ORIGINAL RESEARCH

Comparative Evaluation of Antibacterial Efficacy of Silver and Cadmium Nanoparticles and Calcium Hydroxide against *Enterococcus faecalis* Biofilm

Sonal Singh Arora[1], Rajesh Shetty[2], Manjunath Sushilamma Hemagiriyappa[3], Sonal Singh Thakur[4], Niharika Mishra[5], Nitin Manoharrao Lokhande[6]

Abstract

Aim: The purpose of this study was to evaluate and compare the antibacterial efficacy of calcium hydroxide medicament, silver (AgNPs) and cadmium nanoparticles (CdSNPs) as medicament against the biofilms of *Enterococcus faecalis* on dentin sections. *E. faecalis* is commonly detected in asymptomatic and persistent endodontic infections.

Methods: Twenty standard size dentin sections were prepared. *E. faecalis* was inoculated on these dentin sections for four weeks to form the bacterial biofilm. Twenty dentin sections were segregated into four different groups with five specimens in each group. Group I was kept as control group, and antibacterial efficacy was tested by treating biofilms with $Ca(OH)_2$ medicament, 0.02% AgNP and CdSNP gels for 7 days. The ultrastructure of biofilms from each group was examined under scanning electron microscope and was visually evaluated and compared for different groups.

Results: $Ca(OH)_2$ exhibited a slight disruption of *E. faecalis* biofilm. Both AgNP and CdSNP medicaments disrupted *E. faecalis* biofilm effectively after 7 days of inoculation. AgNPs disrupted the biofilm more effectively than CdSNPs. Biofilms in control group, which was irrigated with saline, did not show any disruption of biofilm, which could be seen as homogenous layer over most of dentin sections.

Conclusions: This study suggests that both AgNP and CdNP gels are effective against *E. faecalis* and can be used as a medicament to eliminate residual bacterial biofilms during root canal disinfection. AgNP medicament is more effective than CdNP, whereas $Ca(OH)_2$ is not effective against *E. faecalis* biofilms.

Clinical significance: Incomplete clearance and the development of antibiotic resistance in *E. faecalis* are the important factors for failure of root canal treatment. When cationic nanoparticles are introduced for the treatment of biofilms, it can interact with both extracellular polymeric substances and bacterial cells. The initial electrostatic attraction between positively charged nanoparticles and negatively charged bacterial surface leads to bacterial killing via the production of reactive oxygen species. Metal nanoparticles that are effective against *E. faecalis* have a significant potential role in the prevention and treatment of such cases, as bacteria do not develop resistance against metal nanoparticles.

Keywords: Antibacterial efficacy, Biofilm, Cadmium, Calcium hydroxide, *Enterococcus faecalis*, Nanoparticles, Silver.

The Journal of Contemporary Dental Practice (2021): 10.5005/jp-journals-10024-3222

Introduction

Root canal treatment (RCT) is one of the most important procedures in dentistry, which lays the foundation for natural teeth preservation. It has been seen that many a times, even a precisely done RCT may fail. The major cause of failure is the survival of microorganisms in the apical portion of the root-filled tooth.[1,2] While most of the primary endodontic infections are polymicrobial in nature, the secondary infections are composed of one or a few bacterial species. *Enterococcus faecalis* has been one of the predominant bacteria isolated from the failed root canal.[2–6]

E. faecalis is a gram-positive facultative anaerobic opportunistic pathogen. It is highly virulent bacteria, which invades and survives within dentinal tubules for prolonged periods.[7] These also form biofilm on dentin, resist disinfecting agents, and can survive challenging environments in the filled root canal.[8,9] The virulence and antibacterial resistance of bacteria result from the ability of microorganisms to form biofilms.[10] Biofilms are bacterial communities attached to a biotic or an abiotic substrate and encased in a matrix that may be composed of carbohydrates, DNA, or proteins. Biofilms not only play an important role in the pathogenesis of several chronic infections but are also central to nosocomial infections.[11] Therefore, successful treatment of infections involves the elimination or significant reduction of

[1,4]Department of Conservative Dentistry and Endodontics, Sri Aurobindo College of Dentistry, Sri Aurobindo University, Indore, Madhya Pradesh, India

[2]Department of Conservative Dentistry and Endodontics, Dr DY Patil Dental College and Hospital, Dr DY Patil Vidyapeeth, Pune, Maharashtra, India

[3]Department of Periodontology, SMBT Dental College and Hospital, Sangamner, Maharashtra, India

[5]Department of Conservative Dentistry and Endodontics, Mansarovar Dental College Hospital and Research Centre, Bhopal, Madhya Pradesh, India

[6]Department of Conservative Dentistry and Endodontics, SMBT Dental College and Hospital, Sangamner, Maharashtra, India

Corresponding Author: Sonal Singh Arora, Department of Conservative Dentistry and Endodontics, Sri Aurobindo College of Dentistry, Sri Aurobindo University, Indore, Madhya Pradesh, India, Phone: +91 9893171126, e-mail: sonal.singh.arora@gmail.com

How to cite this article: Arora SS, Shetty R, Hemagiriyappa MS, *et al*. Comparative Evaluation of Antibacterial Efficacy of Silver and Cadmium Nanoparticles and Calcium Hydroxide against *Enterococcus faecalis* Biofilm. J Contemp Dent Pract 2021;22(12):1438–1443.

Source of support: Nil

Conflict of interest: None

bacterial biofilms. It has been shown in various studies that bacterial biofilm persists in the root canals despite thorough chemo-mechanical disinfection and subsequent root canal obturation.

Thus, the need to develop advanced antibiotics and other disinfection systems is felt, which should help in an effective elimination of biofilms and does not induce bacterial resistance.

The most frequently used intracanal medicament in the treatment of root canal is calcium hydroxide. It releases hydroxyl ions, which are responsible for its antibacterial effects by damaging bacterial cytoplasmic membranes and DNA and protein denaturation.[12] However, the antimicrobial activity of $Ca(OH)_2$ can be deactivated by dentin, periapical exudates, and microbial aggregates, and it does not always eliminate *E. faecalis* biofilms.[6,13]

Recently, various metal nanoparticles have been studied with reference to their antimicrobial properties, e.g., silver, titanium, cadmium, alumina, copper oxide, etc., and were found to be effective antibacterial agents attributed to an electrostatic interaction between nanoparticles with bacterial membrane and accumulation in the cytoplasm.[14,15] "A nanoparticle (10^{-9} m) is defined as a small object that behaves as a whole unit in terms of its transport and properties. Nanoscale materials have emerged up as novel antimicrobial agents owing to their high surface area-to-volume ratio and their unique chemical and physical properties."[15]

Silver nanoparticles (AgNPs) are established effective bactericidal agents and have been applied in many healthcare disciplines. The medical applications of silver as antimicrobial were declined with the advent of antibiotics.[16,17] In the late 1800s, western scientists re-discovered what had been known for thousands of years that silver is a powerful germ fighter. Dr Carl A Moyer, along with Dr Margraf, the biochemist, re-established the antimicrobial role of silver in burn patients in the form of 0.5% colloidal silver, after the invent of nanotechnology in 1959.[18] Cadmium nanoparticles (CdSNPs) are comparatively new entry and are being studied for their bactericidal efficacy against various gram-positive and gram-negative bacteria and were proved to be potent bactericidal agents.[15,19] The studies for bactericidal efficacy of CdSNPs against *E. faecalis* are still scarce in literature, so this study tries to fill the gap and compare the already-proved AgNPs with that of CdSNPs against the most important infected root canal pathogen, i.e., *E. faecalis*.

The aim of this study was to assess and compare the antibacterial effectiveness of calcium hydroxide medicament and AgNPs and CdSNPs against *E. faecalis* biofilms under the scanning electron microscope (SEM).

Materials and Methods

This *in vitro* study was done using 10 human single-rooted mandibular permanent premolar teeth, extracted for orthodontic reasons. Sample size was based on the feasibility of time and resources. Unidentified teeth were collected from the Department of Oral and Maxillofacial Surgery of Dr DY Patil Dental College and Hospital, Pune, as per the study protocol approved by Institutional Ethics Committee. The need for informed consent was waived off in view of *in vitro* study on non-identified samples. The inclusion criteria were permanent mandibular premolar teeth, teeth with intact apices, and no previous restoration, while exclusion criteria were carious teeth, fractured and restored teeth, and teeth with open apex.

Preparation of Dentin Sections

Teeth were decoronated and the apex was removed using a diamond disk. Teeth were then sectioned vertically into two halves in the midsagittal plane. Cementum was removed using a diamond bur. Twenty dentin sections of $4 \times 4 \times 1$ mm (length × width × height) sizes were prepared using diamond disk as described by Wu et al. About 5% sodium hypochlorite (Prime dental, India) and 17% ethylene diamine tetraacetic acid (EDTA) (Dent Wash, Prime dental, India) with ultrasonic activation were used for 4 minutes each to remove the smear layer from dentin sections. Sterile water was used for 1 minute to rinse the dentin sections, which were then autoclaved in brain–heart infusion (BHI) broth (HiMedia, Mumbai, India) at 121°C and 15 lb pressure for 15 minutes. These sections were then incubated for 24 hours at 37°C in BHI broth to confirm no contamination of bacteria.

Inoculation of Dentin with Bacteria

E. faecalis (ATCC 29212) was procured from the Department of Microbiology and plated on blood agar and incubated aerobically for 24 hours at 37°C. *E. faecalis*, a single colony, was suspended at 37°C in sterile BHI broth. These dentin sections were then placed in 2 mL of suspension in each sterile polystyrene vial having 1×10^8 mL^{-1} bacterial concentration. The specimens were incubated at 37°C for 4 weeks. To confirm bacterial viability and to remove dead cells, BHI broth was replaced every fourth day.

The specimens were taken out aseptically after 4 weeks. The sections were then washed with phosphate-buffered saline (PBS) to clear the culture medium and non-adherent bacteria. Two dentin sections were scanned to confirm the growth of *E. faecalis* biofilm on the dentin surface under SEM.

Preparation of AgNP and CdNP Gel

AgNPs and CdSNP gels were prepared in the concentration of 0.02% w/v. AgNPs and CdSNPs were obtained from NANO LAB, Jamshedpur, Jharkhand, India, as 0.1% concentration solution. The solutions were sonicated (Probe Sonicator, PCI Analytics Pvt. Ltd., India) before use and were five times diluted to achieve 0.02% concentration. To convert this solution into gel with 6% w/w hydroxyethyl cellulose (HEC) (Ashland, USA), 18.8 g of nanoparticle solution was mixed with 1.2 g of HEC powder to prepare 20 g of 0.02% nanoparticle gel. Small portions of HEC were mixed to nanoparticle solution while continuously stirring it at low speed by electronic stirrer (Remi Elektrotechnik Ltd., India), till all the HEC powder is mixed thoroughly. The gel was kept in sterile glass containers and autoclaved at 121°C and 15 lb pressure for 15 minutes.

Antibacterial Activity of $Ca(OH)_2$ Medicament, AgNP and CdNP Gels

AgNPs and CdSNP gels were tested in 0.02% concentration each, based on the results obtained with AgNP medicament by Wu et al.

Four groups of five dentin sections each were made out of 20 dentin sections. Group I (control group) received no treatment. Dentin sections of Group I were examined under SEM (Zeiss, EVO LS10) to confirm the presence and extent of *E. faecalis* biofilm. Calcium hydroxide medicament (UltraCal XS, Ultradent, USA), AgNP and CdSNP medicament gels were applied to the surfaces of dentin sections in Group II, III, and IV, respectively, and kept in sterile polystyrene vials. These gels were incubated at 37°C for 7 days in 100% humid environment.

Examination of Dentin Sections under SEM

After 7 days, the samples of Group II, III, and IV were washed with 5 mL sterile PBS to remove the medicament gel. About 0.5% citric acid was used to neutralize the dentin sections treated with calcium hydroxide. The samples of all the four groups (Group I–IV) were dehydrated using ascending grades of ethanol. Gold sputter coating of the samples was done in a vacuum evaporator (Quorum Q150T ES) and examined by a single blinded examiner using SEM (Zeiss, EVO LS10) at × 2500 magnification (Figs 1A and B to 3A and B). All the samples were visualized under the same magnification. As SEM observations were descriptive in nature and did not generate any numerical data, statistical analysis was not done.

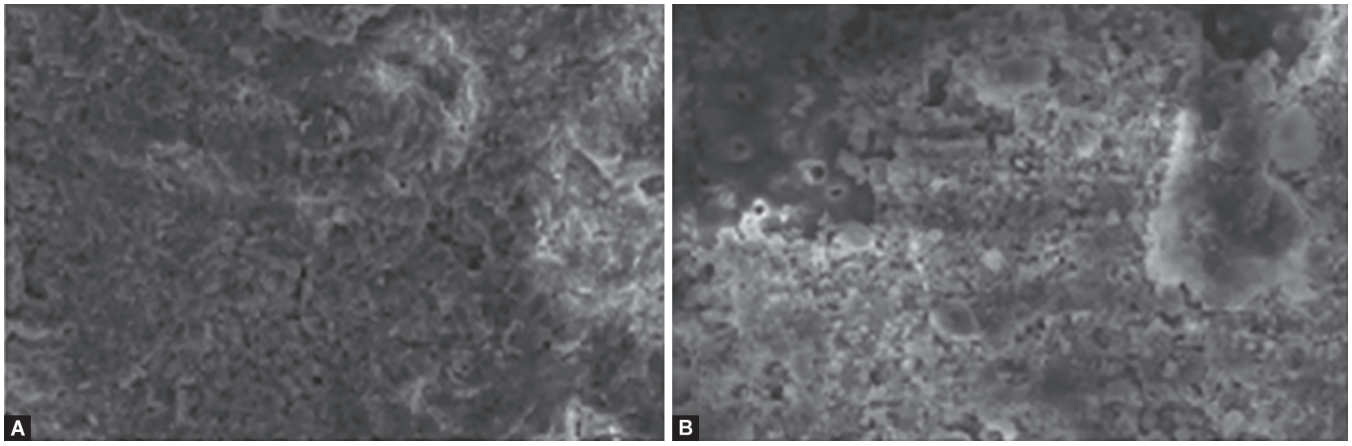
Results

All the dentin sections of four groups were visually evaluated under SEM regarding the extent of structural damage to *E. faecalis* biofilm. The observation of various study groups showed the following:

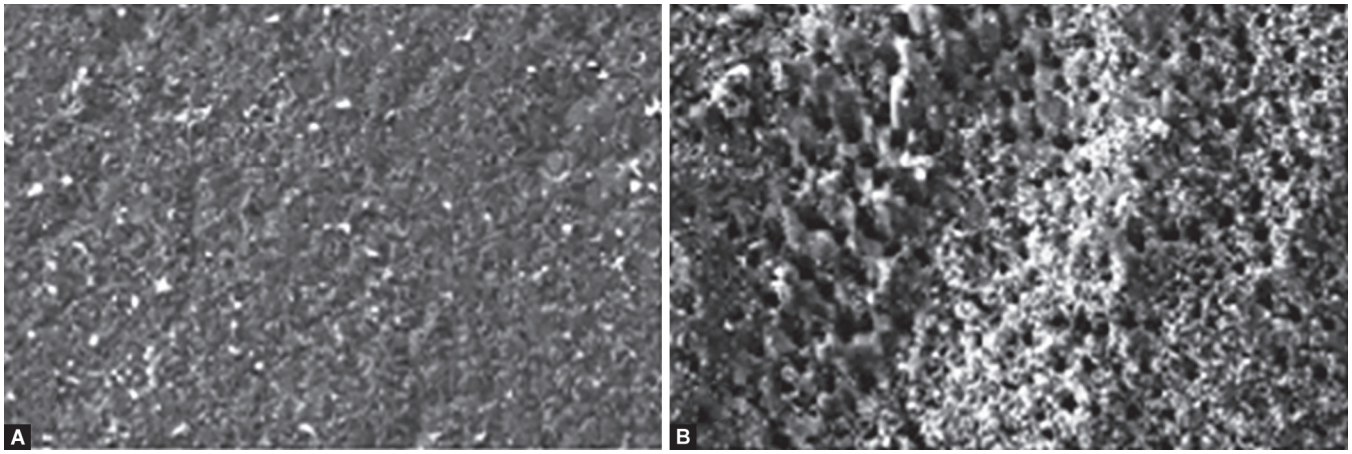
- **Control group:** Figure 1 shows the well-formed *E. faecalis* biofilm over untreated dentin sections after 4 weeks of incubation. The dentin surface can be seen completely covered with biofilm.
- **Calcium hydroxide-treated group:** In Figure 2, *E. faecalis* biofilm can be seen covering the entire surface of dentin sections. The biofilm is intact over most of the dentin sections, except only in Figure 2B, where the biofilm is partly damaged in some areas.
- AgNP gel-treated group: Figure 3 shows SEM images of dentin sections treated with 0.02% AgNP gel. The images show bare dentin surface with clearly visible openings of the dentinal tubules. There is no *E. faecalis* biofilm visible on dentin surfaces, signifying extensive damage to *E. faecalis* biofilm, with whole of dentine sections being clear of biofilm.
- **CdSNP gel-treated group:** Figures 4A and B exhibit dentin sections covered with *E. faecalis* biofilm, showing different degrees of structural damage. Dentin sections are not free of biofilm, except only a small area in picture 1H appears free of biofilm.

Inference

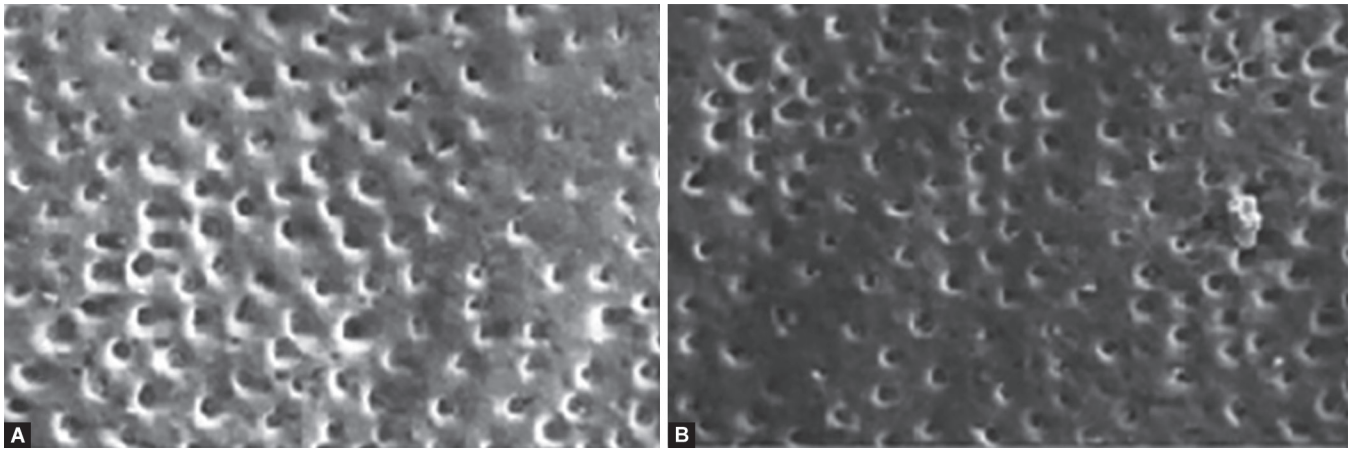
The above findings of visual examination of dentin sections under SEM suggest that AgNP gel is most efficient in causing structural damage to *E. faecalis* biofilm, followed by CdSNP gel, whereas calcium hydroxide is practically ineffective against *E. faecalis*



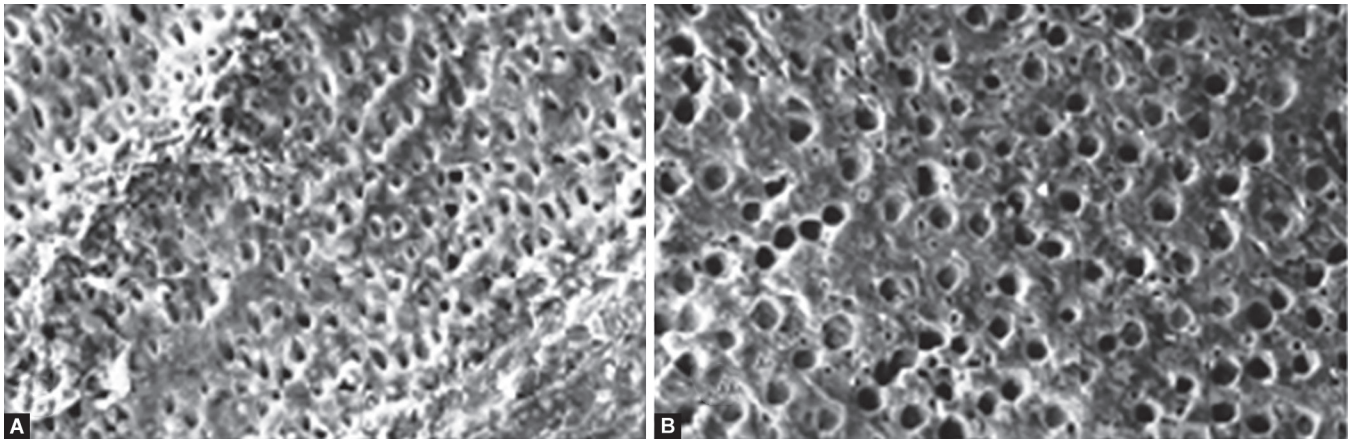
Figs 1A and B: Dentin samples under SEM (magnification 2500×). Group I (Control group) showing the well-formed *E. faecalis* biofilm



Figs 2A and B: Dentin samples under SEM (magnification 2500×). Group II: Showing intact biofilm unaffected by $Ca(OH)_2$ medicament



Figs 3A and B: Dentin samples under SEM (magnification 2500×). Group III: Showing completely clear dentin surface with openings of dentinal tubules and complete clearance of biofilm



Figs 4A and B: Dentin samples under SEM (magnification 2500×). Group IV: Show partially damaged biofilm covering dentin surface

biofilm. Thus, it can be inferred from this study that AgNPs have the highest antibacterial efficacy against *E. faecalis* biofilms, followed by CdSNPs.

Discussion

Researchers have found *E. faecalis* as one of the most important bacteria in teeth with failure of RCT.[4–6] Growing resistance of *E. faecalis* to antimicrobial agents with the formation of biofilms has been a matter of concern.[11] Successful elimination of bacteria from treated root canals is essential for recovery. Metal nanoparticles have been recognized as potent antimicrobial agents without the development of resistance by microbes.[14]

Calcium hydroxide, introduced by Hermann, has been widely used in endodontics due to its various biological properties. Its antimicrobial activity is attributed to high alkalinity (pH 12.5). Lethal effects of calcium hydroxide on bacterial cells have been observed only when the substance is in direct contact with bacteria.[12]

A number of studies have been done to evaluate the antibacterial properties of AgNPs and CdSNPs individually.[15,19–22] Antibacterial properties of silver have been evaluated against many bacteria, including bacteria of significance in endodontic infections, i.e., *E. faecalis*.[2–7,14,20,23] Although cadmium had been evaluated for its activity against many other bacteria (*Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli, Klebsiella pneumoniae, Bacillus licheniformis, Bacillus cereus, Aspergillus flavus, Fusarium oxysporum, Penicillium expansum*), but not against *E. faecalis*.[15,19,21,22] Therefore, this study was taken up to evaluate and compare the antimicrobial activity of calcium hydroxide as standard medicament and AgNP and CdSNP nanoparticles gels against *E. faecalis* biofilm.

In the present study, calcium hydroxide was not found to be effective against *E. faecalis* biofilm on dentin samples. Our finding is well supported by available literature, which shows the inefficiency of calcium hydroxide against *E. faecalis*.[12,13,24] Haapasalo and Orstavik found that calcium hydroxide could not eradicate *E. faecalis* from the innermost zones of dentin even after prolonged incubation.

This study exhibits that both AgNP and CdSNP gels are effective against *E. faecalis* biofilm at a concentration of 0.02%. Lotfi et al. found nanosilver to be effective against *E. faecalis* at a concentration as low as 50 µg/mL.[25] Daming Wu et al. tested the efficacy of AgNP against *E. faecalis* in the form of (0.1%) solution and (0.02 and 0.01%) gels.[20] They found that 0.1% AgNP solution could not destroy the integrity of the biofilm effectively. Although both 0.01 and 0.02% AgNP gel effectively damaged the biofilm, the proportion of live bacterial cells was found to be high in dentin sections treated with 0.01% AgNP gel. Liu et al. observed that AgNPs-PL gel at

the concentration of 16 and 32 µg/mL can effectively eliminate *E. faecalis* biofilm in dentinal tubules.[26] Krishnan et al. studied MIC and minimum bactericidal concentration (MBC) of AgNP (45–50 nm size) against *E. faecalis* and found a bactericidal activity at 5 mg/mL, whereas the present study used AgNPs of 20–30 nm size at 0.2 mg/mL.[27] Halkai et al. also found the activity of AgNPs against *E. faecalis*.[28] Bruniera et al. found that AgNPs exhibit better physical properties in HEC, which is used as vehicle in the preparation of gel in our study.[29]

The antimicrobial potential of CdS nanoparticles against *S. aureus*, *S. typhimurium*, *P. aeruginosa*, *E. coli*, and *K. pneumonia* has been evaluated by Kumar et al.[15] They found these particles are effective from the concentration of 0.078–0.83 mg/mL. In the present study, the concentration of CdSNP falls in the same range at 0.2 mg/mL (0.02%). Shukla et al. effectively used 0.5 and 1.0% concentration of CdO nanoparticles against *E. coli*, which was much higher than that used in the present study.[19] The difference may be due to differences in the methodology and the organism studied.

Comparative evaluation of efficacy of AgNPs and CdSNPs against *E. faecalis* was assessed by visual examination of extent of damage to biofilm. AgNPs gel damaged biofilm more effectively than CdSNP. CdSNPs damaged the biofilm, but remnants of the same could be seen on dentin surface.

Limitations of the Study

This study does not evaluate the extent of live or dead bacteria present in the biofilm. Further, the toxicity of the AgNPs and CdSNPs is not evaluated in the current study.

Conclusion

AgNP and CdSNP gels are effective antibacterial agents against *E. faecalis* and can be used as medicament in RCT to prevent failure due to *E. faecalis* biofilm. Although calcium hydroxide is used very commonly in endodontic practice for its activity against many bacterial strains, it is not effective against endodontically significant bacteria *E. faecalis* for practical purposes.

The authors feel that further *in vitro* and *in vivo* studies are required to establish the efficacy and safety of AgNPs and CdNPs.

Acknowledgments

Support for this research project was received from the Department of Microbiology, Dr DY Patil Medical College, Hospital and Research Centre, Pune, and the preparation of nanoparticle gels was supported by the Department of Pharmaceutics, Dr DY Patil Institute of Pharmaceutical Sciences and Research, Pune.

References

1. Evans M, Davies JK, Sundqvist G, et al. Mechanisms involved in the resistance of *Enterococcus faecalis* to calcium hydroxide. Int Endod J 2002;35(3):221–228. DOI: 10.1046/j.1365-2591.2002.00504.x.
2. Baumgartner JC, Falkler WA. Bacteria in the apical 5 mm of infected root canals. J Endod 1991;17(8):380–383. DOI: 10.1016/s0099-2399(06)81989-8.
3. Molander A, Reit C, Dahlen G, et al. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998;31(1):1–7. PMID: 9823122.
4. Sundqvist G, Figdor D, Persson S, et al. Microbiologic analysis of teeth with failed endodontic treatment and the outcome of conservative re-treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1998;85(1):86–93. DOI: 10.1016/s1079-2104(98)90404-8.
5. Hancock HH, Sigurdsson A, Trope M, et al. Bacteria isolated after unsuccessful endodontic treatment in a North American population. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91(5):579–586. DOI: 10.1067/moe.2001.113587.
6. Stuart CH, Schwartz SA, Beeson TJ, et al. *Enterococcus faecalis*: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32(2):93–98. DOI: 10.1016/j.joen.2005.10.049.
7. Love RM. *Enterococcus faecalis*: a mechanism for its role in endodontic failure. Int Endod J 2001;34(5):399–405. DOI: 10.1046/j.1365-2591.2001.00437.x.
8. 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):867–872. DOI: 10.1097/01.don.0000164855.98346.fc.
9. Rocas IN, Siqueira JF Jr, Santos KR. Association of *Enterococcus faecalis* with different forms of periradicular diseases. J Endod 2004;30(5):315–320. DOI: 10.1097/00004770-200405000-00004.
10. Tendolkar PM, Baghdayan AS, Gilmore MS, et al. Enterococcal surface protein Esp. enhances biofilm formation by *Enterococcus faecalis*. Infec Immun 2004;72(10):6032–6039. DOI: 10.1128/IAI.72.10.6032-6039.2004.
11. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev 2002;15(2):167–193. DOI: 10.1128/CMR.15.2.167-193.2002.
12. Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J 1999;32(5):361–369. DOI: 10.1046/j.1365-2591.1999.00275.x.
13. Happasalo HK, Siren EK, Waltimo TM, et al. Inactivation of local root canal medicaments by dentine: an in vitro study. Int Endod J 2000;33(2):126–131. DOI: 10.1046/j.1365-2591.2000.00291.x.
14. Sachindri R, Kalaichelvan PT. Antibacterial activities of metal nanoparticles. Adv Bio Tech 2011;11(2):21–23.
15. Kumar A, Singh S, Kumar D. Evaluation of antimicrobial potential of cadmium sulphide nanoparticles against bacterial pathogens. Int J Pharm Sci Rev Res 2014;24(2):202–207.
16. Castellano JJ, Shafii SM, Ko F, et al. Comparative evaluation of silver-containing antimicrobial dressings and drugs. Int Wound J 2007;4(2):114–122. DOI: 10.1111/j.1742-481X.2007.00316.x.
17. Chen X, Schluesener HJ. Nano-silver: a nano-product in medical application. Toxicol Lett 2008;176(1):1–12. DOI: 10.1016/j.toxlet.2007.10.004.
18. Uses of silver: history of support to the health [Internet]. Silver Colloids. 2021. Available from: https://www.silver-colloids.com/history-silver/.
19. Shukla M, Kumari S, Shukla S, et al. Potent antibacterial activity of nano CdO synthesized via microemulsion scheme. J Mater Environ Sci 2012;3(4):678–685.
20. Wu D, Fan W, Kishen A, et al. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. J Endod 2014;40(2):285–290. DOI: 10.1016/j.joen.2013.08.022.
21. Salehi B, Mortaz E, Tabarsi P. Comparison of antibacterial activities of cadmium oxide nanoparticles against *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria. Adv Biomed Res 2015;4(105):1–7. DOI: 10.4103/2277-9175.157805.
22. Shivashankarappa A, Sanjay KR. Study on biological synthesis of Cadmium sulphide nanoparticles by *Bacillus licheniformis* and its antimicrobial properties against food borne pathogens. Nanosci Nanotech Res 2015;3(1):6–15. DOI: 10.12691/nnr-3-1-2.
23. Rai MK, Deshmukh SD, Ingle AP, et al. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 2012;112(5):841–852. DOI: 10.1111/j.1365-2672.2012.05253.x.
24. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66(8):1375–1379. DOI: 10.1177/00220345870660081801.
25. Lotfi M, Vosoughhosseini S, Ranjkesh B, et al. Antimicrobial efficacy of nanosilver, sodium hypochlorite and chlorhexidine gluconate against *Enterococcus faecalis*. Afr J Biotech 2011;10(35):6799–6803. DOI: 10.5897/AJB11.240.

26. Liu T, Aman A, Ainiwaer M, et al. Evaluation of the anti-biofilm effect of poloxamer-based thermoreversible gel of silver nanoparticles as potential medication for root canal therapy. Sci Rep 2021;11(1):12577. DOI: 10.1038/s41598-021-92081-7.
27. Krishnan R, Arumugam V, Vasaviah SK. The MIC and MBC of silver nanoparticles against *Enterococcus faecalis*–a facultative anaerobe. J Nanomed Nanotechnol 2015;6(3):1000285. DOI: 10.4172/2157-7439.1000285.
28. Halkai KR, Mudda JA, Shivanna V, et al. Evaluation of antibacterial efficacy of fungal-derived silver nanoparticles against *Enterococcus faecalis*. Contemp Clin Dent 2018;9(1):45–48. DOI: 10.4103/ccd.ccd_703_17.
29. Bruniera JFB, Silva-sousa YTC, Lara MG, et al. Development of intracranial formulation containing silver nanoparticles. Bra Dent J 2014;25(4):302–306. DOI: 10.1590/0103-6440201302431.