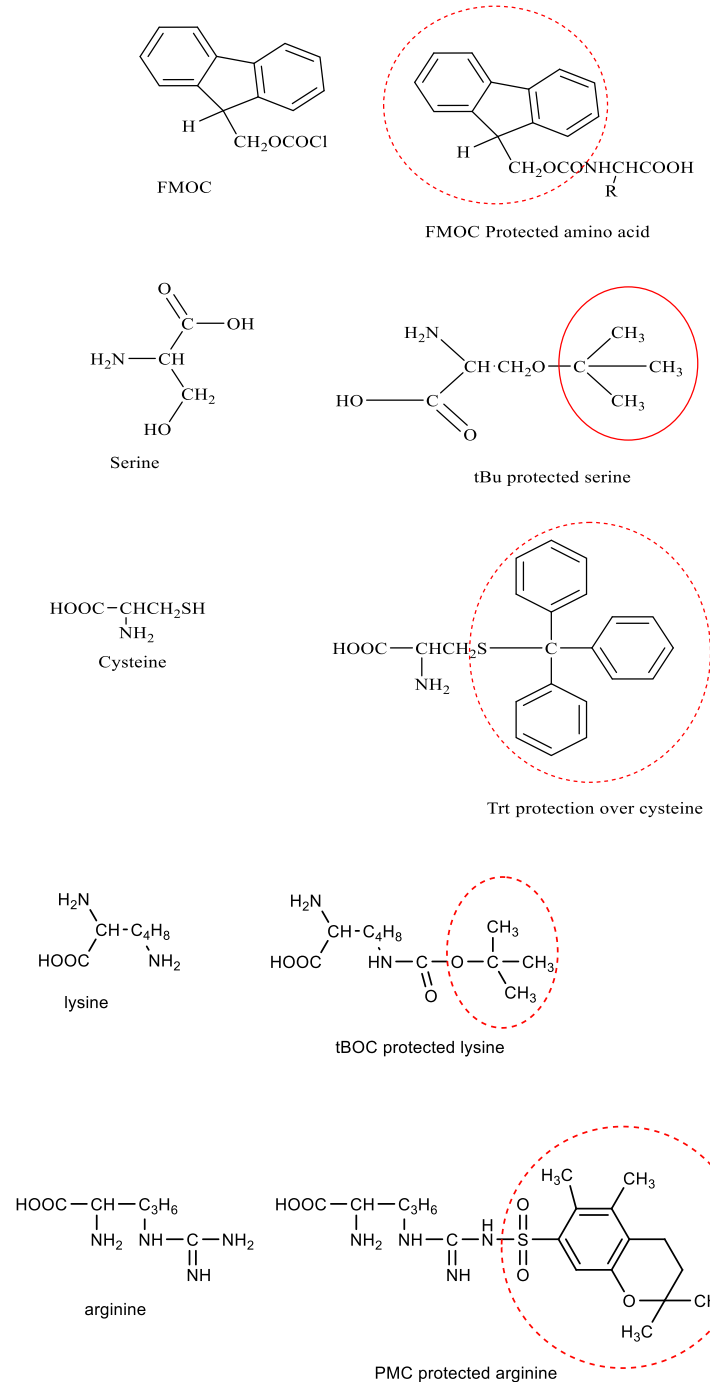

FIGURE 27.6

Different protecting groups involved in the peptide synthesis.

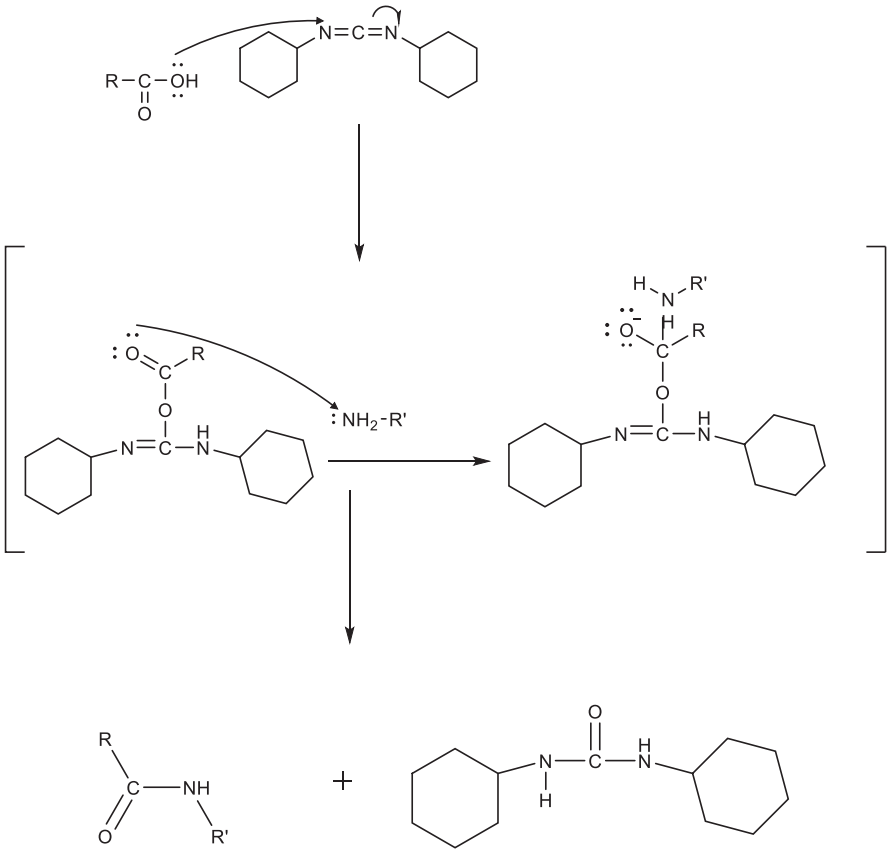

FIGURE 27.7

Amino acid coupling reaction.

Step 5—Polymer removal

The peptide is always eliminated from the polyamide after it is finished. This is accomplished by utilizing 95% TFA to cleave the polyamide-peptide bond. At this point, the side-chain protective groups are likewise eliminated.

27.2.7 Applications of cell-penetrating peptide in the delivery of bioactive(s)

At present, peptides are emerging alternatives owing to their multiple benefits including small size, efficient delivery to the tumor site, rapid renal clearance, decreased nonspecific side effects, and high specificity (Aina et al., 2007). Moreover, they are suitable candidates for the targeted delivery of the bioactive (s) due to their greater tumor-specific binding capacity as compared to other

targeting moieties without affecting the normal cells in the surrounding environment (Qiu et al., 2007). Among peptides, one of the important categories is cell-penetrating peptides (CPP). CPP is a group of peptides that possesses the capacity to translocate within the cell membrane via energy-dependent endocytic mechanisms or energy-dependent direct penetration. Thus, they also act as transporters. They consist of 4−30 amino acids. These peptides are widely employed for the intracellular delivery of the antitumor bioactive(s), imaging and diagnostic agents, proteins, nucleic acids, etc. In contrast to normal cells, cancerous cells possess certain receptors that are upregulated. Employing CPPs, these receptors could be exploited for the intracellular delivery of the bioactive(s). Their cellular uptake is influenced by multiple factors such as types, concentration, size, and CPP conjugated cargos. One of the key challenges in the intracellular delivery of the bioactive(s) is to cross the cell membrane, especially in the case of charged protein molecules with larger sizes. CPPs could be easily altered for effective translocation via plasma membrane (Deshayes et al., 2005; Reubi, 2003; Kaushik et al., 2017; Ramsey & Flynn, 2015; Raucher & Ryu, 2015). CPP-mediated targeted bioactive(s) delivery is feasible by covalent conjugation between peptide and bioactive moiety to form a stable complex. The delivery of CPP conjugated bioactive(s) can be attained via pinocytosis followed by endosomal escape. Vander Waals forces and hydrogen bonding are involved in the internalization of CPP-based drug conjugates (Deshayes et al., 2005; Holm, 2011; Moulton & Moulton, 2004; Ramsey & Flynn, 2015; Torchilin, 2008).

CPPs play a pivotal role in the field of drug delivery. In one of the studies Nam et al. (2021) worked on pH-activatable CPP dimers. In weak acidic conditions at nanomolar concentrations, they can easily penetrate the cells. The dimer was developed with histidine residues. The author developed noncovalent complexes as well as covalent conjugates by using paclitaxel (drug) and CPP-based dimer and extensively characterized them. The cell line studies were performed on MDA-MB-231 cancer cells. The results showed that the drug that is, paclitaxel was effectively delivered by CPP-based conjugates into the triple-negative breast cancer cells, MDA-MB-231. Apart from this, the developed CPP-based dimer demonstrated longer circulation in the body and improved accumulation in the tumors. Animal studies revealed that both the developed carrier modules demonstrated substantial anticancer activity at a very low dosage in a triple-negative breast cancer grafted mouse model (Nam et al., 2021).

Guo et al. (2020) reported CPP-based nanoparticles for the treatment of cancer. To improve the biological specificity of the carrier system, the author synthesized a peptide consisting of GPLGIAG (matrix metalloprotein-triggered peptide for targeted delivery) and r9 (CPP for penetration improvement). The synthesized peptide was utilized in the functionalization of the nanoparticulate formulation. Curcumin-loaded matrix metalloprotein triggered star-shaped CPP-based nanoparticles were developed and extensively characterized. In vitro release, results showed that the curcumin was released in a sustained manner from nanoparticles at pH 7.4. MTT assay demonstrated that the prepared CPP-based nanoparticulate

formulation was efficient toward tumor cell inhibition. The intensity of fluorescence was higher in the case of the CPP-based nanoparticulate system as compared to plain nanoparticle formulation suggesting improved penetration within cancer (tumor) cells. Animal studies showed that the strongest tumor growth inhibition was observed in the case of the CPP-based formulation when A549 tumor xenograft nude mice were treated with it. Thus, the CPP-based nanoparticles demonstrated considerable potential as an ability to affect enhanced/preferential intracellular targeting of the therapeutic agents (Guo et al., 2020).

Deng et al. (2021) developed CPP drug conjugate, and tumor-targeting peptide-based PDCs (LTP-1) for the efficient delivery of paclitaxel to treat cancer. LTP-1 delivered the paclitaxel effectively into the LHRH receptor overexpressed MCF-7 cells. The developed formulation showed two times increased cellular uptake than free paclitaxel. The cytotoxicity value (IC_{50}) was found to be 3.8 nM, however, LTP-1 demonstrated minimum cytotoxicity to noncancerous cells. Moreover, the LTP-1 demonstrated increased antitumor efficacy in vivo without apparent toxicities (Deng et al., 2021).

Yu et al. (2021) worked on pH-dependent CPP-based liposomes for the effective delivery of artemisinin. Liposomes were functionalized by using reversibly activatable CPPs and further characterized. The bioactive that is, artemisinin was loaded into it. In vitro, a drug release study reported a pH-dependent sustained release pattern of the drug. Drug-loaded functionalized liposomes showed maximum internalization and cytotoxicity within the tumor cells at low pH that is, 6.5 as compared to nonfunctionalized liposomes. Apart from this, a longer retention time and greater tumor suppression activity were observed in the case of CPP functionalized liposomes. The strategy seems promising and paves the way for cancer chemotherapy (Yu et al., 2021).

Li et al. (2020) developed mitochondrial-targeted delivery devices based on HPMA copolymers for the treatment of breast cancer. A mitochondrial-targeted hybrid peptide (R8MTS) that comprises CPP and mitochondrial targeting sequence was used for the synthesis of PDCs. R8MTS was employed as a ligand (targeting moiety) here. R8MTS was then conjugated to doxorubicin. pH-dependent release behavior of the doxorubicin was observed when the system entered inside the tumor cells. Moreover, increased reactive oxygen species generation and apoptosis initiation were also observed. In vitro studies exhibited that the developed system not only prevented cell proliferation but also suppressed 4T1 and MDA-MB-231 cells (breast cancer cells). Significant inhibition in the tumor growth and apparent lung metastatic nodules was observed (in vivo) in the case of the developed system (Li et al., 2020). The strategy seems promising and holds the potential for breast cancer therapy.

Zhang et al. (2021) worked on CPP modified redox responsive complex for the codelivery of EGFR and BRD4 siRNA in triple-negative breast cancer cells. The author synthesized GALA- and CREKA-modified PEG-SS-PEI complexes by using the electrostatic interaction phenomenon. The effect of these complexes was studied on MDA-MB-231 cells. The complexes demonstrated good biological

compatibility and excellent transfection efficiency. Apart from this, these complexes offered maximum protection to siRNA from RNA degrading enzymes. The complexes also decreased MDA-MB-231 cells proliferation, invasion, and migration by inhibiting the EGFR/PI3K/Akt and BRD4/c-Myc pathways in a synergistic manner (Zhang et al., 2021).

Farkhani et al. (2016) reported CPP-modified silver nanoparticles for the treatment of cancer. The CPP was synthesized by a solid-phase peptide synthesis method and was utilized in the functionalization of silver nanoparticles. The cell line studies were performed on MCF-7 cells. The CPP-modified silver nanoparticles demonstrated dramatic anticancer activity. The developed carrier module significantly killed the tumor cells indicating that the cellular uptake of the CPP-modified silver nanoparticles was higher. The strategy seems promising and could be further exploited for the treatment of cancer (Mussa Farkhani et al., 2017). In Table 27.1 some of the examples of the theranostic nanomedicine along with the CPPs incorporated.

27.3 Conclusion

Currently, CPP is working as a novel category for the targeting of payload or as well as generating a novel drug delivery system which essentially needs for the carrier system to reach the target site. CPP its self-work as a ligand, as a carrier/cargo which combines with the different nanosystems and achieves the target. On the other hand, the selectivity of the CPP is also suspect because of its penetration mechanism to each type of cell. There were some limitations in the previous research regarding the sequencing of the peptide chain and its mechanism of action for the penetration of the cell membrane, but still, the research is continuing to resolve this. In the future, new peptide sequences have been synthesized and their affinity and targeting can be specified for targeting the newer disease. In the treatment of cancer, the role of the CPP will provide a new scope nanocarrier-based targeting system that not only reduced the toxicity of the drug but increases the affinity of the drug to its active site. Also, the maximum drug will be reached at the site which will reduce the side effect of the drug. CPP-based targeting gives new hope to scientists, researchers, and medical practitioners to effectively go through this nanomedicine-based therapy in the treatment of the different types of cancer.

Acknowledgment

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CHAPTER

28

Development of nanographene oxide/2-hydroxyethyl methacrylate/gelatin/alginate and nanotitanium dioxide/2-hydroxyethyl methacrylate/gelatin/alginate polymeric systems for biomedical applications

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28.1 Graphene oxide-based materials for biomedical applications

Modern research in the field of biomedicine and polymer engineering is in constant search of new, sophisticated materials that have specific performance and show a versatile applications. Therefore, graphene oxide (GO) is one of the most promising functional materials used in various applications like energy storage sensors, photocatalysis, electronics, and biomedicine. The last ten years of research on GO for biomedical applications revealed and confirmed the scope of its potential capabilities as a biomaterial (Ahmad, Fan, & Hui, 2018; Dideikin & Vul, 2019; Singh, Kumar, & Sing, 2016). GO alone and its modified form with different materials (surface functionalization, immobilization of nanoparticles, and composite formation) also proved as a multifunctional candidate for medical biotechnology. GO-based materials are biocompatible and nontoxic to living cells and can be used in biomedical applications. GO as a biomedical agent proved to be biologically

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acceptable and therefore can be used for biomedical applications like targeted drug delivery, cancer therapy and cancer theranostics, bioimaging, and biosensors. GO-based materials are mainly used in biomedicine because of their favorable physico-chemical properties and biocompatibility. GO possesses a unique structure-graphene basal plane is attached with various biocompatible functional groups like carboxylic (COOH) and hydroxyl (OH). The attached functional groups lead to further functionalization and conjugation or immobilization of other nanoparticles on their surface. Further, the size (number of layers, lateral dimension) and shape of GO play an important role in deciding its properties that could be used in various applications. The thickness gradient of the GO sheets showed various functionality and tunable properties.

GO can be incorporated into hydrogels to improve the properties (e.g., mechanical strength) of conventional hydrogels and/or develop new functions (electrical conductivity, drug loading, drug delivery, and release). Unique molecular interactions between GO and various small or macromolecules enable the fabrication of various functional hydrogels appropriate for different biomedical applications (Peppas, Hilt, Khademhosseini, & Langer, 2006).

Hydrogels are three-dimensional hydrophilic polymeric networks (Liu, He, Zhang, & Lee, 2018; Montheil, Echalier, Martinez, Subra, & Mehdi, 2018) with several unique characteristics, including hydrophilicity, swelling, from micro-to nanosized pores, softness, and consistency most similar to living tissue. Hydrogels can absorb considerable amounts of water/fluid ($> 90\%$) from aqueous solutions and swell but do not dissolve in the solution (Ahmed, 2015). The maintenance of hydrogels in aqueous solutions is attributed to the chemical or physical crosslinking of their hydrophilic chains (Bahram, Mohseni, & Moghtader, 2016). Also, hydrogels contain internal micro-and/or nanopores that allow the diffusion of small molecules from and to their environments (Fu & In Het Panhuis, 2019; Martín et al., 2019; Chai, Jiao, & Yu, 2017; Hoffman, 2012). Hydrogels can be mechanically flexible and soft, and their properties can at least partly mimic those of soft living tissue (Caló & Khutoryanskiy, 2015; Moghadam & Pioletti, 2015; Xue et al., 2019). These properties of hydrogels make them very useful for various biomedical applications, such as tissue augmentation, drug delivery, and tissue engineering scaffolds. Hydrogels are typically synthesized with various natural and synthetic polymers. The natural polymers that are commonly used for hydrogel synthesis include hyaluronic acid, alginate, chitosan (CS), collagen, and gelatin. Synthetic polymers include polyethylene glycol (PEG), poly(acrylamide), poly(2-hydroxyethyl methacrylate) (PHEMA), and poly(acrylic acid). The characteristics of hydrogels, such as toughness, elasticity, and water/fluid content, can be tailored by varying the monomer (macromer) component, crosslinker type(s), and crosslinking densities (Akhtar, Hanif, & Ranjha, 2016). Despite the utilities and versatilities of hydrogels, conventional hydrogels still have some limitations in some cases. For example, most pristine hydrogels are mechanically weak (Jiang et al., 2017), whereas hydrogels with high mechanical strength and stability are frequently desired for long-term, load-bearing applications (Dai et al., 2015).

Novel properties, such as electrical conductivity, have been recently introduced to hydrogels to extend their applicability and/or the realization of new functions while maintaining the original properties (hydrophilicity and softness) (Alam et al., 2016; Liu et al., 2017; Navaei et al., 2017; Qazi, Rai, & Boccaccini, 2014; Song, Li, Wang, Liao, & Zhang, 2015). In the field of drug delivery, typical hydrogels often display poor drug loading and drug release abilities for some drug molecules (hydrophobic drugs and therapeutics), which causes some difficulties in the fine-tuning of the drug release profiles (Song et al., 2015). Consequently, many strategies involving the incorporation of the double polymeric network, polyampholyte, and nanocomposites have been developed to enhance the mechanical strength and drug release performances of hydrogels and imbue electrical conductivity to hydrogels (Hu, Vatankhah-Varnoosfaderani, Zhou, Li, & Sheiko, 2015).

Graphene and GO has been widely formulated with hydrophilic polymers to improve the properties of hydrogels, such as mechanical properties, electrical conductivity, and drug loading and release properties. Graphene is a two-dimensional single layer of carbon atoms with a hexagonal lattice structure and hybridized sp^2 orbitals (Allen, Tung, & Kaner, 2010; Pumera, 2013). In practice, GO is obtained from graphite by oxidative exfoliation using strong oxidants and acids. Due to these harsh synthetic procedures, GO possesses defects in its hybridized sp^2 orbitals and diverse functional groups (epoxide, phenolic, hydroxyl, and carboxylic groups). These oxygen-containing functional groups of GO aid its dispersion in aqueous solutions and its miscibility with hydrophilic polymeric chains in hydrogels (Aliyev et al., 2019; Huang, Zhang, & Ruan, 2014; Nath, Chowdhury, & Dolui, 2018). Consequently, most graphene-based biomaterials, including hybrid hydrogels, have been fabricated using GO. GO generally exhibits hydrophilic edges with oxygenated functional groups (carboxylic groups and hydroxyl groups) and a hydrophobic basal plane, which enables its multiple molecular interactions with various small molecules and macromolecules, including van der Waals, hydrophobic interactions, and electrostatic interactions (Li & Shi, 2014). GO can be an effective building block for generating new chemical and physical properties, such as electrical and thermal conductivity, light adsorption, flexibility, and high mechanical strength (Martín et al., 2019; Shin et al., 2016). GO can be modified for the incorporation of additional functional groups and their conjugation and immobilization with other micro-and macromolecules, which can broaden the application of the resulting hydrogels for drug delivery, biosensors, and tissue scaffolding biomaterials (Paik, 2017). It should be noted that a high concentration of GO often results in its assembly to form a gel (Bai, Li, Wang, & Shi, 2011). However, this gelation occurs under specific conditions, such as low pH, a high GO concentration (> 4 mg/mL), and large-sized GO sheets (several microns in lateral sizes). The repulsive forces between GO sheets should be greatly reduced to form self-assembled GO sheets and, in turn, a GO gel. In an acidified solution, the carboxylic groups of GO become protonated and neutral, whereas GO sheets do not form a gel under physiological conditions (neutral pH) due to their

excessive repulsive forces. Also, assemblies of GO in a GO gel slowly change their organization from edge-to-edge binding to basal binding, which eventually results in the formation of GO aggregates and causes instability of the GO gel.

Various studies based on the in vivo behavior and bioactivity of GO-based materials show that the nanocarriers interact with the cell membranes and enter the cells by endocytosis. For targeted drug delivery to the cell nucleus, it is essential that the drug carrier escapes the endosomal compartment and releases the loaded drug into the cytosolic compartments (Liu, Robinson, Sun, & Dai, 2008; Yang et al., 2010a). This process proposed a strategy to reverse cancer drug resistance in doxorubicin (DOX) resistant MCF-7/ADR cells by loading DOX on GO surface via physical mixing (Jing et al., 2012). High pH-dependent release for drug loading with DOX was observed in vitro. GO enhanced accumulations of DOX in MCF-7/ADR cells causing higher cytotoxicity in comparison to free DOX. It is well known that pH is acidic in the cancer microenvironment, intracellular lysosomes, and endosomes. This fact has been exploited to achieve active drug release in the tumor tissue/cells using a chemical modification of graphene (Bai, Li, Wang, & Shi, 2010; Depan, Shah, & Misra, 2011; Zhang, Xia, Zhao, Liu, & Zhang, 2010). For chemotherapeutic efficacy use of graphene-based materials has also been explored for the codelivery of multiple drugs (Zhang et al., 2010). Loading of DOX and camptothecin (CPT) in a controlled way inside the same drug delivery system resulted in remarkably higher toxicity in MCF-7 cells compared with GO-loaded only with DOX or CPT. Thus, graphene and GO-modified magnetic nanoparticles result in various biomedical applications in the field of drug delivery, magnetic resonance imaging, and bioimaging.

Attachment of nanoparticles such as iron oxide with graphene-based nanomaterial makes them superparamagnetic in property and can be useful in drug delivery applications (Yang et al., 2009). The resulting magnetic hybrids dispersed uniformly in an aqueous solution before and after loading of DOX. Magnetic hybrids show agglomeration behavior in acidic medium and redispersion behavior observed in basic medium. This pH-triggered magnetic behavior of GO-Fe_3O_4 nanoparticle hybrids can be helpful in controlled drug delivery. A similar pH-dependent drug release system was reported for a 5-fluorouracil (5-FU) loaded nanohybrid system composed of graphene nanosheets, carbon nanotube (CNT), and Fe_3O_4 (Fan, Jiao, Gao, Jin, & Li, 2013).

GO-based materials can be used for small molecule drug delivery. Small molecule drugs having pH-dependent solubility have been widely used for pH-responsive delivery by using GO as a carrier. DOX-GO complexes showed a pH-responsive release of DOX from GO due to higher solubility of DOX at low pH conditions (Yang et al., 2008). Taking this advantage, pH-responsive codelivery of DOX and camptothecin (CPT) was successfully demonstrated by using folic acid (FA) conjugated-nano-GO for cancer targeting (Zhang et al., 2010). Antiinflammatory drugs with different hydrophilicity (ibuprofen and 5-fluorouracil) were also delivered by using CS-GO complex with the pH-responsive release (Rana et al., 2011).

Gene therapy is used in many expanding areas to treat genetic disorders like Parkinson's disease, cystic fibrosis, and cancer. An effective gene therapy needs efficient and safe gene vectors that also protect DNA from nuclease degradation as well as facilitate DNA uptake with high transfection efficiency (Shen et al., 2012; Yang et al., 2007). Graphene has been reported for wide applications in the field of gene delivery, gene-drug codelivery, and protein delivery. Polyethyleneimine (PEI) has been extensively investigated as a nonviral gene vector having strong electrostatic interactions with negatively charged phosphates of RNA and DNA. Chemical modification is very easy in PEI which offers increased transfection efficiency, cell selectivity, and reduced cytotoxicity however low biocompatibility and high toxicity of PEI limit its use for biomedical applications (Jager, Schubert, Ochrimenko, Fischer, & Schubert, 2012). CS-GO complex is also used for simultaneous drug and gene delivery (Bao et al., 2011). CS-GO converts pDNA into stable nanosized complexes. Amine terminated PEGylated GO was effectively used to deliver high protein payloads due to noncovalent interactions with the surface of PEG-GO (Shen et al., 2012). Bone morphogenic protein-2 (BMP-2) was loaded onto Ti substrate coated with alternate layers of positively ($GO\text{-}NH^{3+}$) and negatively ($GO\text{-}COO^{-}$) charged GO nanosheets with high loading efficacy and conserved bioactivity. Osteogenic differentiation of MSCs was enriched on Ti coated GO surfaces carrying BMP-2 than only on Ti surfaces with BMP-2. In vivo studies in mice also exhibited vigorous new bone formation with Ti-GO-BMP-2 implants compared to Ti or Ti-GO or Ti-BMP-2 implants and made the new composite a very effective carrier for therapeutic drug delivery/release (La et al., 2013). All studies up to date have highlighted the potential of GO-based materials as drug and gene delivery vehicles in in vitro studies. Though there is a necessity to validate their potential in vivo with a particular focus on safety, biodistribution, and efficacy.

GO-based materials can be served as gene delivery vehicles. PEI has been extensively utilized as a surface modifier of GO sheets for gene delivery into cells by complexation through electrostatic interaction (Feng, Zhang, & Liu, 2011) and covalent conjugation for loading of plasmid DNA (pDNA). The covalent GO conjugates with linear (Chen et al., 2011) and branched PEI (Kim, Namgung, Singha, Oh, & Kim, 2011) showed high gene transfection efficiency with low cytotoxicity compared to PEI/pDNA complexes. Sequential delivery of Bcl-2 targeted siRNA and DOX into HeLa cells was also successfully demonstrated by using the PEI-GO complex with enhanced therapeutic efficacy (Zhang et al., 2011a). CS-functionalized GO (CS-GO) complex was synthesized and applied to efficient codelivery of anticancer drug and pDNA loaded respectively by π-π and electrostatic interactions (Bao et al., 2011).

Research on GO-based materials was reported extensively for its uses in drug delivery and cancer therapy. GO played a significant role in sorting out the failure in dealing with cancer treatment. Hydrophobic drugs can also be delivered to the tumor cells using GO. Water insoluble anticancer drug SN38 (hydrophobic aromatic molecules including CPT analog) can be made soluble for treating cancer

cells efficiently by loading them with novel nanocomposite of nano GO with branched PEG. This drug-loaded nanocomposite formulation showed high cancer-killing potency than that of irinotecan (CPT-11) (Liu et al., 2008).

GO-based materials can be used in therapeutic modalities for cancer treatment. For efficient delivery of hydrophobic drugs, nano-GO has been functionalized with six-arm poly(ethylene glycol) (6-arm PEG) and successfully applied to cancer cell lines with high cellular uptake and therapeutic efficacy (Liu et al., 2008; Sun et al., 2008). For applications as a carrier, nano-GO derivatives have been used for photothermal therapy based on their high absorption of near IR. The high therapeutic efficacy of PEG-GO conjugate was demonstrated in mouse cancer xenograft models (Yang et al., 2010b). It was reported that the phototherapeutic effect of nano-GO derivatives originated from the induction of oxidative stress, mitochondrial depolarization, and caspase activation resulting in apoptotic and necrotic cell death (Marković et al., 2011). For the enhancement of therapeutic efficacy by combinational therapy, PEGylated GO has been used as a carrier for DOX for the combination of photothermal and chemical therapy (Zhang et al., 2011b). Porphyrin photosensitizer has also been loaded on FA conjugated PEG-GO for a combination of photothermal and photodynamic therapy with the cancer-targeting ability (Huang et al., 2011). In addition to the small molecule drugs, graphene/TiO_2 nanohybrid composite also showed high therapeutic efficacy based on photothermal and photocatalytic therapy (Hu et al., 2012).

Loading of more amount of drugs is possible due to the surface functionality and high surface area of GO. Tests have shown more amount of drug loading (irinotecan) was possible due to the high surface area of GO in hyaluronic acid/polyaspartamide-based double network. This loaded drug can be driven to the target by changing the pH of the external medium and near-infrared irradiation and can be treated human colon cancer (Fiorica, Mauro, Pitarresi, Scialabba, & Palumbo, 2017). GO blending with alginate and CS derivatives hydrogel is also a potential candidate for the delivery of various small drug molecules. This nanocomposite exhibited a high drug loading efficiency of 82% for small molecule fluorescein sodium and also showed negligible cytotoxicity to hepatic stellate cell lines (Chen et al., 2016). A thermosensitive nanogel of N-isopropylacrylamide modified GO nanocomposite showed potential for high drug loading capacity and excellent drug release behavior at high temperatures. It was also observed that there was no burst release with temperature (Bardajee & Hooshyar, 2017). Magnetically modified GO functionalized with CS and methoxy poly(ethylene glycol) mPEG-NHS nanostructure was used for targeted drug delivery of chemotherapy drugs CPT-11 and DOX. High drug loading and pH-dependent drug release properties are promising for targeted drug delivery and cancer therapy (Huang, Lu, & Chen, 2017). GO-Fe_3O_4 superparamagnetic drug carrier was developed with the loading of 18.6 wt.% of DOX hydrochloride. The loading capacity was as high as 1.08 mg/mg. The loaded and unloaded nanocomposites showed good hydrophilicity. Aggregation under acidic conditions can be regulated by applying external magnetic fields (Yang et al., 2009). Oligodeoxynucleotide loading capacity was

increased on GO-CS nanocomposites and showed lower cytotoxicity compared with GO. The loading of oligodeoxynucleotide was due to the electrostatic interaction between oligodeoxynucleotide and GO-CS (Zhang, Yan, Xu, Feng, & Huang, 2017). FA-modified nano-GO loaded with dual drugs DOX and CPT showed remarkably high toxicity to MCF-7 cells (Michigan cancer foundation-7), while these drugs loaded with pristine nano-GO showed lesser cytotoxicity. The loading ability of this nanostructure for multiple drugs enables its potential use in biomedicine (Zhang et al., 2010).

Multifunctional nanocomposites have also been reported based on the GO for drug loading, cancer treatment based on poly(ethylene glycol) and nano-GO was prepared for loading of photosensitizer zinc phthalocyanine towards treating cancer cells MCF-7 through photodynamic therapy. Though zinc phthalocyanine is hydrophobic, it is internalized in MCF-7 cells with the addition of nano-GO-PEG nanocomposite (HaiQing, ZhiLei, HuiYun, YongYong, & FangFang, 2010). Low molecular weight branched polyethyleneimine (BPEI) attached GO showed an improved DNA binding, condensation, and transfection efficiency compared to high molecular weight BPEI. Due to the tunability of GO, this BPEI and GO-based hybrid nanocomposite could be extended to SiRNA delivery (Kim et al., 2011). Light controllable oligodeoxynucleotide delivery was proposed and showed excellent photothermal and immunological effects on the cancer cell tumor reduction in GO/PEG and PEI nanocomposites (Tao, Ju, Ren, & Qu, 2014). Through electrostatic self-assembly of functionalized GO with CS and sodium alginate, a nanocomposite was formed and loaded with DOX and showed pH-dependent drug release behavior. Remarkable inhibition of MCF-7 cancer cells was also reported using this nanocomposite (Lei et al., 2016). GO and sodium alginate nanohybrid showed potential for DOX loading and exhibited profound cytotoxicity to HeLa cells. A very high loading capacity of 1.843 mg/mg was obtained for this nanocomposite under the physiological conditions of pH 6.5 and 7.4 (Fan, Ge, Zou, Xiao, & Wen, 2016).

GO-based materials are also promising candidates for antibacterial activities. Several types of GO-based nanocomposites evidenced better antibacterial activities compared to the existing material. CS, nanosilver, and GO-based nanocomposite exhibited highly efficient antibacterial activity towards the bacteria (methicillin-resistant *Staphylococcus aureus*) strains. This nanocomposite showed better antibacterial activity than the AgNPs or GO-AgNP materials (Marta, Potara, Iliut, Jakab, & Radu, 2015). GO-zinc oxide (ZnO) nanofillers filled poly (lactic acid) (PLA) matrix nanocomposite showed multiple improved properties like mechanical strength and antibacterial activity (Huang, Wang, Zhao, Wang, & Zhou, 2015).

GO-based materials can be used as substrates with antibacterial effects. The GO-based materials showed an inhibition effect of bacterial growth on their surfaces (Hu et al., 2010). The inhibition effect was demonstrated on GO nanowalls with both Gram-positive and Gram-negative bacteria. The antibacterial effect of GO nanowalls was high because of the efficient charge transfer of GO with

bacterial cells (Akhavan & Ghaderi, 2010). The antibacterial effect of GO-based materials was derived from oxidative stress induced by membrane disruption (Liu et al., 2011). On the other hand, one report suggested that bacterial growth was enhanced, rather than inhibited, on the graphene surfaces (Ruiz et al., 2011). The controversial results indicated that the bacterial growth on GO could be varied under experimental conditions.

GO-based materials were utilized as a substrate for mammalian cell culture. The behavior of NIH-3T3 fibroblasts was investigated on various carbon nanomaterial-coated substrates such as GO, and CNTs. The carbon nanomaterial-coated substrates showed high biocompatibility and enhanced gene transfection efficiency (Ryoo, Kim, Kim, & Min, 2010). Graphene/CS hybrid films also showed promising applicability to tissue engineering to repair and improve tissue functions (Fan et al., 2010). Interestingly, neurite sprouting and outgrowth were also promoted on graphene surfaces compared to conventional tissue culture plates made of polystyrene (Li et al., 2011, 2013).

Tissue engineering is an interdisciplinary field that endeavors to develop biological substitutes to resolve, retain or enhance the functionality of tissue or whole organ (Langer & Vacanti, 1993). Recently, graphene-based materials have been used to treat wound healing, stem cell engineering, regenerative medicine, and tissue engineering. Hydrogels have viscoelastic and transport properties mimicking natural tissues (Sant, Hancock, Donnelly, Iyer, & Khademhosseini, 2010), but their weak mechanical properties can limit their use in many tissue engineering applications. Graphene has a platform for tailoring various functionalities on flat surfaces with outstanding mechanical properties like high elasticity, strength, and flexibility. Potentially, graphene has a wide range of applications in the field of hydrogels, biodegradable films, electrospun fibers, and other tissue engineering scaffolds. When GO is incorporated into PVA-based hydrogels it potentially increases tensile stability (132%) and compressive strength (36%) of composite hydrogels without altering their cytoaffinity (Zhang et al., 2011c). According to Lu et al. (2012), graphene-based composite materials are applicable for wound healing by formulating graphene-containing CS-PVA nanofibrous scaffolds. These three groups, CS-PVA-graphene electrospun fibers, and CS-PVA fibers were also studied without graphene and control (no scaffold), to check wound healing affinity in mice and rabbits (Lu et al., 2012). Graphene-containing samples healed the wound completely at a faster rate in comparison to those without graphene-based samples in both mice and rabbits. Graphene-based materials also have applications in the area of musculoskeletal tissue engineering using mouse myoblast C2C12 cell lines (Ku & Park, 2013). Cellular behavior on graphene derivatives is enhanced by the surface roughness and surface oxygen content and adsorption of serum proteins. Thus, graphene-based materials can be useful in reinforcing tissue engineering scaffolds due to their mechanical and electrical properties. Graphene materials have properties like a large surface area which adsorb proteins/DNA and can be useful in many therapeutic applications. Mahmoudi et al. (Mahmoudi, Akhavan, Ghavami, Rezaee, & Ghiasi, 2012)

reported a protective role of GO and protein-coated GO surfaces in the amyloid beta fibrillation process, which is implicated in various neurodegenerative disorders. However, along with detailed in vitro characterization of scaffolds, more emphasis should be placed on their evaluation in vivo concerning inflammatory responses, biocompatibility, and regenerative potential.

28.2 Design of hybrid polymeric hydrogels platform based on nanographene oxide, alginate, gelatin, and 2-hydroxyethyl methacrylate

The series of the novel, porous, and degradable hybrid polymeric hydrogels were successfully synthesized by combining natural polymers-alginate and gelatin, and synthetic monomer 2-hydroxyethyl methacrylate (HGA) with inorganic agent-nanographene oxide using modified porogen leaching method followed by freeze-drying treatment (Chen, Park, & Park, 1999). The synthesis route of the hydrogels is shown in Fig. 28.1.

The porous and mechanically strong polymeric network was obtained by free-radical crosslinking polymerization of 2-hydroxyethyl methacrylate in the presence of the activating system. The additional polymeric network was created by the crosslinking of gelatin while the alginate chains are loaded to intertwine in the networks. The porosity of the hydrogels and dispersion of the nanographene oxide evenly through the polymeric network was achieved during foaming reactions, by CO_2 gas bubbles generated from the thermal decomposition of sodium bicarbonate. The technique based on CO_2 gas bubbles for the first time was

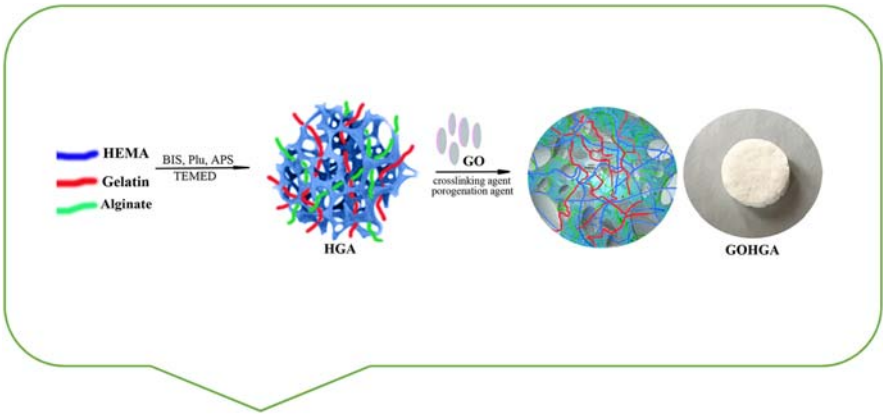

FIGURE 28.1

Schematic representation of the formation of the hybrid polymeric hydrogels composed of 2-hydroxyethyl methacrylate, gelatin, and alginate nanographene oxide.

proposed as a successful way to disperse the inorganic agent evenly through the composition of the hydrogel. The polymerization/crosslinking reactions are fast and easy to conduct.

28.2.1 Biocompatibility of GOHGA hydrogels

To serve the function as a biomaterial, hybrid hydrogels should match the biocompatibility of natural tissue/cells (Cartmell, 2009). The biocompatibility of GOHGA hydrogels was examined in a cytotoxicity test on normal human fibroblasts (MRC5). The results of this assay for GOHGA samples are shown in Fig. 28.2. GOHG50A50 and GOHA extracts were not significantly cytotoxic in vitro under tested conditions, moreover, lower doses of extracts induced higher proliferation in MRC5 cells (in comparison to untreated control which was 100%) (Fig. 28.2A). All materials, even when supplied directly to cells were shown to support the accumulation of cells on its surface (Fig. 28.2B).

28.2.2 Structural characteristics of GOHGA hybrid hydrogels

Fourier transform infrared spectroscopy gave an insight into structural data for hybrid polymeric materials based on nanographene oxide, 2-hydroxyethyl

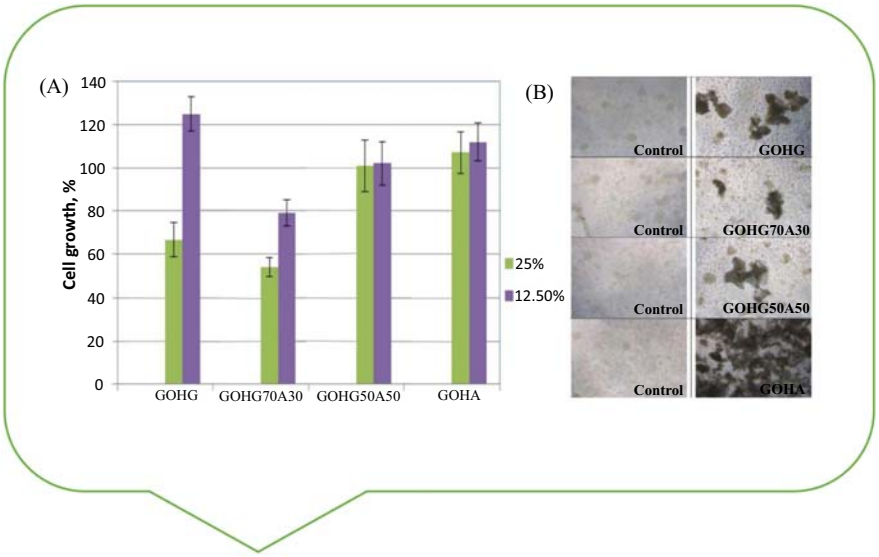

FIGURE 28.2

(A) Cell viability of GOHGA hybrid polymeric hydrogels and (B) accumulation of cells on its surface.

methacrylate, gelatin, and alginate. Obtained FTIR spectra of PHEMA, GOHA, and GOHG hydrogels are presented in Fig. 28.3.

The FTIR spectrum obtained for poly(2-hydroxyethyl methacrylate) (PHEMA) displays typical signals for O-H stretching vibration at approximately 3432 cm^{-1}, C-H stretching vibrations at 2930 and 2886 cm^{-1}, and strong C = O vibration at 1717 cm^{-1} (Tomić et al., 2014). FTIR spectra of the synthesized multicomponent materials (GOHA and GOHG) presented combinations of typical HEMA, gelatin, and alginate vibrations, while the intensity of the peaks depended on the ratio and type of the component. The FTIR spectrum of GOHA demonstrates the characteristic absorption bands of HEMA and polysaccharide structure of alginate (1278 cm^{-1} C-O stretching, 1160 cm^{-1} C-C stretching, 1017 cm^{-1} C-O-C stretching) (Vieira, Cestari, Airoldi, & Loh, 2008). The absorption bands at around 1482 and 1406 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of the carboxylate group of alginate. Similarly, GOHG exhibits several characteristic spectral bands for HEMA and gelatin. The most characteristic vibrations of gelatin are N-H stretching vibration around 3354 cm^{-1}, C-H stretching at 3003 cm^{-1}, C = O stretching at 1701 cm^{-1} for amide I, N-H definition at 1630 cm^{-1} for the amide II (Chang & Tanaka, 2002). These results prove that the novel hybrid hydrogels possess a homogeneous distribution of the components.

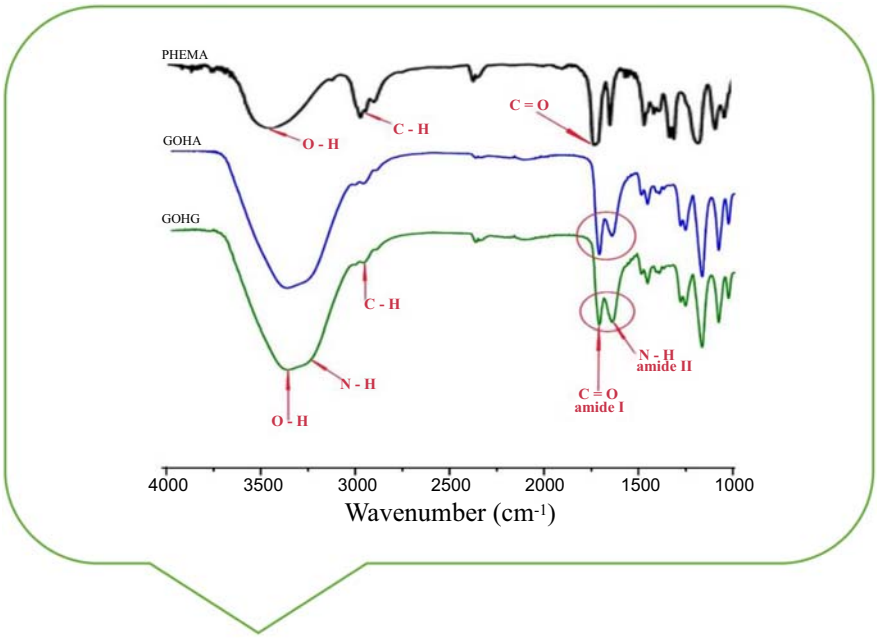

FIGURE 28.3

FTIR spectra of PHEMA, GOHA, GOHG hybrid hydrogels.

28.2.3 Morphology of GOHGA hybrid hydrogels

To detail understand the microstructure of the hybrid hydrogels, their cross-sectional and surface morphology was analyzed by scanning electron microscopy (SEM). Obtained SEM micrographs are displayed in Fig. 28.4. As can be seen, the interior of all samples was found to be highly porous, with open, interconnected pores, with spherical to elliptical shapes. The pore size was found to vary between 10 and 100 μm. The SEM micrographs of the hybrid hydrogels containing alginate showed a more asymmetrical and disoriented porous structure whereas the GOHG hydrogel exhibited a fine porous structure with fairly uniform distribution. Withal, the pore size increased with the increasing content of alginate in hydrogels. The surface morphology of GOHGA hybrid hydrogels can be described as rough and porous.

28.2.4 Mechanical properties of GOHGA hybrid hydrogels

The mechanical properties of hydrogels are their essential characteristic due to their relatively poor mechanical properties is a key limitation with the use of hydrogels in many applications. The mechanical properties of the hybrid hydrogels based on nanographene oxide, alginate, gelatin, and 2-hydroxyethyl methacrylate, were analyzed by values of Young's modulus (E). The obtained results of E values of GOHGA hydrogels in the range of 1.69−4.78 MPa illustrate the

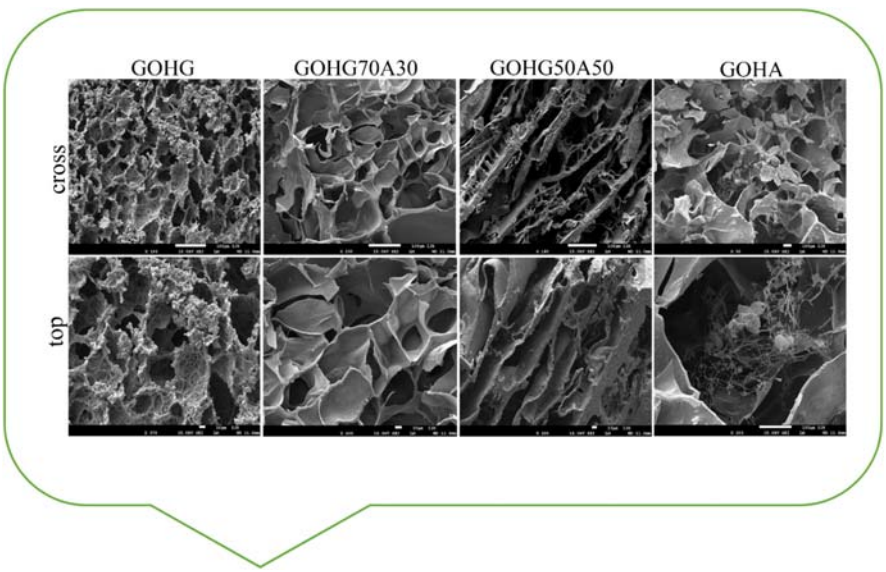

FIGURE 28.4

SEM micrographs of GOHGA hybrid hydrogels.

effects of the chemical composition of the hybrid hydrogels on their mechanical properties. The values of Young's modulus decreased with increasing content of alginate in hydrogels. Hydrogels containing alginate (GOHG50A50 and GOHA) show lower values of Young's modulus (2.68 and 1.69 MPa), due to their higher porosity and larger pore size compared to hydrogels based on gelatin GOHG (4.78 MPa). The presence of crosslinked gelatin resulted in a more compact, mechanically stronger structure. Obtained results of values of Young's modulus indicate that the mechanical properties can be tuned by controlling the chemical composition of hybrids.

28.2.5 The porosity of GOHGA hybrid hydrogels

The porosity of hydrogels is a very important parameter that determines their applications, especially in the biomedical field. The porosity of hybrid hydrogels based on nanographene oxide, alginate, gelatin, and 2-hydroxyethyl methacrylate was evaluated by the bulk density method. The obtained results of the percentage of porosity for GOPHGA hydrogels are in the range of 52.80%−76.36% indicating the influence of the hydrogel composition on the porosity values. The highest porosity showed in the GOHA sample (76.36%), while the lowest value has the GOHG sample (52.80%). The presence of crosslinked gelatin slightly decreased the porosity, most probably due to the more crosslinks of gelatin. Results indicate that the porosity of the GOHGA hydrogels can be designed and adapted for various applications by varying hybrid hydrogels composition and components ratio.

28.2.6 The hydrophilicity of GOHGA hydrogels

The surface hydrophilicity of the hydrogels based on nanographene oxide, alginate, gelatin, and 2-hydroxyethyl methacrylate was assessed by measuring the contact angle through the water spread of a droplet on a surface. The results obtained from the static water contact angle measurements are presented in Fig. 28.5. As can be seen, all hybrid hydrogels with measurements performed at 0 and 1 s are fully hydrophilic, water completely wetted their surface and drop immediately disappeared after putting on the surface of the samples.

28.2.7 In vitro degradation properties of GOHGA hybrid hydrogels

The favorable degradation performance of hydrogels is an important factor for their applications in the biomedical field. As it is known, PHEMA is biocompatible but not biodegradable. Accordingly, we develop the potential to improve the degradability of HEMA-based hydrogels. The degradation behavior of the hybrid hydrogels was evaluated in in vitro controlled conditions-simulated buffer fluid of pH 7.40 and at a temperature of 37°C. The weight loss, as an indicator of degradation of hybrid hydrogel matrices based on nanographene oxide, alginate, gelatine, and 2-hydroxyethyl methacrylate was investigated for 6 months. The tested

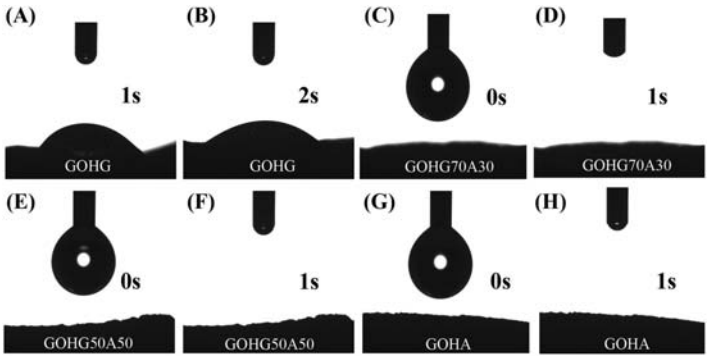

FIGURE 28.5

Water contact angle of GOHGA hybrid hydrogels. (A) GOHG; (B) GOHG; (C) GOHG70A30; (D) GOHG70A30; (E) GOHG50A50; (F) GOHG50A50; (G) GOHA; (H) GOHA.

hydrogels degraded up to 41% after 6 months. The highest weight loss was detected for the GOHA hydrogel (44.29%), while the lowest weight loss exhibited GOHG (40.91%) indicating the dependence of the chemical composition of the hydrogels and their degradation behavior. The decrease of alginate content and the increase of gelatin in hydrogels resulted in decreased biodegradability. As it is assumed, the degree of gelatin crosslinking is one of the most important parameters for tuning the degradation of hydrogels.

28.2.8 Evaluation of GOHGA hybrid hydrogels as controlled drug release systems

28.2.8.1 Drug loaded and entrapment efficiency for curcumin

An additional motive of this study is to evaluate the applicability of hybrid hydrogels as adequate systems for loading and controlled release of various drugs and active agents. The obtained results for curcumin as active agent loading (*DL*, mg drug/g hydrogel) and entrapment efficiency (*EE*, %) of hybrid hydrogels are in the range of 47.99−49.86 mg drug/g hydrogel and 95.97%−99.72%. As can be seen, values of *DL* and *EE* were affected by the composition of hydrogels. GOHG hydrogel was able to load a lesser amount of curcumin (47.99 mg drug/g hydrogel) due to the degree of crosslinking of gelatin compared to GOHA hydrogel which absorbs a higher amount of the active agent (49.86 mg drug/g hydrogel). All hybrids showed *EE* of curcumin above 95%. The highest *EE* of curcumin (99.72%) was achieved by GOHA hydrogel while GOHG showed a slightly lower value (95.97%). Obtained *DL* and *EE* data suggest that hydrophobic drugs could be efficiently loaded into the hydrogels, and the desired drug formulation can be achieved by varying the composition of the hydrogels.

28.2.8.2 Controlled release properties of the GOHGA hybrid hydrogels

To evaluate hydrogels as efficient systems for drug delivery, in vitro curcumin-controlled released study from the hydrogels was performed in a phosphate buffer of pH 7.40, at 37°C. The release profiles of curcumin (Fig. 28.6) showed that the composition of the hydrogels has a significant effect on the release rate of curcumin. About 98% of released curcumin was achieved approximately within 25, 30, 38, and 48 h of releasing in a buffer pH 7.40, at 37°C from GOHA, GOHG50A50, GOHG70A30, and GOHG, respectively. The slower and prolonged release has appeared for GOHG and GOHG70A30 hydrogels which could be related to the degree of gelatin cross-linking. These results indicate that the release rate of curcumin increased with the increasing content of alginate in the hydrogels. Among that, at a pH of 7.40, the carboxylic groups from gelatin, alginate, and HEMA are ionized. In the pH range of 7.00−8.50, it is postulated that curcumin exists in equilibrium in three forms H_3A, H_2A^- and HA^{2-} (Tonnesen & Karlsen, 1985). This leads to mutual electrostatic repulsion, which facilitates the curcumin release from hydrogels containing a higher amount of alginate. As previously reported, the amount of loaded drug may affect the drug release rate, also (Ruan & Feng, 2003). We found that the hybrids loaded with a higher amount of curcumin showed faster release. This phenomenon could be attributed to the change in the drug diffusivity caused by the different drug loading levels.

28.2.8.3 Analysis of drug transport mechanism

For a more detailed investigation of the drug release process, five various kinetic models (Higuchi, Ritger-Peppas, Peppas Sahlin, and Peppas Sahlin model when

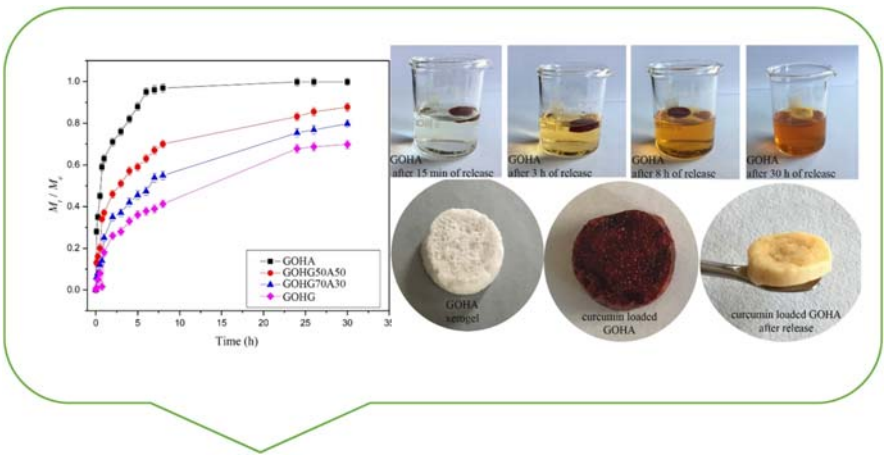

FIGURE 28.6

Curcumin release profiles from GOHGA hybrid hydrogels.

$m = 0.5$) were applied to study the release kinetics of active agent-curcumin from the hybrid hydrogels (Caccavo, 2019; Gouda, Baishya, & Qing, 2017; Vigata, Meinert, Pahoff, Bock, & Hutmacher, 2020a; Vigata, Meinert, Hutmacher, & Bock, 2020b). The in vitro obtained curcumin release data were used to fit the kinetic models. Calculated parameters are presented in Fig. 28.7. Analyzing the Akaike information criterion values, it turns out that Peppas-Sahlin (Fig. 28.7C) and Peppas-Sahlin when $m = 0.5$ (Fig. 28.7D) models best describe release phenomena in the investigated curcumin/hybrid systems.

Using the Peppas-Sahlin equation the ratio between the relaxation (R) and the diffusional (F) contribution during the curcumin release was calculated and presented (Fig. 28.7E). We found that the GOHG hydrogel shows a lower *R/F* ratio than hybrids containing alginate. These results are confirmation that the curcumin transport mechanism from GOHG70A30, GOHG50A50, and GOHA hydrogels is anomalous and the importance of the relaxation contribution for curcumin release is more pronounced in these hybrids compared to the GOHG hydrogel.

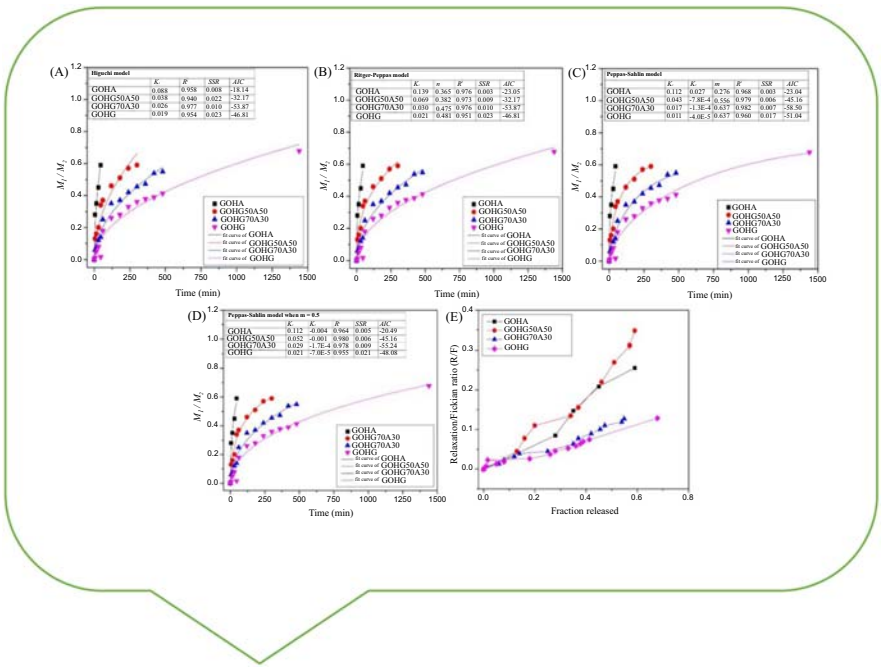

FIGURE 28.7

Obtained experimental data of curcumin release from hybrids fitted by Higuchi (A), Ritger-Peppas (B) Peppas Sahlin (C), and Peppas Sahlin model when $m = 0.5$ (D) the ratio *R/F* vs the fraction of curcumin released from the GOHGA hydrogels (E).

28.3 Titanium dioxide-based materials for biomedical applications

Titanium dioxide (TiO_2) has attracted much attention since the discovery of its excellent photocatalytic performance (Fujishima & Honda, 1972; Fujishima, Rao, & Tryk, 2000; Tryk, Fujishima, & Honda, 2000). It has been followed by extensive research on the fabrication, structure, and applications of nanoTiO_2-based materials (Buot, 1993; Fréchet, 2003; Whitesides, 2005). As a result, nanoTiO_2-based materials with various morphologies of different hierarchical nanostructures have been produced by different techniques (Chen & Mao, 2007). The materials have been applied in a myriad of areas involving energy and environmental research as well as biomedical engineering due to their unique characteristics, comprising low density, large strength-to-weight ratio, photochemical stability, high catalytic efficiency, excellent biocompatibility, good corrosion resistance, as well as excellent mechanical properties (Bao, Lei, Xu, Cai, & Jia, 2012; Barbora, Acharya, & Verma, 2009; Biswas & Becker, 2013; Brammer, Frandsen, & Jin, 2012; Guo et al., 2012; Hu, Kienle, Guo, & Maier, 2006; Ren et al., 2012; Wang, Wen, & Li, 2006; Wang, Liu, Wu, & Lu, 2012a).

Functionalized nanostructured TiO_2-based materials have positive effects in many biomedical applications such as bone scaffolding materials, vascular stents, and drug delivery systems. It turned out that nanoTiO_2 scaffolding biomaterials accelerate the rate of apatite formation and enhance osteoblast adhesion, proliferation, and differentiation (Brammer et al., 2011; Dong et al., 2007; Gongadze et al., 2013; Nazari, Riahi, Riahi, Shamekhi, & Khademno, 2010; Tan, Pingguan-Murphy, Ahmad, & Akbar, 2013). Possessing good blood compatibility and anticoagulation characteristics, TiO_2 nanotube arrays are promising for vascular implants, and nanostructured TiO_2 has been widely reported as drug carriers as well (Weng et al., 2011; Yang et al., 2010). Especially, TiO_2 nanotubes have proven to be a superior platform for local drug delivery due to their excellent biocompatibility, controllable dimensions, surface chemistry, and large surface-to-volume ratio (Lai et al., 2013; Peng et al., 2009; Shokuhfar, Sinha-Ray, Sukotjoc, & Yarin, 2013; Vasilev et al., 2010). By changing the nanotube diameter, wall thickness, and length, the release kinetics of specific drugs can be tailored to achieve stable and sustained release (Popat, Eltgroth, LaTempa, Grimes, & Desai, 2007a).

TiO_2-based materials make good scaffolding materials because they can satisfy many requirements for bone implant biomaterials. TiO_2 component is biocompatible (Crane, Ishaug, & Mikos, 1995), enhances the ingrowth of bone and vascular tissues (Jokinen et al., 1998), possesses antibacterial properties (Rincon & Pulgarin, 2004; Yuranova et al., 2006) and delivers good osteoconductive performance (Forsgren, Svahn, Jarmar, & Engqvist, 2007; Tsukimura et al., 2008). Osteoconductivity is important for scaffolds as this property affects the integration between the scaffold and bone tissues (Albrektsson & Johansson, 2001;

Ducheyne & Qiu, 1999). Therefore, synthetic TiO_2 scaffolds show a high porosity, excellent interconnectivity, and sufficient mechanical strength boding well for load-bearing orthopedic and dental applications (Fostad et al., 2009; Tiainen, Lyngstadaas, Ellingsen, & Haugen, 2010). Owing to the inherently high compressive strength of ceramic TiO_2 in comparison to other osteoconductive materials such as calcium phosphate ceramics (CaP), bioactive glass, and CaP/polymer composites, TiO_2 can provide better mechanical strength to the scaffold even at high porosity. Compressive strength of 2.5 MPa has been observed for TiO_2 scaffolds with a porosity of 85%, and the strength can be retained after implantation due to the nonresorbable nature of TiO_2 (Tiainen et al., 2010). In contrast, the values obtained for CaP and CaP/polymer composite scaffolds with a similar porosity are generally in the range of 0.1−1 MPa (Rezwan, Chen, Blaker, & Boccaccini, 2006; Wagoner Johnson & Herschler, 2011; Miao & Herschler, 2011; Miao, Tan, Li, Xiao, & Crawford, 2008) and below 2 MPa, which is the minimum value of trabecular bone (Carter & Hayes, 1976).

Nanostructured TiO_2 has been combined with inorganic and organic materials, such as different types of polymers (Jayakumar et al., 2011; Rani et al., 2011; Zhao et al., 2011a; Liu, Slamovich, & TWebster, 2006; Wu et al., 2012), to produce bone scaffolding biomaterials that have excellent biocompatibility, good mechanical properties, and enhanced osteoconductivity. TiO_2/polymer composites, such as TiO_2/PLA, TiO_2/poly(lactic-*co*-glycolic acid) (PLGA), and TiO_2/poly(ether-ether ketone) show controllable swelling and degradation compared to the pure polymeric scaffolds, and in vitro tests indicate that osteoblasts attach well to the exposed area and grow into the inner pores of the scaffolds (Jayakumar et al., 2011; Liu et al., 2006; Rani et al., 2011; Wu et al., 2012).

TiO_2-based biomaterials can be used for therapeutic delivery applications. Therapeutic vehicles based on TiO_2 nanomaterials have been developed to deliver small molecules, proteins, and genes to target tissues and organs in the body. Increasing the surface area by generating pores within the nanomaterials maximizes therapeutic loading compared to their nonporous counterparts. Research has shown that the charge interactions between the therapeutic agents and nanomaterials mainly facilitate physical adsorption (Patra, Das, Fraceto, Campos, & Rodriguez-Torres, 2018). Furthermore, controlled release kinetics are also favorable for drug delivery systems. The release rate can be tuned using different strategies including polymeric capping or coatings on the surface of TiO_2 nanostructures (Genchi, Cao, & Desai, 2018; Wang, Li, & Liu, 2019). The controlled filling of nanotubes with drug-loaded polymeric micelles can generally slow down the release rate (Aw, Addai-Mensah, & Losić, 2012).

A stimuli-responsive drug release system for precise chemotherapy to minimize side effects is achievable through the conjugation of pH-, thermo-, and enzyme-responsive polymers on the surface (Kafshgari, Voelcker, & Harding, 2015). Multifunctional mesoporous TiO_2 nanocarriers that had been conjugated with PEI and FA were prepared for a drug delivery system based on the

near-infrared laser-controlled drug release system (Wang et al., 2015). X-ray illumination of TiO_2 nanomaterials can create electron-hole pairs within the structure (degrading organic linkers) and generate a triggered release (Schmidt-Stein et al., 2009). A combined strategy for a stimuli-responsive drug release has also been reported by allocating a hydrophobic cap for amphiphilic TiO_2 tubular arrays sensitive to UV light irradiation (Song, Schmidt-Stein, Bauer, & Schmuki, 2009). The multifunctional porous TiO_2 nanoparticles, conjugated with PEI and FA, have also been developed for UV-responsive drug release as well as targeted drug delivery. The "burst" effect release of loaded anticancer drug paclitaxel from TiO_2-based nanocarriers was controlled by the PEI capping, and the exposure to UV light irradiation, which accelerated the degradation of PEI on the surface by the generation of free-radicals, released the entrapped anticancer drugs.

The targeted therapeutic delivery system is a key approach to accumulating therapeutics into the site of action to boost therapeutic efficacy. The postfabrication of nanocarriers by using biomolecules and ligands (FA, hyaluronic acid, and antibody) is a promising strategy, which can precisely accumulate nanomaterials in a specific tissue (Cheng et al., 2019; Liu et al., 2015; Wang et al., 2015). Correspondingly, successful delivery of DOX to the orthotopic breast tumor has been achieved by the administration of poly(ethylene glycol) (PEG) coated TiO_2 nanoparticles based on the enhanced permeability and retention effects (Du et al., 2015).

Compared to traditional drug delivery, TiO_2-based nanostructured materials can control drug release to improve the therapeutic efficacy and reduce the side effects as well as the pain caused by multiple doses. TiO_2/polymer composite nanoparticles have been used as drug carriers in cancer therapy. FA/poly(ethylene glycol)/TiO_2 (FA-PEG-TiO_2) is synthesized by grafting FA onto poly(ethylene glycole). (PEG)ylated TiO_2 nanoparticles can be used for the targeted delivery of paclitaxel, an anticancer drug (Venkatasubbu, Ramasamy, Ramakrishnan, & Kumar, 2013). The FA-PEG-TiO_2 system can target cancer cells and is also capable of evading the reticuloendothelial system. The TiO_2 nanocarriers possess a higher adsorption capability, and the in vitro release profile of paclitaxel from the FA-PEG-TiO_2 nanoparticles shows fast release initially followed by a sustained release phase (Venkatasubbu et al., 2013).

Organisms often encounter unwanted foreign or mutated DNA that may negatively affect their health. Traditional modes of diagnosis are often unable to detect the presence of deleterious DNA. Besides, conventional treatments do not address the underlying cause of the diseases and do not discriminate well between the target and healthy cells, thereby resulting in low therapeutic efficacy. Hence, both imaging and elimination of unwanted genes are major goals in molecular biology (Gonzalez-Alegre, 2007; Kami et al., 2011; Scherer, Rossi, & Weinberg, 2007). On the heels of rapid development in genetic engineering, it is possible to directly alter a genome and insert or remove a chunk of DNA. Nanotitanuim dioxide-based materials hold great promise in gene therapy by

facilitating the targeted delivery of DNA into tissues and cells (Brown et al., 2008; Paunesku et al., 2003; Paunesku et al., 2007).

The human body's self-healing process is slow when the injury is severe. However, the body indeed accepts external aids from implanted biological tissues and organs grown in the laboratory as a means to accelerate the healing process. Scaffolds or implants in the body must communicate with the surrounding microenvironment since the recipient's immune system may very likely cause rejection. Biocompatible TiO_2-based nanomaterials are one of the greatest implantable materials for tissue regeneration owing to their properties of high tensile strength, flexibility, and corrosion resistance. The morphology of TiO_2 nanomaterials is, nevertheless, the most important factor in improving cell adhesion, proliferation, and differentiation (Bauer et al., 2009; Park et al., 2012). Scaffolding materials composed of PLGA and TiO_2 nanoparticles, as well as decorated glass with TiO_2 nanoparticles, can improve the amount of precipitated calcium for bone regeneration compared to the scaffold without TiO_2 nanoparticles (Rasoulianboroujeni, Fahimipour, Shah, Khoshroo, & Tahriri, 2019; Vercellino, Ceccarelli, Cristofaro, Balli, & Bertoglio, 2016). The adhesion and spreading of osteoblast cells with a complete integration can also be attained with composites made of PLA, poly-ε-caprolactone, and TiO_2 particles or nanofiber meshes mimicking the bone regeneration properties (Nájera, Michel, Kyung-Hwan, & Kim, 2018; Wang et al., 2012b). Compared to functionalized nanomaterials, it was observed that mesenchymal stem cells prefer to migrate without the interfering features of bare TiO_2 nanoparticles. It was shown that bare TiO_2 nanoparticles with different sizes can induce negative impacts on viability, adhesion, migration, proliferation, and differentiation of mesenchymal stem cells in a size- and dose-dependent manner in vitro; however, small bare TiO_2 nanoparticles can activate the migration of mesenchymal stem cells compared to larger bare nanoparticles. The alkaline phosphatase activity, which determines an early mineralization-related protein marker for osteogenesis of osteoblasts, was also increased in the mesenchymal stem cells treated with TiO_2 nanoparticles (14 nm in diameter) after 2 weeks compared to those treated with bigger nanoparticles (108 and 196 nm in diameter) (Hou et al., 2013). Biomolecule-TiO_2 nanohybrids can be an advantage due to improving antibacterial and antiinflammatory features and biocompatibility without increasing the content of TiO_2 in vital organs.

Nevertheless, either bone and wound infections or dental abscesses are highly likely because of possible contamination of implants and systemic diseases; infections can be minimized by the physical adsorption of antiinflammatory drugs or silver nanoparticles onto TiO_2 nanostructures (Popat, Eltgroth, LaTempa, Grimes, & Desai, 2007b; Zhao, Wang, Huo, Cui, & Zhang, 2011b). Moreover, it has been proved that the antimicrobial activity of TiO_2 nanomaterials eliminates infections and accelerates the proliferation of cells in the wound area compared to other materials (Lapa, Cresswell, Campbell, Jackson, & Goldmann, 2019; Limongi et al., 2016; Unalan et al., 2019a, 2019b).

28.4 Design of hybrid polymeric hydrogels platform based on nanotitanium dioxide, alginate, gelatin, and 2-hydroxyethyl methacrylate

To obtain novel, porous and degradable hybrid hydrogels, the polymeric networks were successfully synthesized by synergistically combining synthetic monomeric component 2-hydroxyethyl methacrylate, and natural polymers-gelatin and alginate (HGA) with inorganic agent nanotitanium dioxide using modified porogen leaching method followed by freeze-drying treatment (Chen et al., 1999). The synthesis route of the hydrogels is shown in Fig. 28.8.

The mechanically strong polymeric network was prepared by free-radical polymerization/crosslinking of 2-hydroxyethyl methacrylate in the presence of the activating system. The additional polymeric network was created by the crosslinking of gelatin while the alginate chains are loaded to intertwine in the networks. Incorporation of the nanotitanium dioxide evenly through the polymeric network was achieved during foaming reactions, by CO_2 gas bubbles generated from the thermal decomposition of sodium bicarbonate. The technique based on CO_2 gas bubbles was proposed as a successful way to achieve the porosity of hybrid hydrogels and to disperse inorganic agents evenly through their composition.

28.4.1 Biocompatibility of TiHGA hybrid hydrogels

The focus of attention in biomaterials science is how the mutually acceptable coexistence of biomaterials and tissues/cells is developed and sustained. The single most important factor that distinguishes a biomaterial from other materials is its biocompatibility with natural tissues/cells (Williams, 2008). The biocompatibility of TiHGA hydrogels was examined in a cytotoxicity test on

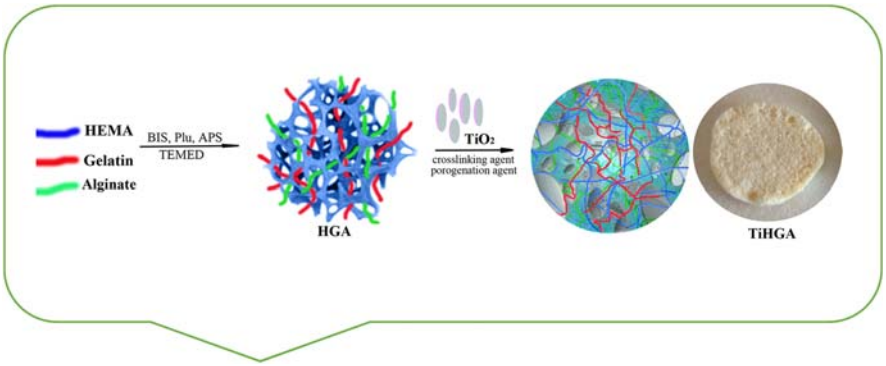

FIGURE 28.8

Schematic representation of the formation of the hybrid hydrogels composed of nanotitanium dioxide, 2-hydroxyethyl methacrylate, gelatin, and alginate.

normal human fibroblasts (MRC5). The results of this assay for TiHGA samples are shown in Fig. 28.9. TiHG and TiHG70A30 extracts were not significantly cytotoxic in vitro under tested conditions, moreover, lower doses of extracts induced higher proliferation in MRC5 cells (Fig. 28.9A). All materials, even when supplied directly to cells were shown to support the accumulation of cells on its surface (Fig. 28.9B).

28.4.2 Structural characteristics of TiHGA hybrid hydrogels

The chemical structure and functional groups in the hydrogels based on nanotitanium dioxide, 2-hydroxyethyl methacrylate, gelatin, and alginate were determined by Fourier transform infrared spectroscopy (FTIR). Obtained FTIR spectra of PHEMA, TiHA, and TiHG samples are presented in Fig. 28.10.

To confirm the presence of a 2-hydroxyethyl methacrylate (HEMA) component in the hydrogels, the PHEMA sample was screened concerning the functional group attributes for HEMA. As can be seen, FTIR spectrum obtained for PHEMA display typical signals for O-H stretching vibration at approximately 3432 cm^{-1}, C-H stretching vibrations at 2930 cm^{-1} and 2886 cm^{-1}, and strong C = O vibration at 1717 cm^{-1} (Tomić et al., 2014). On the other hand, FTIR spectra of the synthesized multicomponent hydrogels (TiHA and TiHG) presented combinations of typical HEMA, gelatin, and alginate vibrations, while the intensity of the peaks

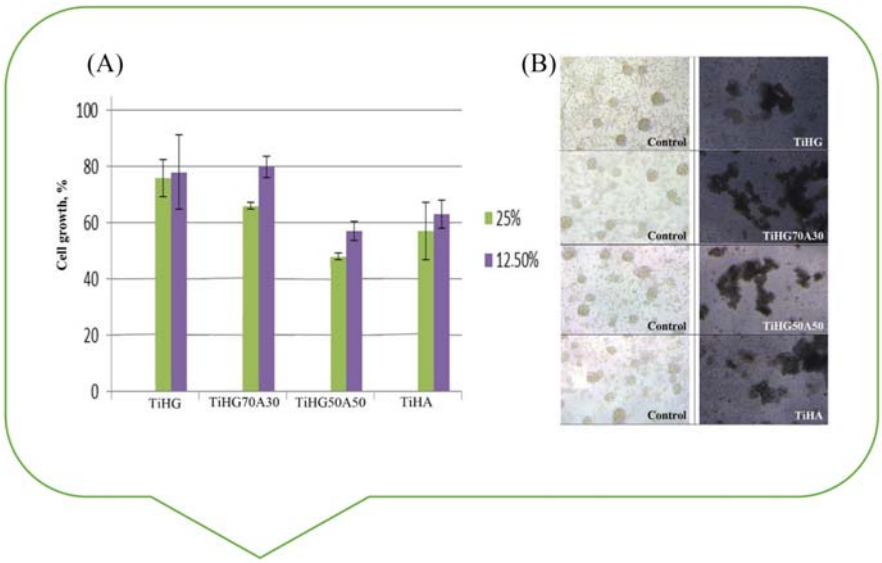

FIGURE 28.9

(A) Cell viability of TiHGA hybrid polymeric hydrogels and (B) accumulation of cells on its surface.

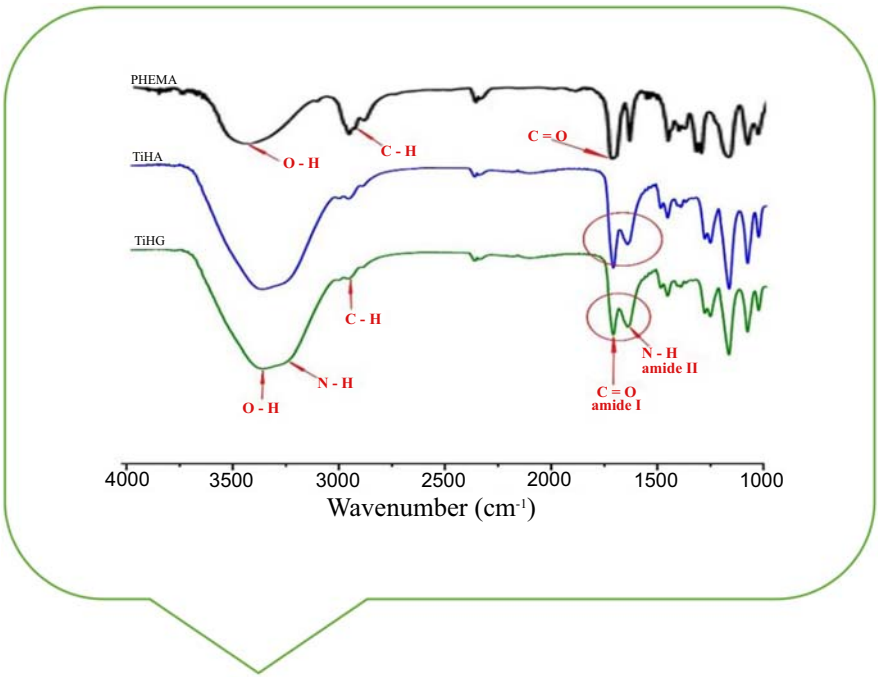

FIGURE 28.10

FTIR spectra of PHEMA, TiHA, TiHG hybrid hydrogels.

depended on the ratio and type of the component. The spectrum of TiHG exhibits several characteristic spectral bands for HEMA and gelatin. Functional groups of gelatin are N-H stretching vibration around 3350 cm^{-1}, C-H stretching at 3003 cm^{-1}, C = O stretching at 1701 cm^{-1} for amide I, N-H definition at 1630 cm^{-1} for the amide II (Chang & Tanaka, 2002). Similarly, TiHA shows signals originating from the polysaccharide structure of alginate: 1276 cm^{-1} C-O stretching, 1159 cm^{-1} C-C stretching, 1015 cm^{-1} C-O-C stretching and absorption bands of HEMA (Vieira et al., 2008). The absorption bands at around 1480 and 1400 cm^{-1} are assigned to asymmetric and symmetric stretching peaks of the carboxylate group of alginate.

28.4.3 Morphology of TiHGA hybrid hydrogels

Due to the importance of the porous microstructure for various applications, SEM was used to evaluate the morphology of the synthesized TiHGA hydrogels after the freeze-drying treatment. The homogeneity of the micro-architecture, orientation, interconnectivity, size, and shape of pores are the most important features of hydrogels for their applications as biomaterials (Dragusin et al., 2012). To detail

understand the microstructure of the hydrogels, their cross-sectional and surface morphology was analyzed by SEM, and obtained SEM micrographs are displayed in Fig. 28.11.

The presented micrographs (Fig. 28.11) of all samples revealed a highly porous structure with interconnected pores of different sizes, shapes, and orientations, and the manifestation of any phase separation phenomenon no observed. This confirms the good compatibility between components of hybrid hydrogels. The interior of all samples was found to be highly porous, with open, interconnected pores, with spherical to elliptical shapes. The pore size was found to vary between 10−100 μm. The surface of the TiHGA hybrid hydrogels displayed open and filled pores, crinkles, and cracks. These phenomena increased the surface porosity and roughness and could affect the fluid absorption capacity as well as hydrogel application.

28.4.4 Mechanical properties of TiHGA hybrid hydrogels

The mechanical properties of the hydrogels presented a very important physical property that determines their applications, especially in the biomedical field. Values of Young's modulus (E) obtained for hydrogels based on alginate, gelatin, 2-hydroxyethyl methacrylate, and nanotitanium dioxide were used to analyze the mechanical properties of the TiHGA hydrogels. TiHGA hydrogels show E values in the range of 2.81−8.94 MPa indicating the strong dependence of the chemical

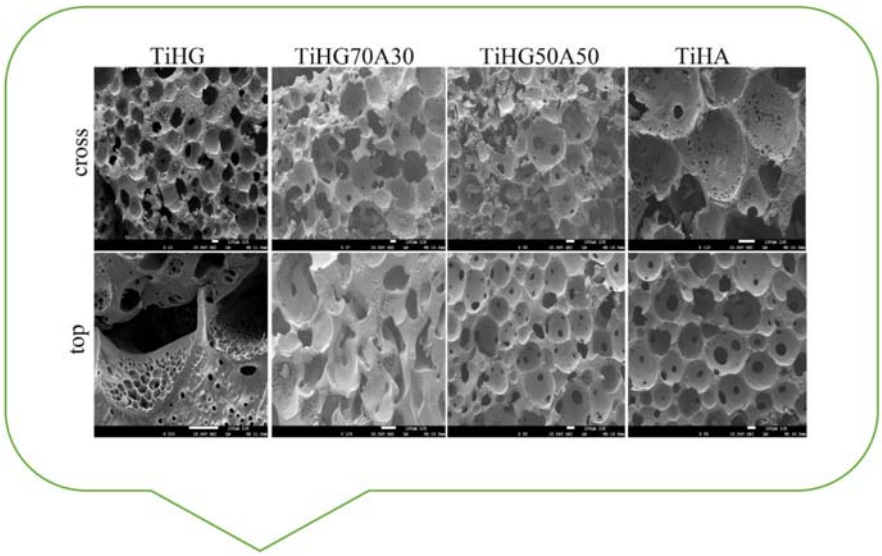

FIGURE 28.11

SEM micrographs of TiHGA hybrid hydrogels.

composition of the hydrogels and their mechanical properties. Young's modulus of TiHG (8.94 MPa) is higher than the other hydrogels. The increasing content of gelatin in the hybrids increases the values of Young's modulus due to the presence of crosslinking in gelatin-based hydrogels leads to a more compact structure resulting in better mechanical properties of these hydrogels. The hybrid hydrogels containing alginate (TiHG50A50 and TiHA) show lower values of Young's modulus (3.50 and 2.81 MPa). The desired mechanical strength can be adjusted by optimizing the synthesis process and tuning the component ratios.

28.4.5 The porosity of TiHGA hybrid hydrogels

The porosity of hydrogels is a fundamental parameter to characterize the hydrogels and their possible applications in different fields. For systems designed to transport fluids or active agents, as is the usual case of hydrogels, a high level of porosity is desirable. The porosity of the hybrid hydrogels based on nanotitanium dioxide, alginate, gelatin, and 2-hydroxyethyl methacrylate was evaluated by the bulk density method. The obtained results of the percentage of porosity for the TiHGA hybrids are in the range of 52 to 66% indicating their dependence on the chemical composition of the hydrogels. The increasing content of gelatin in the hydrogels decreases the level of their porosity caused by the presence of more crosslinks of gelatin. The highest porosity showed in the TiHA sample (66%). Results indicate that the porosity of the TiHGA hydrogels can be adjusted and adapted by modifications of hydrogel composition and variations of components ratio.

28.4.6 The hydrophilicity of TiHGA hybrid hydrogels

Due to the water affinity is a key parameter influencing the biological performance of hydrogels, the surface hydrophilicity of the hybrid hydrogels based on nanotitanium dioxide, alginate, gelatin, and 2-hydroxyethyl methacrylate was assessed by measuring contact angle through water spread of a droplet on a surface. The results obtained from the static water contact angle measurements are presented in Fig. 28.12. As can be seen, all hydrogels with measurements performed at 0 and 1 s are fully hydrophilic, water completely wetted their surface and drop immediately disappeared after putting on the surface of the samples.

28.4.7 In vitro degradation properties of TiHGA hybrid hydrogels

The in vitro degradation of the hydrogels is of great importance for their applications as biomaterials since the degradation behavior determines key in vivo properties such as the stability of the biomaterial in a living body, and the efficiency with the respect to the release of different active agents. The in vitro degradation process was monitored in controlled conditions-simulated buffer fluid of pH 7.40 and at a temperature of 37°C. The weight loss, as an indicator of degradation of

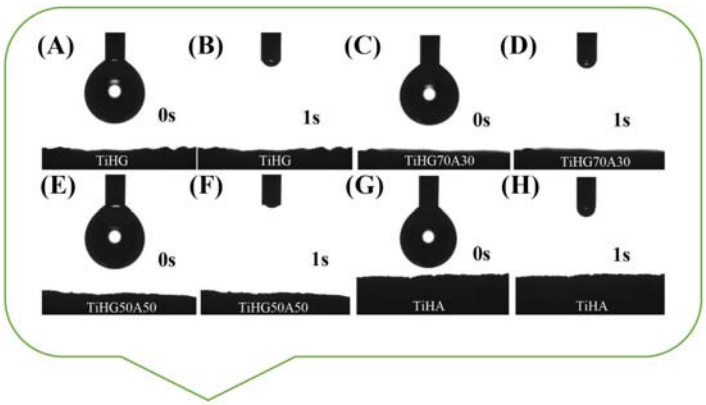

FIGURE 28.12

The water contact angle of TiHGA hybrid hydrogels. (A) TiHG; (B) TiHG; (C) TiHG70A30; (D) TiHG70A30; (E) TiHG50A50; (F) TiHG50A50; (G) TiHA; (H) TiHA.

hybrid hydrogels based on nanotitanium dioxide, alginate, gelatine, and 2-hydroxyethyl methacrylate was investigated for 6 months. The tested hydrogels degraded up to 40% after 6 months. Significant differences in the degradation behavior of the hydrogels depending on their compositions were expected. The highest weight loss was detected for the TiHA hydrogel (45.89%), while the lowest weight loss exhibited TiHG (39.28%). The obtained results indicate that the degradation degree of the hybrid hydrogels is strongly influenced by the gelatin content of the hydrogels. The presence of crosslinked gelatin generates a lower degradation degree of hybrid hydrogels.

28.5 Summary and future perspectives

GO-based materials' potential applications in the field of biomedicine have been revealed based on the reported works in the past few years. GO's structure, size and shape played an important role in various applications. Because of the high surface area, a large number of nanoparticles or biomolecules were immobilized on it together leading to multifunctional nanocomposites. The various functional groups attached to GO increase its dispersity in various biofluids. Also, hydrophobic drug-loaded GO-based materials can easily be delivered into the cancer cells for enhancing the efficiency of treatment. GO-based materials can be tuned to be nontoxic and biocompatible. These findings in the literature enable one to recognize and use GO's tremendous applicability in biomedicine.

The unique and fascinating properties of graphene derivatives such as functionalized surfaces, strong UV absorption, and fluorescence and fluorescence quenching ability make them one of the most promising materials for

therapeutics, and tissue engineering. Although several challenging issues remain, the biological applications of graphene derivatives have significant potential because many attempts have shown promising results regarding biofunctionalization and standardization of graphene derivatives by fractionation based on size, the number of layers, and chemical functionalities. Furthermore, there are many unique advantages and still many chances to discover fascinating properties and potential applications. Efforts with interdisciplinary approaches among chemistry, biology, and engineering will accelerate mechanistic understanding of graphene-based platforms for bioapplications and make current successful demonstrations more routinely implemented in diverse applications.

TiO_2-based materials are a promising class of biomaterials for decoding a wide variety of limitations present in nanomedicine, also thanks to its different and available ways of fabrication, postfabrication, and biocompatibility. Various types of TiO_2-based materials have been fabricated with high precision and post-fabricated with adjustable physiochemical properties. Biocompatible TiO_2-based materials are unique due to a wide range of features (a tunable geometry, dimension, porosity, as well as quantum effect, photoactivity, and well-established surface chemistry) that generate less toxic biological responses. There has also been substantial progress in the fabrication and postfabrication of TiO_2-based materials to obtain the best performance for different biomedical applications in vivo. Many promising studies show the successful development of biomaterials based on nanoTiO_2 structures for therapies in vitro and in vivo. Therefore, an elaborate design of TiO_2-based materials based on biological microenvironments and responses is still needed to minimize long-term cytotoxicity. Recent advances in nanostructured TiO_2-based materials in important areas are reviewed, namely bone scaffolding biomaterials, intravascular stents, drug delivery systems, and tissue healing and regeneration. Clinical-use implants based on orthopedic TiO_2 nanomaterials, offering significant osseointegration and greatly imitating the strength of the bone structure, are well known. However, cellular responses to nanoscale TiO_2-based biomaterials used to directly or indirectly support cellular differentiation and proliferation are ambiguous due to unknown long-term biocompatibility. Multicomplex nanohybrid systems composed of smart biocompatible hydrogels and nanoTiO_2 structures still need to be developed to successfully regenerate and repair tissues, hereby extending human life. All things considered, most of the studies as proof of principle have demonstrated that TiO_2-based materials have the potential to overcome challenges in certain aspects associated with the biomedical field.

The review is elaborate on two novel platforms of multifunctional, biocompatible, porous, and degradable hybrid hydrogels based on synthetic monomer 2-hydroxyethyl methacrylate and natural polymers-gelatin and alginate integrated with nanomaterials-nanographene oxide (GOHGA) and nanotitanium dioxide (TiHGA). These hybrids were successfully synthesized by the simple but effective method-modified porogenation. To achieve a balance between the biological and mechanical properties of the hydrogels to satisfy their final applications in

different fields, especially as an efficient drug delivery carrier, the chemical composition of the hybrid hydrogels was varied in all samples. All samples showed favorable biocompatibility, water uptake, morphology, mechanical properties, porosity, hydrophilicity, and degradation behavior.

28.6 GOHGA hybrid hydrogels

We have successfully developed hybrid hydrogels based on alginate, gelatin, 2-hydroxyethyl methacrylate, and nanomaterial-nanographene oxide by a modified porogenation method. The results of the biocompatible behavior test suggested that the GOHGA hydrogels do not show significant changes in cell viability with the change in composition and have proper biocompatibility to be used in biomedical applications. The spectroscopic characteristics of the GOHGA hydrogels demonstrated the presence of bands indicating that the polymeric networks were constructed of alginate, gelatin, and 2-hydroxyethyl methacrylate components. These multicomponent biomaterials exhibit good swelling capacity, porous structure with interconnected pores, porosity in the range of 52.80%−76.36%, and degradation rate up to 41% after 6 months. The highest weight loss was detected for the GOHA hydrogel, while the lowest weight loss exhibited GOHG. Water uptake behavior for GOHGA hydrogels demonstrated a time-favorable hydration profile, which is suitable for use as biomaterials. Obtained data showed favorable hydrophilicity of all GOHGA hybrid hydrogels. The morphology of GOHGA hybrid hydrogels was found to be highly porous, with open, interconnected pores, spherical to elliptical shapes, with pore size in the range of 10−100 μm. The mechanical properties of GOHGA hydrogels, represented by the modulus of elasticity, are in the range of 1.69−4.87 MPa, and depend on the composition of the hydrogels. Among that, the hydrophobic drug, curcumin, was efficiently loaded into the GOHGA hydrogels with an efficiency of over 96%. Obtained results of in vitro release study showed that about 98% of released curcumin was achieved approximately within 25, 30, 38, and 48 h of releasing in a buffer pH 7.40, at 37°C from GOHA, GOHG50A50, GOHG70A30, and GOHG, respectively suggesting that the GOHGA hydrogels are promising systems for controlled curcumin release with high potential for releasing of other hydrophobic drugs. These promising results support the potential application of GOHGA hybrid hydrogels as a successful biological alternative in biomedical and drug delivery applications.

Owing to the unique properties of graphene, numerous researchers have focused on developing materials with graphene as a dispersed nanofiller in various matrices, including ceramics, metals, and polymers. A large majority of these efforts focus on developing the method to homogeneously disperse nanographene in polymeric matrices. Thus, the presented synthesis strategy and the innovative design of suitable porous, biocompatible GOHGA hybrid hydrogels are significant steps for developing a new class of biomaterials with incorporated nanographene oxide.

Further, the cytotoxic effects of graphene-polymeric materials represent one of the major challenges that may have a great impact on the future exploitation of graphene-based polymeric materials, especially in the field of technology and biomedicine.

28.7 TiHGA hybrid hydrogels

We have successfully developed hybrid hydrogels based on gelatin, alginate, and 2-hydroxyethyl methacrylate and nanomaterial-nanotitanium dioxide by the modified porogenation method. The biocompatible assay for TiHGA hybrid hydrogels shows satisfied cell viability to be used in biomedical applications. The spectroscopic characteristics of the TiHGA hybrid hydrogels demonstrated the presence of bands indicating that the polymeric networks were built of alginate, gelatin, and 2-hydroxyethyl methacrylate components. Water uptake behavior for TiHGA hydrogels displayed a time-favorable hydration profile. All TiHGA hybrid hydrogels were found to be fully hydrophilic. The morphology of TiHGA hybrids indicates highly porous structures with open, interconnected pores, spherical to elliptical shapes, with pore sizes in the range of 10−100 μm. The surface of the TiHGA hybrid hydrogels displayed open and filled pores, crinkles, and cracks. The mechanical properties of TiHGA hydrogels represented by the modulus of elasticity are in the range of 2.81−8.94 MPa and depend on the composition of the hydrogels. The porosity data of TiHGA hybrids is in the range of 52% to 66%. This feature can be tuned and adapted by modifications of hydrogels and variations of components ratio. The tested TiHGA hydrogels degraded up to 40% after 6 months. The highest weight loss was detected for the TiHA sample (45.89%), while the lowest weight loss exhibited TiHG (39.28%) suggesting that the degradation degree of the TiHGA hybrids is strongly influenced by the gelatin content of the hydrogels.

Recent years have attracted a lot of interest in employing a tool to disperse nanotitanium into polymeric biomaterials. Thus, the presented synthesis strategy and the innovative design of suitable porous, biocompatible TiHGA hybrid hydrogels will be of pragmatic significance for developing a new class of biomaterials with incorporated nanotitanium dioxide. Being versatile and practical, we believe that the described multifunctional TiHGA hybrid hydrogels can find applications in different areas, especially as biomaterials, and that the safe and effective clinical implementation of TiHGA polymeric systems is expected to accelerate and expand shortly.

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VOLUME THREE

ADVANCED NANOFORMULATIONS

Theranostic Nanosystems

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Provides a thorough insight into the discussions about the preparation, properties, and theranostic applications of advanced nanoformulations.

- Contains in-depth discussions of the advanced formulations using nanosystems including high-quality graphics, flowcharts, and graphs for enhanced understanding
- Reviews the literature on advanced formulations while also suggesting new avenues
- Includes contributions in all areas of advanced formulations, providing a thorough and interdisciplinary work

Advanced Nanoformulations: Theranostic Nanosystems, Volume 3 is an interdisciplinary work examining the applications of nanotherapeutic systems as well as nanodiagnostics in relation to advanced nanoformulations. In the last decade, numerous biopolymers have been utilized to prepare polymeric nanosystems for therapeutic applications. Several advanced and specialized nanoformulations have been developed for bioactivity, site/target-specific delivery of therapeutic agents, and/or diagnostic purposes. These advanced and specialized theranostic nanoformulations includes nanocrystals, nanosuspensions, self-nanoemulsifying systems, nanoemulsions, polymeric nanomicelles, nanoliposomes, dendrimers, nanogels, niosomes, solid lipid nanoparticles, nanostructured lipids, stimuli-responsive smart nanosystems, nanoconjugates, nanocomposites, nanofibers, nanophytomedicines, and nanovaccines. This book explores these advanced nanoformulations to understand therapeutic potentials. This book will be useful to pharmaceutical scientists, including industrial pharmacists and analytical scientists, health-care professionals, and regulatory scientists actively involved in pharmaceutical product and process development of tailor-made polysaccharides in drug delivery applications.

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