Spring 2-1-2022

The antibacterial effect and the incidence of post-operative pain after the application of nano-based intracanal medications during endodontic retreatment: a randomized controlled clinical trial

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Clinical Oral Investigations
https://doi.org/10.1007/s00784-021-04196-w

ORIGINAL ARTICLE

The antibacterial effect and the incidence of post-operative pain after the application of nano-based intracanal medications during endodontic retreatment: a randomized controlled clinical trial

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Received: 28 May 2021 / Accepted: 21 September 2021

Abstract

Objective This clinical trial aimed to evaluate the effect of nano-silver and nano-calcium hydroxide intracanal medicaments (ICM) during retreatment regarding their antibacterial effect and their effect on post-operative pain and flare-ups.

Materials and methods Sixty-nine patients scheduled for endodontic retreatment were included in this randomized clinical trial and randomly allocated to 3 equal groups ($n=23$) according to the type of ICM used. The first microbial sampling (S1) representing the original microbiota was obtained after the removal of the old canal filling. After chemo-mechanical debridement, another sample (S2) was obtained representing the microbial state before ICM application. Patients were randomly allocated to receive either nano-silver (nano-Ag), nano-calcium hydroxide (nano-CH), or calcium hydroxide (CH) as ICM. Patients rated their pain pre-operatively and then after 6, 12, 24, 48, and 72 h. During the second visit (7 days later), the last microbial sample (S3) was obtained after removal of the ICM. Reduction of total bacterial and total *E. faecalis* counts and the biofilm-forming capability of the existing microbiota were determined.

Results Results showed reduction in total bacterial count, total *E. faecalis* count and the biofilm-forming,capability of the existing microbiota after chemo-mechanical debridement (S1-S2) and after the application of ICM (S3-S2). However, the reduction after cleaning and shaping was significantly more pronounced ($p<0.001$) compared to the effect of ICM application, with no difference between the 3 ICM ($p>0.05$). Post-operative pain was significantly reduced at the 48- and 72-h intervals after the application of nano-Ag and nano-CH only ($p<0.001$), with no significant difference between these two ICM ($p>0.05$). The incidence of flare-ups in all groups was similar ($p>0.05$).

Conclusions The antibacterial effect of the nano-Ag and nano-CH was equivalent to that of CH, but they contributed to better pain control.

Clinical relevance.

Nanoparticles may have a positive impact on post-endodontic pain.

Keywords Bacterial reduction · Calcium hydroxide · Endodontic retreatment · Flare-ups · Post-operative pain · Nano-silver · Nano-calcium hydroxide · Intracanal medications

Introduction

A growing interest in endodontic retreatment has been noticed recently due to the increased demand to preserve teeth. Intraradicular bacterial infection is considered to be the main cause of post-treatment apical periodontitis [1–3] whether these bacteria are persisting due to inadequate primary treatment or reintroduced to the canal system due to inadequate apical or coronal seal [4]. Bacteria harboring the root canals are very diverse in their species, shapes and virulence factors [5]. Among the bacterial strategies that contribute to its pathogenicity is their ability to colonize and organize themselves in biofilm structure. This can be defined as a sessile multicellular microbial community characterized by cells that are firmly attached to a surface in a self-produced matrix of polysaccharides [6]. Biofilms were found to be responsible for chronicity of diseases [7], as it makes

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Published online: 25 October 2021

the bacteria within its resistant to antibiotics, enables them to evade the immune system, and also enables exchange of genetic material between biofilm bacteria resulting in higher resistance and virulence. Furthermore, anatomic complexities of the root canals as isthmuses, fins, ramifications, lateral canals,and dentinal tubules make the situation much more difficult as such areas can harbor bacteria making them inaccessible to mechanical instrumentation [8]. Retreatment requires the removal of the original root canal filling, further disinfection, and followed by three-dimensional obturation of the root canal system [9].

Intracanal medicaments (ICM) are sometimes applied between visits to prevent regrowth of the remaining microbiota within the root canal system and also to increase the level of disinfection [10]. ICM in the nano-form can achieve optimal therapeutic activity through their interaction with microorganisms at both the sub-cellular and molecular levels. Because of their small size, they can penetrate well into the complex anatomy of the root canal system [11]. Nanoparticles (NPs) exclusive features include smaller sizes, increased surface area to volume ratio, and higher chemical reactivity and charge density leading to greater interaction with the environment and negatively charged bacterial cells, compared to their bulk counterparts [12–14]. These advantages may be exploited to design highly anti-microbial agents with maximal therapeutic efficacy and minimal side effects [15]. NPs of titanium, gold, zinc, copper, and chitosan have attracted particular attention with different physical properties and spectra of antimicrobial effects [16–19].

Calcium hydroxide NPs (nano-CH) have been synthesized as active catalysts in chemical reactions [20]. Their application as ICM might overcome the limitations of regular calcium hydroxide which are culture reversal [21], limited penetration inside the dentinal tubules [22], and bacterial resistance [23]. However, this is not yet explored clinically.

Determination of bacterial count via plate technique is laborious and tedious, but it represents an important approach for assessment of viable bacterial burdens in microbiologically relevant studies. Investigations of biofilm-related infections in various clinical settings require fast and accurate quantitative assays. Accordingly, different high-throughput assays have been utilized for quantitative determination of bacterial biofilms including crystal violet (CRV) assay, safranine (SAF) assay, ATP bioluminescence (BLM) assay, and resazurin (RES) assay. The CRV technique can estimate bacterial burden as well as microbial matrix of the biofilm [24, 25].

Post-operative pain and flare ups which are occasionally associated with root canal retreatment procedures represent a relevant concern for both patients and clinicians. The origin of post-operative pain is multifactorial and the development is mostly influenced by mechanical, chemical, and microbial factors. These factors are interrelated and directly interdependent, for example, failure of correctly estimating the working length of the root canal is a mechanical factor causing a damaging effect of the apical periodontal tissue. Development of the flare-up after root canal treatment procedures can be also influenced by demographics, general state of health, condition of the pulp and apical periodontal tissue, clinical symptoms, type of tooth which is being treated, number of visits during the treatment, and intracanal medicaments [26, 27].

Therefore, the primary aim of this study was to evaluate the antibacterial effect (as a primary outcome) and the incidence of post-operative pain or flare -ups (as a secondary outcome) after the application of nano-based ICM during endodontic retreatment. The null hypotheses of no significant differences in the anti-biofilm effect and the incidence of post-operative pain and flare-ups were postulated.

Materials and methods

Subjects and methods

This was a randomized, parallel, double-blinded, clinical trial performed using comparative tests. Ethical approval was obtained from the ethical committee of the Faculty of Dentistry, Ain Shams University, Cairo, Egypt (FDASU-REC49392017). This study was designed according to CONSORT guidelines for reporting randomized clinical trials.

Patients signed a printed informed consent form after explanation of the treatment procedure and associated possible adverse effects of the proposed interventions such as pain or swelling at this stage. Recruitment and completion of the operative procedures for the study participants were done by the principal investigator (MMF) at the outpatient clinic of the Endodontic Department, Faculty of Dentistry, Ain Shams University in Egypt, from January 2020–January 2021.

The inclusion criteria of the participants were healthy males and females (Category: American Society of Anesthesiologists class 1) aged 21–49 years, with no physical disability or psychological problems, presenting with single-rooted teeth, with signs and/or symptoms of post-treatment disease manifested by recurrent acute and/or chronic periapical abscess, pain on palpation and/or percussion at least after 1 month of primary treatment, and/or radiographic evidence of bone loss either as a new developing lesion or an increase in the size of a pre-existing one. The exclusion criteria were pregnant women, patients who took antibiotics within the period of 1 month or intake of analgesics within a period of 72 h pre-operatively, or those with known sensitivity to the medicaments or pharmaceuticals used in this trial. Non-restorable teeth, or those with former mishaps as

perforations or separated instruments, root fractures, and/or advanced periodontal involvement were also excluded.

A power analysis was designed to have adequate power to apply a 2-sided statistical test of the null hypotheses. According to the results of Rôças and Siqueira [25] and Relvas et al. [26], using an alpha (α) level of 0.05 (5%) and a Beta (β) level of 0.20 (20%), i.e., power = 80%, the predicted sample size (n) was a total of (39) cases to study the anti-biofilm effect and a total of (69) cases to study the incidence and severity of post-operative pain and flare-ups. Sample size calculation was performed using G*Power version 3.1.9.2.

Participants ($n = 69$) were randomly allocated into 3 groups (23 each) according to the ICM placed between visits using simple randomization procedure using IBM (IBM Corporation, NY, USA.) SPSSV25 statistical analysis software (SPSS, Inc., IBM Company). Random codes were generated and concealed in opaque envelopes until use. The clinical procedure of this study was performed by a single endodontist (MMF), and evaluation also was done by a single microbiologist (WF), both have more than 10 years of clinical experience, and it is considered a double-blinded study as neither the participant nor the evaluator knew the kind of the treatment received by the participant.

Each patient was given a numeric rating scale (NRS), to record the presence and severity of pain using a numerical score from 0 to 10 before treatment, with 0 indicating no pain and 10 reflecting the worst possible pain. This was clarified visually, verbally, and numerically to the patients.

Clinical procedure

The first visit

The treatment plan was discussed with the patients and their acceptance to participate in the study was confirmed. Teeth were anesthetized using Articaine 4% and 1:200,000 Epinephrine (Septanest, Septodont, Saint-Maur-Des-Fosses, France). Following rubber dam isolation (Sanctury, Sanctury health Sdn Bhd, Perak, Malaysia), any defective coronal restoration was removed, and the access cavity preparation completed. Before entering the root canals, teeth were disinfected using 2.5% sodium hypochlorite (Clorox; Nobel Wax Factories for Chemicals, Cairo, Egypt), and then the antimicrobial effect of sodium hypochlorite was inhibited using sodium thiosulphate 5% (El Gomhorya, Cairo, Egypt). Old gutta-percha was removed from the coronal two thirds of the root canal using a Gates Glidden bur #3 (Mani, Tochigi, Japan) without using a solvent. In the presence of sterile saline, patency was obtained using manual K-files #10 and 15 (Mani). The working length (WL) was determined using an electronic apex locator; J Morita Root ZX (Morita, Fushimi-ku, Kyoto, Japan) and confirmed radiographically. The remaining gutta-percha was removed using rotary NiTi files #25 0.06 (Fanta AF Blue S One, Shanghai Dental Materials Co, Shanghai, China), and then gradually increasing sizes of manual K-files were used to clean as much as possible of the apical canal circumference. Scrubbing of the walls using manual files was done to soak the irrigant (sterile saline) with parts of the bacterial film on the canal walls, then a sterile paper point equivalent to the size of largest file reaching the working length was placed in the canal for 1 min to collect the first sample (inoculum) for bacteriological assessment (S1). Cleaning and shaping of the canal were completed using manual K-files and 2.5% sodium hypochlorite. No attempts have been made to activate the irrigant (e.g., passive ultrasonic activation) in order to avoid any confounding effects. The master apical file was determined according to each case where white clean dentine chips were evident at the flutes of the apical 3 mm of the enlarging file, the file apical preparation size was between #50 and #70 in all cases, then the action of sodium hypochlorite was inhibited using 10 ml of 5% sodium thiosulfate, and another sample (inoculum) was obtained in the same way as the previous one (S2). Apical patency was maintained throughout the entire chemo-mechanical debridement.

Then, according to the participant number and the randomization table, the ICM used was identified as either nano-Ag paste (Nanogate, Cairo, Egypt), nano-CH paste (Nanogate), or CH paste (MetaBiomed, Chungcheongbuk-do, Korea) in its regular form. The relevant details of the used nano-materials are: nano-CH: contain CH NPs 27% w/v, size: 80–90 nm, pH 12; nano-Ag: contain Ag NPs 200 ppm, size:8–12 nm, pH 5. The canals were dried with sterile paper points, ICM were slowly injected under magnification and the access cavity was sealed with a sterile cotton pellet and temporary filling (Coltosol F, Coltene/Whaldent, Altstätten, Switzerland) for 1 week.

The presence and severity of post-operative pain were recorded after 6, 12, 24, 48, and 72 h from the first visit. Patients were instructed not to take any pain killers, and those who did were excluded from the study. Flare-up was defined as the development of swelling or the occurrence of severe pain (NRS ≤ 7) after the first visit. The incidence of flare-ups was also recorded.

The second visit

After tooth isolation, the temporary filling was removed and 20 ml of sterile saline were used to remove the ICM until the irrigant got out clear from the canal. Then, sterile paper points were placed in the canal 1 mm from the WL to take the last sample (inoculum) (S3). This was followed by master cone verification and obturation using the warm vertical compaction technique in all groups.

Microbiologic evaluation

Sterile paper points of the three samples (S1, S2, and S3) were placed each in a sterile falcon tube labeled according to the type of ICM used and the order of the sample. Each falcon tube contained 1 ml of tryptic soy broth (TSB) (HiMedia, Mumbai, India) supplemented with 0.5% w/v glucose (TSBG), and all tubes were transferred to the microbiological lab within 2–3 h from sampling procedure in an ice box with refrigerated ice bag in contact with it. All microbiological steps were carried in the vicinity of trilaminar flow cabinet (Telstar Manufacturing, Corporation, Manila, Spain) to avoid air contamination of the samples.

For determination of the total bacterial count, a total of 100 µl from each inoculum were aspirated using a sterile micropipette from each tube. A sterile inoculum spreader was used to evenly distribute the inoculum over the surface of the nutrient agar medium (HiMedia). The plate was then labeled according to the tube of the same sample and incubated in an incubator for 24 h at 37^0C. After incubation, the total numbers of bacterial counts were then estimated.

For determination of the *E. faecalis* count, another 100 µl of each inoculum was aspirated using a sterile micropipette from each tube. A sterile inoculum spreader was used to evenly distribute the inoculum over the surface of the bile esculin azide agar medium (HiMedia). The plate was then labeled as the tube of the same sample and incubated in an incubator for 24 h at 37^0C. After incubation, the total numbers of *E. faecalis* counts were then estimated.

Crystal violet (CRV) assay (24)

Microbial cultures of the collected samples (S1, S2, and S3) were performed in TSBG to be ready for quantitative assessment of its ability to develop biofilm of microbial consortium [18]. Overnight cultures grown in TSBG at 37 °C were diluted in sterile TSBG (MP Biomedicals, LLC) to match 0.5 McFarland turbidity standard (Thermo Scientific Remel™, Kansas, USA) which is equivalent to 1.5×10^6 CFU/ml. These bacterial suspensions were further diluted 1:100 in TSBG to give a final cell count of 1.5 * 10^8 CFU/ml. A total of 200 µl of the prepared cell suspensions were aseptically transferred to each of three parallel wells of a 96-well, non-treated polystyrene microtiter plate (Costar, Corning Inc., New York, USA) followed by incubation at 37 °C for 24 h under static conditions. After incubation, the culture was removed, and plates were carefully rinsed three times with 200 µl of tryptone water (saline with 0.1% Bacto-Tryptone; Difco, Detroit, MI, USA) to remove non-adherent cells. The 96-well microtiter plate was subsequently air dried in an upright position. The established biofilms were stained with 100 µl/well of 0.1% w/v membrane filtered CRV solution (Sigma-Aldrich) at room temperature for 2 min. After biofilm staining, the CRV solution was aspirated and the biofilms were washed twice with 200 µl phosphate buffered saline (PBS). In order to elute the bound CRV, 100 µl of a mixture containing 80% v/v ethanol and 20% v/v acetone were introduced to each of the wells and the plates were incubated for 20 min at room temperature. The CRV eluted mixture was diluted with 80% ethanol–20% acetone mixture to 1:10 v/v. Finally, the optical density (OD) of the elute was determined at λ562 nm [18] using BioTek Microplate Reader (BioTek, Vermont, USA) to assess the biofilm-forming capability of the existing microbiota.

Statistical analyses

Numerical data were explored for normality by checking the data distribution using Kolmogorov–Smirnov and Shapiro–Wilk tests and were presented as mean and standard deviation values. Parametric data for bacterial reduction were analyzed using one-way ANOVA followed by Tukey's post hoc test for intergroup comparisons and one-way ANOVA followed by multiple pairwise t-tests with Bonferroni correction for intragroup comparisons. Ordinal data for pain scores were presented as median and interquartile range values and were analyzed using Kruskal–Wallis test followed by multiple pairwise comparisons using Mann Whitney U test with Bonferroni correction for intergroup comparisons and Friedman's test followed by Wilcoxon signed-rank test with Bonferroni correction for intragroup comparisons. The significance level was set at $p < 0.05$ within all tests. Statistical analysis was performed with IBM SPSS Statistics Version 26 for Windows.

Results

All patients were included in the statistical analysis, and the process of patients' enrollment and progress through each phase of the trial is shown in Fig. 1. On nutrient agar, the first sample from all cases showed bacterial growth ranging from 200 to 11×10^7 colony per ml after incubation for 24 h 37 ^{0}C. This means that 100% of the cases were infected with different bacterial species. While on bile esculin azide agar, only 14 cases out of all samples showed bacterial growth ranging from 100 to 2×10^6 colony per ml after incubation for 24 h at 37 ^{0}C. This means that 20.2% of cases were infected with group D streptococci (Enterococci).

Table 1 shows the inter- and intra-group comparisons between the microbial samples after cleaning and shaping (S1-S2) and after application of the ICM (S3-S2). Reduction in total bacterial count, total *E. faecalis* count, and the biofilm-forming capability of the existing microbiota occurred after cleaning and shaping as well as after application of the ICM. The reduction after cleaning and shaping

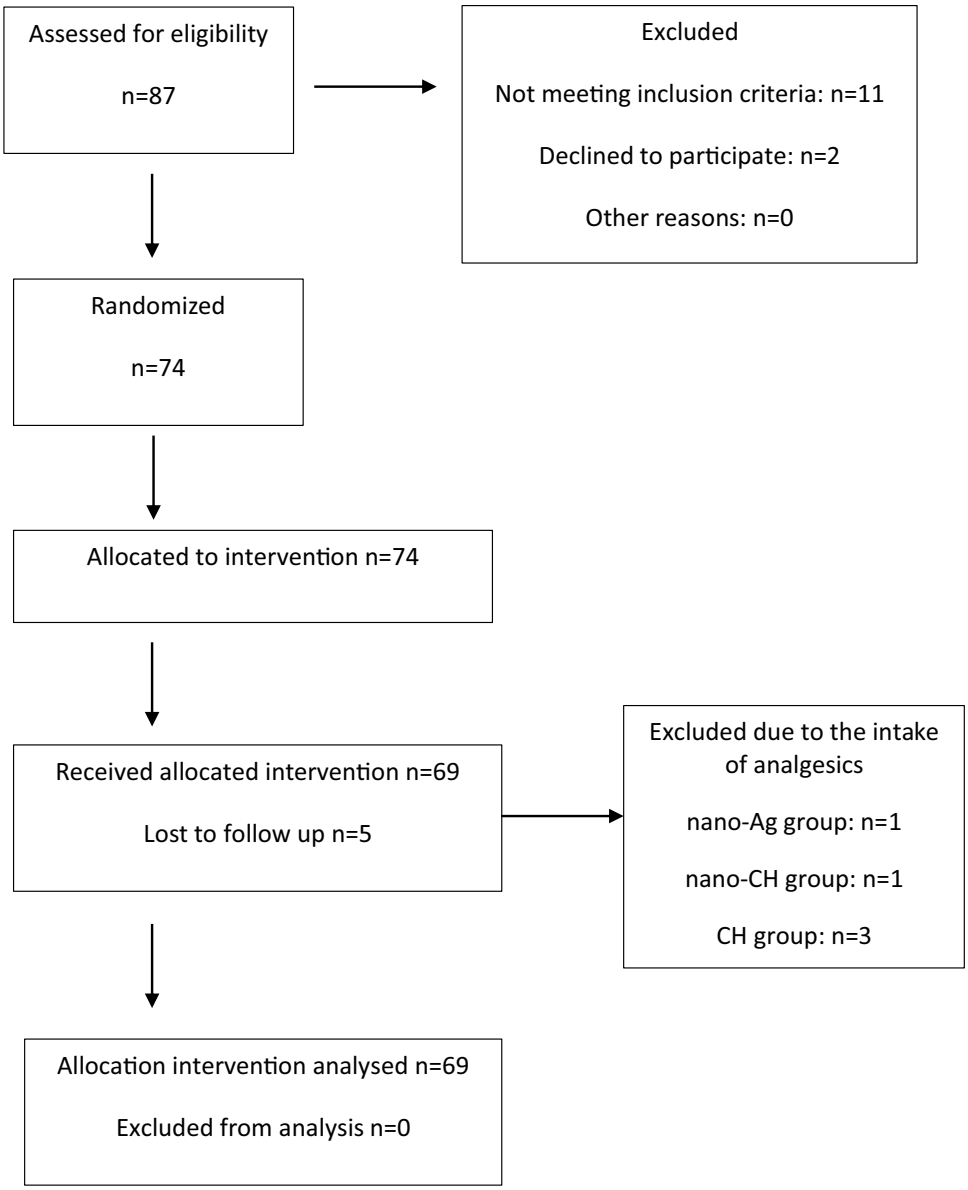

Fig. 1 Flow diagram representing enrollment, allocation, follow-up, and analysis of the patients participating in the clinical trial

was significantly more pronounced ($p < 0.001$) compared to after the effect of ICM application. However, the differences between nano-Ag, nano-CH, and CH as ICM were not statistically significant ($p > 0.05$).

Table 2 shows the median pain scores recorded at all time intervals. Inter- and intra-group comparisons showed that the post-operative pain was significantly reduced at the 48- and 72-h intervals after the application of nano-Ag and nano-CH only ($p < 0.001$). However, the difference between these two ICM was not statistically significant ($p > 0.05$).

The overall incidence of flare-ups was 7.2%. The highest incidence was after application of CH in 3 cases (13%), and only one case after application of either nano-Ag or nano-CH (4.3%). However, the difference between the three ICM was also not statistically significant ($p > 0.05$).

There was a weak negative correlation between degree of pain and total bacterial count ($r_s = -0.322$, $p = 0.046$), but no significant correlation between degree of pain and biofilm forming capability of the existing microbiota ($r_s = –0.215$, $p = 0.188$).

Different upper and lowercase superscript letters indicate a statistically significant difference within the same horizontal row and vertical column respectively; ns; not significant ($p > 0.05$).

Table 1 Inter- and intragroup comparisons of the reduction (%) in bacterial count, *E.faecalis* count, and biofilm forming capability before and after the application of intracanal medicaments (ICM)

Difference	*Biofilm reduction (%) (Mean ± SD)*			*Bacterial count reduction (%) (Mean ± SD)*			*E. faecalis reduction (%) (Mean ± SD)*		
	nano-Ag	nano-CH	CH	nano-Ag	nano-CH	CH	nano-Ag	nano-CH	CH
S1-S2	40.31 ± 21.76^{Aa}	43.78 ± 2.05^{Aa}	49.67 ± 7.48^{Aa}	72.56 ± 23.48^{Aa}	57.41 ± 26.04^{Aa}	75.36 ± 26.22^{Aa}	96.30 ± 6.42^{A}	85.71 ± 20.20^{A}	92.92 ± 11.20^{A}
S2-S3	19.04 ± 13.33^{Ab}	24.32 ± 12.20^{Ab}	19.34 ± 13.95^{Ab}	28.21 ± 12.99^{Ab}	20.28 ± 12.35^{Ab}	48.39 ± 19.16^{Ab}	75.00 ± 5.70^{Ab}	76.00 ± 4.90^{Ab}	81.00 ± 2.44^{Ab}
p value	**< 0.001***	**< 0.001***	**< 0.001***	**< 0.001***	**< 0.001***	**< 0.001***	**< 0.001***	**< 0.001***	**< 0.001***

Table 2 Inter- and intragroup comparisons of pain scores recorded pre-operatively and at the follow-up periods

Follow-up	Pain score [Median (IQR)]			*p* value
	Nano-Ag	*Nano-CH*	*CH*	
Pre-operative	$2.00(1.50)^{Aab}$	$2.70(0.75)^{Aab}$	$2.00(1.00)^{Aab}$	**0.240 ns**
6 h	$4.40(1.70)^{Aa}$	$4.10(1.25)^{Aa}$	$4.00(1.00)^{Aa}$	**0.641 ns**
12 h	$4.00(1.25)^{Aa}$	$4.30(1.50)^{Aa}$	$4.00(2.50)^{Aa}$	**0.567 ns**
24 h	$3.00(1.50)^{Aab}$	$4.00(1.50)^{Aa}$	$3.00(2.50)^{Aab}$	**0.489 ns**
48 h	$0.50(0.25)^{Ab}$	$0.70(0.30)^{Ab}$	$2.70(1.50)^{Bab}$	**0.02***
72 h	$0.00(1.00)^{Ab}$	$0.00(1.00)^{Ab}$	$1.50(0.50)^{Bb}$	**0.04***
p value	**< 0.001***	**< 0.001***	**< 0.001***	

Different upper and lowercase superscript letters indicate a statistically significant difference within the same horizontal row and vertical column, respectively; ns; not significant ($p > 0.05$).

Discussion

Microorganisms harbored within the root canal system are the primary cause of post treatment disease [4]. Therefore, all possible antimicrobial strategies should be implemented during endodontic retreatment. This includes the use of various irrigation regimens and ICM in combination with root canal instrumentation [28]. Nano-based ICM are capable of reducing bacterial biofilm, disrupting biofilm constitution and furthermore, retaining a sustained antibacterial effect [19]. Therefore, this study sought to investigate and compare the antibacterial effect as a primary outcome, and the incidence of post-operative pain or flare-ups as a secondary outcome after the application of nano-based ICM during endodontic retreatment.

This study compared the antibacterial effect of nano-Ag, nano-CH, and CH through several approaches. The culture method was primarily used to evaluate changes in total bacterial count and *E. faecalis* count. Although this method has low sensitivity and specificity compared to molecular techniques [25], yet it is considered to be a useful primary investigation method to rapidly quantify cultivable microorganisms in samples or make a correlation of some bacteria to certain clinical findings, while other molecular techniques can detect uncultivable or difficult-to-grow bacteria or examine more specific effects [28]. The other method used was the CRV assay to quantify the biofilm-forming capability of the microbiota existing before instrumentation, after instrumentation, and after ICM application. This method allows for cultivation and quantification of bacterial biofilms [29]. It has the advantages of being simple and reliable and offers a quick throughout screening of 96 isolates at a time. However, a main disadvantage of this assay is the nonspecific nature in that it does stain the biofilm matrix without distinguishing between live and dead cells [30].

E. faecalis is an extensively evaluated biological indicator. It has been purported that this species is a secondary opportunistic colonizer in treated root canals rather than a persister from unsuccessfully treated primary infections [31]. The role of *E. faecalis* in persistent/secondary infections has been challenged by next-generation sequencing (NGS) reports. Unlike classical studies that reported prevalence values of *E. faecalis* in cases of treated root canals reaching up to 90%, the species was detected in significantly lower frequencies (~30%) by 16S-sequencing in persistent/secondary infection [32]. This agrees with the results of the present study, as *E. faecalis* was detected in only 20% of the S1 samples. However, from an ecological standpoint, every taxon in a mixed consortium is important to detect regardless of its abundance, as any taxa may act as keystone species and potentiate the pathogenicity of the entire community [33].

Results of this study showed a reduction in total bacterial count, total *E. faecalis* count, and the biofilm-forming capability of the existing microbiota after cleaning and shaping as well as after application of the ICM. However, the reduction after cleaning and shaping was significantly more pronounced ($p < 0.001$) compared to after the effect of ICM application. This highlights the fact that cleaning and shaping are major strategies during microbial control of apical periodontitis.

All ICM used in this study showed an antibacterial effect. The antibacterial effect of CH is attributed to the release of hydroxyl ions, which lead to disruption of the bacterial cell wall and its DNA [4]. Several studies have demonstrated that after placement of CH in the root canal system, the hydroxyl ions diffuse through the dentinal tubules to the outer surface of the root [34]. On the basis of these reports, it is possible that CH exerts an immunomodulatory effect by local denaturation of inflammatory mediators, possibly via alkaline hydrolysis of amide bonds [35] or denaturation of some proinflammatory mediators [36]. Additional advantages for nano-CH over conventional CH have been demonstrated in ex vivo studies in terms of increased antimicrobial activity [37], significantly deeper penetration into dentinal tubules [22], better preservation of dentin microhardness [38], and similar cytotoxicity [39].

Silver nanoparticles have several antibacterial effects such as interaction with bacterial DNA sulfhydryl groups, unwinding bacterial DNA, interference with cell-wall synthesis/cell division, and production of reactive oxygen species [14]. These collective effects account for their broad-spectrum bactericidal activity and the rare existence of bacterial resistance to it [40]. Moreover, the positive charge on the silver nanoparticles interacts electrostatically with the negatively charged bacterial cells and can bind to the negatively charged dentin, thus inhibiting bacterial adherence [41].

Differences between ICM tested in the present study were not statistically significant ($p > 0.05$). This finding disagrees with that of Louwakul et al. [42] who showed, by using a different methodology, namely, CLSM of dentin blocks, a significantly more pronounced elimination of E. faecalis in the deeper parts of dentin by nano-CH over CH and calcium oxide. Also, Wu et al. [41] demonstrated that the application of a 0.02% nano-Ag gel as an ICM resulted in a significant disruption of *E. faecalis* biofilm compared with CH. A possible explanation for these differences compared to the present study could be related to the vehicle of nano-Ag (gel vs paste) that might affect the nanoparticulate size and consequently the biological actions of the material.

Despite the effectiveness of nano-Ag in root canal disinfection, clinical concerns for discoloration and cytotoxicity have been raised, especially after its long-term application as an ICM [43]. Tooth discolorations were not observed in the present study, probably because the ICM was placed for only 1 week. However, Afkhami et al. [44] demonstrated that a mixture of nano-Ag and CH did not cause any significant change in tooth color compared with the application of CH alone when used as ICM. Also, another study confirmed that the cytotoxicity of nano-Ag is concentration and size dependent [45].

The design of this study did not include a negative control group (canals left empty) as it was reported that bacteria in instrumented, unfilled canals can multiply and reach their pretreatment numbers in 2 to 4 days [46].

The NRS was used as an outcome measure to evaluate pain intensity. It was chosen due to its simplicity and ease of communication with the patients, as it is easier to use than a visual analogue scale and more sensitive than a verbal rating scale [47]. Pain assessment continued up to 3 days in this study in order to assess the incidence of flare-up. In this study, the post-operative pain was significantly reduced at the 48- and 72-h intervals after the application of nano-Ag and nano-CH only ($p < 0.001$). This can be attributed to the higher diffusivity and reactivity provided by the nanoparticles that result in faster inhibition of microorganisms and their virulence factors, which are responsible for the initiation, progression, and persistence of the inflammatory reactions in the periapical tissues.

It has been demonstrated that even when the highest standards of treatment are followed, flare-up can still occur, but with no negative effect on treatment outcome [48]. The overall incidence of flare-ups in this study was 7.2%, with the highest incidence was after application of CH in 3 cases. All of these patients were females above 40 years of age. This finding agrees that of Azim et al. [49].

In this study, a weak correlation was found between the degree of postoperative pain and intracanal total bacterial count. This corroborates the findings of Emara et al. [50]

and could be attributed to the low sensitivity and specificity of the culturing technique, which is a limitation of this study and requires further investigations to examine the correlation of both outcomes. Also, the absence of long-term clinical and radiographic follow-up is another limitation of this trial. Regarding future investigations, it would be of interest to correlate culture results with periapical healing in the long-term observation.

In conclusion, within the limitations of this study, both the chemo-mechanical debridement of the root canals and the application of ICM resulted in a reduction of the total bacterial count, total *E. faecalis* count, and the biofilm-forming capability of the existing microbiota. However, chemo-mechanical debridement was more effective than the application of ICM. The application of nano-Ag and nano-CH resulted in significantly reduced post-operative pain at the 48- and 72-h intervals. Further clinical trials are warranted to assess the impact of nanoparticles on post-endodontic pain.

Declarations

Ethical approval Ethical approval was obtained from the research ethics committee of the faculty of dentistry, Ain Shams University. All procedures were in full accordance with ethical principles including the world medical association declaration of Helsinki and the additional requirements dictated in Egypt.

Informed consent Informed consent has been obtained from participants or their legal guardians prior to enrolment in the study.

Conflict of interest Mahmoud Mohamed Fahim declares that he has no conflict of interest. Shehabeldin Mohamed Saber declares that he has no conflict of interest. Walid Faisal ElKhatib declares that he has no conflict of interest. Mohamed Mokhtar Nagy declares that he has no conflict of interest. Edgar Schäfer declares that he has no conflict of interest.

References

1. Rocas IN, Siqueira JF Jr (2012) Characterization of microbiota of root canal-treated teeth with posttreatment disease. J Clin Microbiol 50:1721–1724
2. Antunes HS, Rocas IN, Alves FR, Siqueira JF Jr (2015) Total and specific bacterial levels in the apical root canal system of teeth with post-treatment apical periodontitis. J Endod 41:1037–1042
3. Rodrigues RC, Antunes HS, Neves MA et al (2015) Infection control in retreatment cases: in vivo antibacterial effects of 2 instrumentation systems. J Endod 41:1600–1605
4. Sjogren U, Figdor D, Persson S, Sundqvist G (1997) Influence of infection at the time of root filling on the outcome of endodontic treatment of teeth with apical periodontitis. Int Endod J 30:297–306
5. Munson MA, Pitt-Ford T, Chong B et al (2002) Molecular and culture analysis of the microflora associated with endodontic infections. J Dent Res 81:761–766
6. Hall-stoodley L, Costerton JW, Stoodley P (2004) Bacterial biofilms: from the natural environment to infection diseases. Nat Rev Microbiol 2:95–108
7. Jhajharia K, Parolia A, Shetty V et al (2015) Biofilm in endodontics: A review. J Int Soc Prev Comm Dent 5:1–12
8. Vera J, Siqueira JF, Rucicci D et al (2012) One versus two visit endodontic treatment of teeth with apical periodontitis: a histobacteriologic study. J Endod 38:1040–1052
9. Ørstavik D (2003) Root canal disinfection: a review of concepts and recent developments. Aust Endod J 29:70–74
10. Siqueira JF, Paiva SS, Rocas IN (2007) Reduction in cultivable bacterial populations in infected root canals by chlorhexidine based antimicrobial protocol. J Endod 33:541–547
11. Sireesha A, Jayasree R, Vidhya S et al (2017) Comparative evaluation of micron- and nano-sized intracanal medicaments on penetration and fracture resistance of root dentin - an in vitro study. Int J Biol Macromol 104:1866–1873
12. Kishen A, Shi Z, Shrestha A, Neoh KG (2008) An investigation on the antibacterial and antibiofilm efficacy of cationic nanoparticulates for root canal disinfection. J Endod 34:1515–1520
13. Barros J, Silva MG, Rocas IN et al (2014) Antibiofilm effects of endodontic sealers containing quaternary ammonium polyethylenimine nanoparticles. J Endod 40:1167–1171
14. Shrestha A, Kishen A (2016) Antibacterial nanoparticles in endodontics: A systemic review. J Endod 42:1417–1426
15. Siqueira JF, Rocas IN, Favieri A et al (2002) Incidence of postoperative pain after intracanal procedures based on an antimicrobial strategy. J Endod 28:457–460
16. Venugopal J, Prabhakaran MP, Low S et al (2008) Nanotechnology for nanomedicine and delivery of drugs. Curr Pharm Des 14:2184–2200
17. Mohammadi Z, Jafarzadeh H, Shalvi S, Palazzi F (2017) Recent advances in root canal disinfection: A review. Iran End J 12:402–406
18. Barreras US, Méndez FT, Martínez RE, Valencia CS, Rodríguez PR, Rodríguez JP (2016) Chitosan nanoparticles enhance the antibacterial activity of chlorhexidine in collagen membranes used for periapical guided tissue regeneration. Mater Sci Eng C Mater Biol Appl 58:1182–1187
19. Seil JT, Webster TJ (2012) Antimicrobial applications of nanotechonology: methods and literature. Int J Nanomedicine 7:2767–2781
20. Heidar S, Saber SE, Eissa S, El-Ashry S (2020) Antibacterial potential of nano-particulate intracanal medications on a mature E. faecalis biofilm in an ex vivo model. Italian Endod J 34:100–109
21. Astruc D (2008) Transition-metal nanoparticles in catalysis: from historical background to the state-of-the art. In: Astruc D (ed) Nanoparticles and catalysis, 1st edn. Wiley, Germany, pp 1–48
22. Waltimo T, Trope M, Haapasalo M, Ørstavik D (2005) Clinical efficacy of treatment procedures in endodontic infection control and one-year follow-up of periapical healing. J Endod 31:863–866
23. Zand V, Mokhtari H, Hasani A, Jabbari G (2017) Comparison of the penetration depth of conventional and nano-particle calcium hydroxide into dentinal tubules. Iran Endod J 12:366–370
24. Haapasalo HK, Sirén EK, Waltimo TM, Ørstavik D, Haapasalo MP (2000) Inactivation of local root canal medicaments by dentine: an in vitro study. Int Endod J 33:126–131
25. Elkhatib W, Khairalla A, Ashour H (2014) Evaluation of different microtiter plate-based methods for the quantitative assessment of *Staphylococcus aureus* biofilms. Fut Microbiol 9:725–735

26. Rocas IN, Siqueira JF Jr (2011) In vivo antimicrobial effects of endodontic treatment procedures as assessed by molecular microbiologic techniques. J Endod 37:304–310
27. Relvas JB, Bastos MM, Marques AA, Garrido AD, Sponchiado EC Jr (2016) Assessment of postoperative pain after reciprocating or rotary NiTi instrumentation of root canals: a randomized, controlled clinical trial. Clin Oral Investig 20:1987–1993
28. Sipavičiūtė E, Manelienė R (2014) Pain and flare-up after endodontic treatment procedures. Stomatol Balt Dent Maxillofac J 16:25–30
29. Saber S, El-Hady SA (2012) Development of an intracanal mature Enterococcus faecalis biofilm and its susceptibility to some antimicrobial intracanal medications; an in vitro study. Eur J Dent 6:43–50
30. Stepanović, Srdjan, et al (2007) Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 115:891–899
31. Wilson C, Lukowicz R, Merchant S et al (2017) Quantitative and qualitative assessment methods for biofilm growth: A mini-review. Res Rev J Technol 6:1–25
32. Zoletti GO, Siqueira JF Jr, Santos KR (2006) Identification of *Enterococcus faecalis* in root-filled teeth with or without periradicular lesions by culture-dependent and-independent approaches. J Endod 32:722–726
33. Siqueira JF Jr, Antunes HS, Rocas IN, Rachid CT, Alves FR (2016) Microbiome in the apical root canal system of teeth with post-treatment apical periodontitis. PLoS ONE 11:e0162887
34. Hajishengallis G, Lamont RJ (2016) Dancing with the stars: how choreographed bacterial interactions dictate nososymbiocity and give rise to keystone pathogens, accessory pathogens, and pathobionts. Trends Microbiol 24:477–489
35. Nerwich A, Figdor D, Messer HH (1993) PH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. J Endod 19:302–306
36. Voel D, Voel JG, Pratt CW (1999). Fundamentals of biochemistry.1st ed. Hoboken, NJ: John Wiley & Sons, 93–123.
37. Khan AA, Sun X, Hargreaves KM (2008) Effect of calcium hydroxide on proinflammatory cytokines and neuropeptides. J Endod 34:1360–1363
38. Dianat O, Saedi S, Kazem M, Alam M (2015) Antimicrobial activity of nanoparticle calcium hydroxide against enterococcus faecalis: an in vitro study. Iran Endod J 10:39–43
39. Naseri M, Eftekhar L, Gholami F et al (2019) The effect of calcium hydroxide and nano–calcium hydroxide on microhardness and superficial chemical structure of root canal dentin: an ex vivo study. J Endod 45:1148–1154
40. Dianat O, Azadnia S, Mozayeni MA (2015) Toxicity of calcium hydroxide nanoparticles on murine fibroblast cell line. Iran Endod J 10:49–54
41. Shrestha A, Zhilong S, Gee NK, Kishen A (2010) Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod 36:1030–1035
42. Wu D, Fan W, Kishen A, Gutmann JL, Fan B (2014) Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod 40:285–290
43. Louwakul P, Saelo A, Khemaleelakul S (2016) Efficacy of calcium oxide and calcium hydroxide nanoparticles on the elimination of *Enterococcus faecalis* in human root dentin. Clin Oral Investig 21:865–871
44. Balto H, Bukhary S, Al-Omran O, BaHammam A, Al-Mutairi B (2020) Combined effect of a mixture of silver nanoparticles and calcium hydroxide against *Enterococcus faecalis* biofilm. J Endod 46:1689–1694
45. Afkhami F, Elahy S, Mahmoudi A (2017) Spectrophotometric analysis of crown discoloration following the use of silver nanoparticles combined with calcium hydroxide as intracanal medicament. J Clin Exp Dent 9:e842–e847
46. Takamiya AS, Monteiro DR, Bernabe DG et al (2016) In vitro and in vivo toxicity evaluation of colloidal silver nanoparticles used in endodontic treatments. J Endod 42:953–960
47. Bystrom A, Sundqvist G (1981) Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Scand J Dent Res 89:321–328
48. Sjogren U, Hagglund B, Sundqvist G, Wing K (1990) Factors affecting the long-term results of endodontic treatment. J Endod 16:498–504
49. Azim AA, Azim KA, Abbott PV (2017) Prevalence of interappointment endodontic flare-ups and host-related factors. Clin Oral Investig 21:889–894
50. Emara RS, Gawdat SI, El-Far HMM (2021) Effect of XP-endo Shaper versus conventional rotary files on postoperative pain and bacterial reduction in oval canals with necrotic pulps: a randomized clinical study. Int Endod J (in press). https://doi.org/10.1111/iej.13494

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