EGYPTIAN DENTAL JOURNAL

VOL. 68, 1789:1796, APRIL, 2022

PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360

CONSERVATIVE DENTISTRY AND ENDODONTICS

Submit Date : 2021-12-13 • Accept Date : 2021-12-30 • Available online: 2022-03-30 • DOI : 10.21608/edj.2021.110832.1906

THE ANTIMICROBIAL EFFICACY OF NANOPARTICLES INTRACANAL MEDICAMENTS AGAINST ENTEROCOCCUS FAECALIS BIOFILM

Rami Zeinelabdeen M. Elkillany *, Salma El-Ashry**, Nihal Sabet*** *and* Mohamed Fakhr****

ABSTRACT

Title of manuscript: The Antimicrobial Efficacy of Nanoparticles Intracanal Medicaments Against *Enterococcus Faecalis* Biofilm.

Introduction: Bacteria can create biofilms that resist antimicrobials and cause persistent infections. With the introduction of nanotechnology in dentistry, intracanal medicaments could become more effective against bacteria. The purpose of this study was to compare the efficacy of normal sized calcium hydroxide ($CaOH_2$) and chlorohexidine (CHX) against their nano counterparts in the elimination of *Enterococcus faecalis* biofilm.

Methods: Sixty-six human mandibular molars were contaminated with *E. faecalis* then treated with $CaOH_2$, $CaOH_2$ nanoparticles (np), CHX, CHX loaded by silver nanoparticles (Ag-np) and CHX loaded by chitosan nanoparticles (CH-np). Two periods of contact time were chosen; two-days and seven-days. Comparison was based on colony forming units (CFU) and Scanning electron microscope (SEM) was used for biofilm confirmation and examining the effect of the medicaments on bacterial cells and biofilm.

Results: CHX\Ag-np had the highest percentage reduction with a significant difference of <0.05. CHX and $CaOH_2$ had the lowest percentage reduction. Seven-days period showed more bacterial reduction than the two-days period in all groups.

Conclusion: All tested medicaments reduced bacterial counts. Nanosized medicaments were more effective than normal sized medicaments. Seven-days period is better than the two-days period regarding the antibacterial efficacy.

KEY WORDS: Calcium hydroxide, chlorohexidine, Enterococcus faecalis, Intracanal medicaments, Nanotechnology.

* Msc Student, Endodontics, Dentistrty, Misr International University
** Professor, Faculty of Oral and Dental Medicine, Department of Endodontics, Ain Shams Univerisity
*** Professor, Endodontics, Dentistrty, Cairo University
**** Assistant Professor, Endodontics, Dentistry, Misr International University

INTRODUCTION

The treatment of infected canals is much more challenging than non-infected ones [1]. Bacteria in such cases can escape cleaning and shaping into dentinal tubules, reaching as deep as 300 microns [2]. It can also create biofilms which make them more immune to chemo mechanical preparation and are much more resistant when protected in biofilms than in the planktonic state [2,3].

E. Faecalis is a dominant type of bacteria that appears inside root canals [5]. They have the ability to penetrate deep inside the dentinal tubules [2]. They can create biofilms and have resistance to high alkalinity and can become dormant for long periods of time until nutrition is available again [1].

The most readily used medicament in root canals is calcium hydroxide ($CaOH_2$). It releases hydroxyl ions which provides a high alkaline medium, it has tissue dissolving capabilities and acts as a physical barrier [6]. Chlorohexidine (CHX) is another readily used intracanal medicament. It has an antibacterial effect plus high substantivity that leads to a prolonged action [7]. $CaOH_2$ and CHX intra canal medicaments however aren't able to eradicate bacteria completely from root canals [8].

Recently nanotechnology was found to improve the antibacterial efficacy of intracanal medicaments. Nano-sized particles can increase the contact surface area of medicaments to bacterial biofilms and increase the pH of $CaOH_2$. A smaller size means better penetration inside the bacterial cells and an easier delivery to contaminated areas [9]. Nanotechnology paved the way for the use of chitosan (CH) which has an excellent antiviral, antifungal and antibacterial properties [10]. It also allowed the use of silver (Ag) nanoparticles which are known for their ability to destabilize the bacterial cell membrane and increase its permeability which leads to bacterial death [11]. Hence this study was designed to evaluate the antimicrobial efficiency of nanosized intracanal medicaments against the *E.faecalis* biofilm.

MATERIALS AND METHODS

Teeth preparation

Sixty-six extracted mandibular molar teeth were cleaned and stored in sterile saline. Six teeth were randomly assigned as a positive control group. Three of these teeth were used to obtain the average colony forming units (CFU) after three weeks incubation (initial colony number) [12] and the other three teeth were used for biofilm confirmation using the scanning electron microscope (SEM) [13]. The remainder sixty teeth were randomly divided into five groups, group A, B, C, D and E, each group was comprised of twelve teeth. Then each group was further subdivided into two subgroups (subgroup I and II) according to the bacterial contact time which was two- and seven-days period [14]. Crowns were flattened with a diamond disc to a standardized length of 15 mm [15]. Canals were negotiated by K-file #10. Working length was calculated and verified by periapical radiograph. Edge files (EdgeEndo Albuquerque, NM, USA) rotary files were used for cleaning and shaping in a crown down technique using E-connect endo motor (Changzhou Sifary Medical Technology Co., Ltd, China) in torque 2.5 and speed 450 rpm according to the manufacturer instructions. Starting with the orifice opener size 17 taper 4% to enlarge the coronal third of the canal then rotary files size 20 taper 4% followed by size 25 taper 6% to reach an apical preparation of size 25 with taper 6%. Then K-file size 30,35 and 40 were used for final apical preparation. Between each file 2 ml of 2.5% NaOCl solution was used to irrigate the canals with a size 30- gauge needle in a plastic syringe for 30 seconds. Then final irrigation using 3 ml of 17% EDTA was done for 30 seconds, followed by 5 ml of NaOCl for one minute to remove the smear layer. Paper points size 40 taper 4% was then used to dry the canals. After completion of the Chemo mechanical preparation, the roots were autoclaved at 121°C for 30 minutes to ensure the absence of any microorganisms. Two layers of nail polish were used to seal the root external surface [15].

Bacterial growth and infection of samples

*E. faecalis (*ATTCC19433) was incubated in a brain and heart infusion (BHI) broth culture plate. After discharging sterile broth, this bacterial suspension was used to infect the teeth using sterile syringes. Then the teeth were incubated for three weeks at 37˚C in sealed vials. Every three days of these three weeks fresh bacterial suspension was used to remove the bacterial byproducts [15]. Two positive control group teeth were used after the three weeks to identify the mean CFU of E. faecalis which was 3 x 10^7 CFU/ml. one tooth was scanned at magnification 2000x using scanning electron microscope to confirm biofilm formation (figure 1).

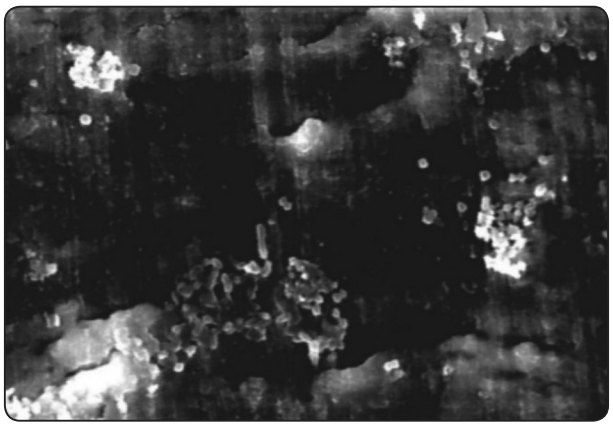

Fig. (1): Scanning Electron micrograph showing biofilm formation of *E. faecalis* (magnification 2000x)

Application of medicaments

All medicaments used were in gel form (nano gate, Cairo, Egypt). Medicaments were $CaOH_2$, nano\$CaOH_2$, CHX, CHX\Ag-np and CHX\ CH-np which were assigned to groups A,B,C,D and E respectively. Teeth were injected with intracanal medicaments in gel form using a plastic syringe with 30'' gauge needle until the canal was filled. After applying the intracanal medicaments, the orifice was sealed with sterile cotton. Then Nucavfil temporary restoration (PSP dental Co. Ltd, Belvedere, Kent. UK) was used to seal the teeth and all samples were incubated at 37°c under anaerobic conditions. Each group was subdivided equally into 2 subgroups. Subgroup I (n=6) was evaluated after two days. Subgroup II (n=6) was evaluated after seven days for CFU. A sterile k-file size 15 was used to circumferentially abrade the canal walls. Debris were then collected in a physiologic saline using a sterile paper point, then applied to an agar plate and CFU was then counted [15].

Data analysis

Numerical data were explored for normality by checking the distribution of data and using tests of normality (Kolmogorov-Smirnov and Shapiro-Wilk tests). Logarithmic transformation of bacterial count data was performed due to the high range of bacterial counts. All data showed normal (parametric) distribution. Data were presented as mean and standard deviation (SD) values. Repeated measures ANOVA test was used to compare between the groups as well as to study the changes by time within each group. One-way ANOVA test was used to compare the percentage reduction in bacterial counts between different groups. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 23.0.

RESULTS

Data collected for E. faecalis percentage reduction by the different examined medicaments were tabulated and statically analyzed in table (1) and figure (2).

A) Effect of intracanal medicaments on percentage reduction in bacterial CFU count:

After two-days the maximum mean value of bacterial percentage reduction was presented by CHX-Agnp followed by CHX-Chnp, $CaOH_2$-np, $CaOH_2$ then finally CHX, with mean and standard deviation values 99.997 ± 0.008, 99.983 ± 0.381, 99.973 ± 0.008, 99.87 ± 0.005, 98.333 ± 0.005 respectively. Using ANOVA (two-way) test a

significant difference was observed between the groups with P-value <0.001 (Table 1).

After seven-days the maximum mean value of bacterial percentage reduction was presented by CHX-Agnp, CaOH2-np and CHX-Chnp followed by CHX then finally CaOH2. With mean and standard deviation values 100 ± 0, 100 ± 0, 100 ±0, 99.984 ± 0.005, 99.933 ± 0.004 respectively. Using ANOVA (two-way) test a significant difference was observed between the groups with P-value <0.001 (Table 1).

Table (1): Descriptive statistics and results of one-way ANOVA test for comparison between percentage reductions in bacterial counts in the five groups

Group	2 days		7 days	
	Mean	SD	Mean	SD
A	99.872 ^{A}	0.055	99.933 ^{C}	0.004
B	99.972 ^{A}	0.008	100 ^{A}	0
C	98.333 ^{B}	0.005	99.984 ^{B}	0.005
D	99.997 ^{A}	0.008	100^{A}	0
E	99.983 ^{A}	0.381	100^{A}	0
P-value	<0.001*		<0.001*	
Effect size (Eta Squared)	0.937		0.837	

: Significant at P ≤ 0.05, Different superscripts in the same column are statistically significantly different.

B) Effect of contact time of each intracanal medicament on CFU:

With increase in contact time up to seven-days bacterial CFU count decreased in all groups.

In group A CFU bacterial count was reduced from 4.55 ± 0.19 to 3.34 ± 0.23. In group B CFU bacterial count was reduced from 3.91 ± 0.11 to 1.48 ± 0.28. In group C CFU bacterial count was reduced from 5.65 ± 0.12 to 3.65 ± 0.12. In group D CFU bacterial count was reduced from 3.06 ± 0.25 to 1.09 ± 0.2. In group E CFU bacterial count was reduced from 3.64 ± 0.17 to 1.67 ± 0.13.

By applying repeated measures ANOVA test there was a significant decrease in bacterial CFU count observed in all groups with P.value <0.001 (Figure 2).

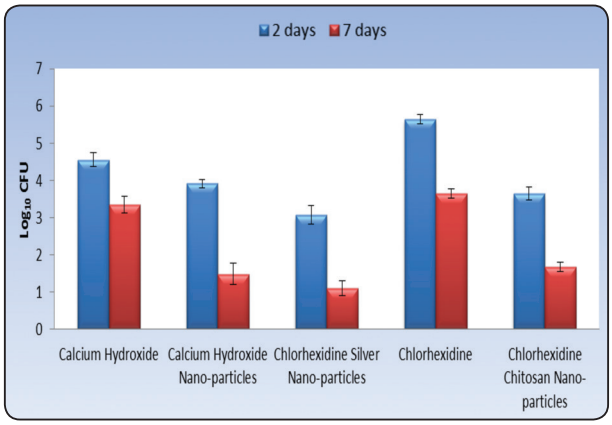

Fig. (2). Bar chart representing mean and standard deviation values for Log_{10} CFU of bacterial counts after two and seven days within each group.

DISCUSSION

Once the pulp becomes necrotic the tooth becomes devoid of blood supply which would mean that neither immune cells nor antibodies can reach deep inside the canal [16]. Bacteria starts to colonize the pulpal space creating a biofilm on the dentin surface. Biofilm is a structure that is dynamic and its main benefits for bacteria is increasing the bacterial resistance against antimicrobials and it acts as a storage to supply the bacteria with nutrition in case of starvation. It also provides cellular communication, protects against environmental stress and increases adhesion plus cohesion capabilities [17]. Eventually bacteria reach the apex and invades the periapical tissues causing acute or chronic apical periodontitis. In this case a root canal treatment is done to eliminate, entomb and overcome bacterial infections. So, the main goal in root canal treatment is to eliminate the microorganisms from the canals, prevent infection and reinfection through

chemo mechanical treatment of the canal. Irrigants are used during chemo mechanical preparation to increase efficiency of bacterial elimination [18]. However, optimum root canal disinfection is not easy to achieve. Some examples that can make such a goal hard to achieve are the complex canals anatomy, persistent and long-standing infections that gives time for the bacteria to penetrate deep inside dentinal tubules reaching as deep as 300 microns [16,18]. Bacterial species found in failed endodontic cases that need retreatment are different than the bacterial species found in cases that have not been treated before. *E.faecalis* was found to be predominant in most failed endodontically treated cases [19]. For this reason, different approaches are used to eradicate such bacterial species especially in retreatment cases. One of the main approaches is the use of intracanal medicaments in order to overcome the highly resistant and massive bacterial loads within the canals [20]. Intracanal medicaments are applied inside the canal and left for a specific period of time to reduce the bacterial load. Historically, materials that were used in the past were based on the release of vapors. These materials are not advocated to be used nowadays for their several side effects. The intracanal medicaments that are readily used are calcium hydroxide, chlorohexidine and antibiotic pastes.

In the present study, a comparison was done between intracanal medicaments and their nano counterparts regarding their antibacterial effect on the biofilm of E. faecalis. E. faecalis can create biofilms after 24 hours [21], but for a more resistant and mature biofilm, three weeks is sufficient. Yang etal found, that mature biofilms are more resistant to disinfections compared to young biofilms [22]. Thus, the incubation period used to grow the *E. faecalis* biofilm in this study was three weeks in order to make sure that a mature biofilm was present.

All groups except the positive control group were divided into two subgroups based on the duration of intracanal medicament application. The duration was two and seven days [14]. A seven-day period is the most commonly used period for intracanal medicament application [14]. In the current literature some researchers advocated a short contact time for intracanal medicaments [14,20] while others advocated a longer time. Due to this discrepancy in intracanal medicament application contact time, two different times ; two and seven days were used in this study.

Calcium hydroxide ($CaOH_2$) paste is a widely used intracanal medicament. It was found that $CaOH_2$ was effective against *E. faecalis* through acting as a physical barrier and through its hydroxyl ions which causes DNA and protein denaturation [23,24]. Since *E. faecalis* is highly resistant to alkaline medium, $CaOH_2$ has less efficacy on such bacteria [25]. Previous studies showed a limited antibacterial efficacy of normal sized $CaOH_2$ as an intracanal medicament against *E.faecalis* [8,25,26]. Chlorohexidine is used as an intracanal medicament and irrigation [7]. CHX as an intracanal medicament showed a limited antibacterial efficacy in failed root canal treatment [27]. Thus, a need for the development of a new intracanal medicament or a modification of $CaOH_2$ and CHX to make them more effective is needed.

Nano technology was introduced in dentistry in many fields. In endodontics it was used in sealers, medicaments and irrigation materials. The idea of converting normal medicament particles to nano sized particles was to increase the density of the materials and increase their ability to penetrate deep inside the dentinal tubules for a better antibacterial efficacy [9]. Calcium hydroxide can be converted into nano particles, but chlorohexidine cannot. In order for chlorohexidine to become nano sized, it is loaded with other nanosized materials like silver and chitosan nanoparticles [28]. Both chitosan and silver have a profound antibacterial effect, which leads to a doubling effect regarding the antibacterial efficacy when they are added to another medicament [10]. The recommended concentration of silver nanoparticles is 50 ppm [29]. All materials used in this study were made in the gel form for standardization and easier application and removal.

Silver nanoparticles presented a high antibacterial action especially in the one-week duration [12]. Their antibacterial action is based on the release of cationic silver which has an oxidative potential [30]. Silver nanoparticles can inhibit gram negative bacteria more than gram positive bacteria [31]. If applied in higher concentrations it would lead to higher antibacterial action and prevent biofilm formation[28,32].

Chitosan nanoparticles are antibacterial and are effective especially against planktonic one [10]. It can prevent bacterial adhesion through chelating calcium from the smear layer, tooth structure and biofilm [33] which means that the addition of chitosan nanoparticles to other antibacterial products would inhibit bacterial adhesion leading to less biofilm formation [10]. Therefore, chitosan nanoparticles are effective against planktonic and biofilm bacterial phenotypes [10]. Chitosan nanoparticles were effective in eliminating bacteria in short and long contact times of application [28]. However, it was found that the toxicity of chitosan nanoparticles increased with time and that its antibacterial efficacy is affected by the vehicle transporting it [34].

The antibacterial effect of the examined intracanal medicaments were evaluated through Colony forming units (CFU) and the Scanning electron microscope (SEM). CFU is the most convenient and standard method that can be used to properly identify and compare between the antibacterial efficacies of different materials through comparing the number of colonies before and after the test [12]. CFU is based on counting the number of colonies formed by the growth of the bacteria which shows the estimation of the concentration of viable bacteria in a test sample. The number of visible colonies present on an agar plate were multiplied by the dilution factor to provide a CFU/ml result [35]. This was the best method for sample collection according to the literature to ensure sufficient collection of bacteria present on all dentin surfaces in addition the physiologic saline has no antibacterial properties that might interfere with the results [12,15]. The SEM was used to confirm biofilm formation and to examine the effect of intracanal medicaments in bacterial cells and biofilms. The SEM was especially chosen because it can identify fine details and can provide information about the size and shape of different structures like dentin surface, bacterial cells and biofilms [13].

In this study, the nano sized intracanal medicaments presented a higher antibacterial efficacy compared to the normal sized medicament. This is attributed to the particle size which improved the ability of the medicaments to penetrate deep inside the dentinal tubules. Also, the increased cytotoxicity and antiadhesive properties of the nano medicaments against the various bacteria is another reason for that higher efficacy [29]. Chlorohexidine silver nanoparticles had the highest efficacy in both periods which is due to the dual antibacterial effect of silver nanoparticles and chlorohexidine. [28,29] Calcium hydroxide and Chlorohexidine showed a lower efficacy compared to the nanosized groups and this is due to the lower penetration and cytotoxicity of both intracanal medicaments [10]. Some studies showed that regular sized intracanal medicaments had the same antibacterial efficacy compared to the nanosized medicaments. However this can be attributed to the lower bacterial contact time; thirty and sixty seconds [36]. Other studies used *E.faecalis* incubated for a shorter duration of only forty-eight hours [37] Also copper nanoparticles don't have the same high antibacterial efficacy as the nano particles used in the present study [38].

When examining the effect of contact time on the antibacterial efficacy of the intracanal medicaments, higher reduction in microbial loads was observed in the seven-day period. This is attributed due to the higher contact time between the medicament and bacteria leading to increased cytotoxicity and a greater eradication of *E.faecalis* [10,12,34]. However, this was against some studies that showed no difference in the increased contact time and this is due to the fact that they have used other types of microorganisms like *Candida albicans*, *Prophyromonas gingivalis* and *Prevotella intermedia* [39,40].

Within the limitation of this study it can be concluded that; Nanoparticles enhanced the effectiveness of the used intracanal medicaments Longer contact time succeeded to boost the antimicrobial efficacy of intracanal medicaments In both contact times the chlorohexidine silver nanoparticles showed the highest antibacterial efficacy. However, in the normal sized medicaments, chlorohexidine was more efficient compared to the calcium hydroxide.

REFERENCES

1. Ørstavik D. Virulence Factors of E Nterococcus Faecalis: Crit Rev Oral Biol Med. 2004;15(5):308–20.
2. George S, Kishen A, Song KP. The role of environmental changes on monospecies biofilm formation on root canal wall by Enterococcus faecalis. J Endod. 2005; 31(12):867–72.
3. Bjørndal L, Darvann T, Thylstrup A. A Quantitative Light Microscopic Study of the Odontoblast and Subodontoblastic Reactions to Active and Arrested Enamel Caries without Cavitation. Caries Res. 1998;32(1):59–69.
4. Rocas I, Siquera J, Santos K. Association of Enterococcus faecalis with different forms of periradicular. J Endod. 2004;30(19):315–20.
5. Love RM. 2001IEJ - E faeculis.pdf. 2001; 399–405.
6. Hiraishi N, Yiu CKY, King NM, Tagami J, Tay FR. Antimicrobial efficacy of 3.8% silver diamine fluoride and its effect on root dentin. J Endod [Internet]. 2010; 36(6): 1026–9. Available from: http://dx.doi.org/10.1016/j.joen.2010.02.029
7. Barbosa CAM, Gonçalves RB, Siqueira JF, De Uzeda M. Evaluation of the antibacterial activities of calcium hydroxide, chlorhexidine, and camphorated paramonochlorophenol as intracanal medicament. A clinical and laboratory study. J Endod. 1997;23(5):297–300.
8. Lima RKP, Guerreiro-Tanomaru JM, Faria-Júnior NB, Tanomaru-Filho M. Effectiveness of calcium hydroxide-based intracanal medicaments against Enterococcus faecalis. Int Endod J. 2012;45(4):311–6.
9. Chogle S, Kinaia BM, Goodis HE. Scope of nanotechnology in endodontics [Internet]. Nanobiomaterials in Clinical Dentistry. Elsevier Inc.; 2019. 517–539 p. Available from: http://dx.doi.org/10.1016/B978-0-12-815886-9.00021-8
10. Kishen A, Shi Z, Shrestha A, Neoh KG. An Investigation on the Antibacterial and Antibiofilm Efficacy of Cationic Nanoparticulates for Root Canal Disinfection. J Endod [Internet]. 2008;34(12):1515–20. Available from: http://dx.doi.org/10.1016/j.joen.2008.08.035
11. Afkhami F, Akbari S, Chiniforush N. Entrococcus faecalis Elimination in Root Canals Using Silver Nanoparticles , Photodynamic Therapy, Diode Laser, or Laser-activated Nanoparticles : An In Vitro Study. J Endod [Internet]. 2016;10–3. Available from: http://dx.doi.org/10.1016/j.joen.2016.08.029
12. Javidi M, Afkhami F, Zarei M, Ghazvini K, Rajabi O. Efficacy of a combined nanoparticulate/calcium hydroxide root canal medication on elimination of Enterococcus faecalis. Aust Endod J. 2014;40(2):61–5.
13. Bergmans L, Moisiadis P, Meerbeek B Van, Quirynen M, Lambrechts P. Microscopic observation of bacteria : review highlighting the use of environmental SEM. 2005;775–88.
14. Valverde ME, Baca P, Ceballos L, Fuentes MV, Ruiz-Linares M, Ferrer-Luque CM. Antibacterial efficacy of several intracanal medicaments for endodontic therapy. Dent Mater J. 2017;36(3):319–24.
15. Abdelrahman MYT, El-Ashry S, El-Batoty K, El-Hady S. Efficiency of Triple Antibiotic Mixture and Propolis as Intracanal Medication in Revascularization process in immature apex: A clinical study. Glob J Med Clin Case Reports. 2019;6(2):019–25.
16. Costerton W, Veeh R. The application of biofilm science to the study and control of chronic bacterial infections. J Clin … [Internet]. 2003;112(10):12. Available from: http://www.jci.org/cgi/content/abstract/112/10/1466
17. Yang Y, Shen Y, Wang Z, Huang X, Maezono H, Ma J, et al. Evaluation of the Susceptibility of Multispecies Biofilms in Dentinal Tubules to Disinfecting Solutions. J Endod [Internet]. 2016;42(8):1246–50. Available from: http://dx.doi.org/10.1016/j.joen.2016.05.011
18. Wilson CE, Cathro PC, Rogers AH, Briggs N, Zilm PS. Clonal diversity in biofilm formation by Enterococcus faecalis in response to environmental stress associated with endodontic irrigants and medicaments. Int Endod J. 2015;48(3):210–9.
19. Prada I, Micó-Muñoz P, Giner-Lluesma T, Micó-Martínez P, Collado-Castellano N, Manzano-Saiz A. Influence of microbiology on endodontic failure. Literature review. Med Oral Patol Oral y Cir Bucal. 2019;24(3):e364–72.

20. Siqueira JF, Magalhães KM, Rôças IN. Bacterial Reduction in Infected Root Canals Treated With 2.5% NaOCl as an Irrigant and Calcium Hydroxide/Camphorated Paramonochlorophenol Paste as an Intracanal Dressing. J Endod. 2007;33(6):667–72.

21. Lima KC, Fava LRG, Siqueira JF. Susceptibilities of Enterococcus faecalis biofilms to some antimicrobial medications. J Endod. 2001;27(10):616–9.

22. Pladisai P, Ampornaramveth RS, Chivatxaranukul P. Effectiveness of different disinfection protocols on the reduction of bacteria in Enterococcus faecalis biofilm in teeth with large root canals. J Endod [Internet]. 2016;42(3):460–4. Available from: http://dx.doi.org/10.1016/j.joen.2015.12.016

23. Siqueira JF, Uzeda M De. Influence of Different Vehicles on the Antibacterial Effects of Calcium Hydroxide. October. 1998;24(10):5–7.

24. Zehnder M, Luder HU, Schätzle M, Kerosuo E, Waltimo T. A comparative study on the disinfection potentials of bioactive glass S53P4 and calcium hydroxide in contra-lateral human premolars ex vivo. Int Endod J. 2006;39(12):952–8.

25. Sathorn C, Parashos P, Messer H. Antibacterial efficacy of calcium hydroxide intracanal dressing: A systematic review and meta-analysis. Int Endod J. 2007;40(1):2–10.

26. Hiraishi N, Yiu CKY, King NM, Tagami J, Tay FR. Antimicrobial efficacy of 3.8% silver diamine fluoride and its effect on root dentin. J Endod [Internet]. 2010;36(6):1026–9. Available from: http://dx.doi.org/10.1016/j.joen.2010.02.029

27. Siqueira JF, Rôças IN, Paiva SSM, Guimarães-Pinto T, Magalhães KM, Lima KC. Bacteriologic investigation of the effects of sodium hypochlorite and chlorhexidine during the endodontic treatment of teeth with apical periodontitis. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;104(1):122–30.

28. Bapat RA, Chaubal T V., Joshi CP, Bapat PR, Choudhury H, Pandey M, et al. An overview of application of silver nanoparticles for biomaterials in dentistry. Mater Sci Eng C [Internet]. 2018;91(2017):881–98. Available from: https://doi.org/10.1016/j.msec.2018.05.069

29. Takamiya AS, Monteiro DR, Bernabé DG, Gorup LF, Camargo ER, Gomes-Filho JE, et al. In Vitro and in Vivo Toxicity Evaluation of Colloidal Silver Nanoparticles Used in Endodontic Treatments. J Endod. 2016;42(6):953–60.

30. Fernandez CC, Sokolonski AR, Fonseca MS, Stanisic D, Araújo DB, Azevedo V, et al. Applications of silver nanoparticles in dentistry: Advances and technological innovation. Int J Mol Sci. 2021;22(5):1–21.

31. Gomaa EZ. Silver nanoparticles as an antimicrobial agent: A case study on Staphylococcus aureus and escherichia coli as models for gram-positive and gram-negative bacteria. J Gen Appl Microbiol. 2017;63(1):36–43.

32. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against Enterococcus faecalis biofilm. J Endod [Internet]. 2014;40(2):285–90. Available from: http://dx.doi.org/10.1016/j.joen.2013.08.022

33. Del Carpio-Perochena A, Kishen A, Shrestha A, Bramante CM. Antibacterial Properties Associated with Chitosan Nanoparticle Treatment on Root Dentin and 2 Types of Endodontic Sealers. J Endod. 2015;41(8):1353–8.

34. Elgendy AA, Fayyad DM. Cell viability and apoptotic changes of dental pulp stem cells treated with propolis, chitosan, and their nano counterparts. Tanta Dent J [serial online] 2017 [cited 2021 Oct 31];14:198-207.

35. Me V, H-p H, Conrads G, Feres M, Bpfa G. Comparative analysis of endodontic pathogens using checkerboard hybridization in relation to culture. 2008;(39):282–90.

36. Aguiar AS, Guerreiro-Tanomaru JM, Faria G, Leonardo RT, Tanomaru-Filho M. Antimicrobial Activity and pH of Calcium Hydroxide and Zinc Oxide Nanoparticles Intracanal Medication and Association with Chlorhexidine. J Contemp Dent Pract. 2015;16(8):624–9.

37. Alabdulmohsen Z, Saad A. Antibacterial effect of silver nanoparticles against Enterococcus faecalis. Saudi Endod J. 2017;7(1):29–35.

38. Mardones J, Ml G, Díaz C, Galleguillos C, Covarrubias C. In Vitro Antibacterial Properties of Copper Nanoparticles as Endodontic Medicament against Enterococcus faecalis. J Dent Oral Disord. 2018;4(6):1–5. Wang X. Preparation, synthesis and application of Sol-gel method University Tutor : Pr . Olivia GIANI Internship Tutor : Mme . WANG Zhen. 2020;(October).

39. Neelakantan P, Sanjeev K, Subbarao C V. Duration-dependent susceptibility of endodontic pathogens to calcium hydroxide and chlorhexidene gel used as intracanal medicament: an in vitro evaluation. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007; 104(4):138–41.

40. Awawdeh L, AL-Beitawi M, Hammad M. Effectiveness of propolis and calcium hydroxide as a short-term intracanal medicament against Enterococcus faecalis: A laboratory study. Aust Endod J. 2009;35(2):52–8.