Original Article

In vitro comparison to study the antimicrobial effect of silver nanoparticles gel and its various combinants as an intracanal medicament against *Enterococcus faecalis*

Swaty Jhamb, Ruchi Singla, Amandeep Kaur, Jyoti Sharma[1], Jagat Bhushan

Departments of Conservative Dentistry and Endodontics and [1]Microbiology, Dr. H. S. J. I. D. S and Hospital, Panjab University, Chandigarh, India

Abstract

Background: Endodontic infections have been clearly described as biofilm-mediated infections. Bacteria and their by-products have been known to cause these infections. With the introduction of new drugs and the use of nanoparticles in recent times, there has been a significant reduction in the bacterial load in endodontic infections.

Aims and Objectives: The *in vitro* study focuses on checking the antibacterial efficacy of silver nanoparticles and its combination with other medicaments against the root canal pathogen – *Enterococcus faecalis* (*E. faecalis*).

Methodology: In the present study, 140 extracted human teeth were used. The teeth were sectioned, and biomechanical preparation was done. The root canals of the extracted teeth were inoculated with the culture of *E. faecalis*. The teeth were divided into six groups based on the intracanal medicament used:

- Group 1 – Silver nanocure gel
- Group 2 – Silver nanocure gel + Cavisept gel (1:1)
- Group 3 - Silver nanocure gel + Aveu-Cal gel (1:1)
- Group 4 – Silver nanocure gel + Cavisept gel + Aveu-Cal gel (1:1:1)
- Group 5 - Positive control (specimens were inoculated with *Enterococcus faecalis* and left untreated to confirm the presence of infection)
- Group 6 - Negative control (no bacterial contamination of specimens).

The colony-forming units were recorded after 48 h of incubation.

Results: The statistical analysis of the colony-forming units was done using the Kruskal–Wallis tests. Silver nanocure gel + Cavisept gel + Aveu-Cal gel (1:1:1) showed the least colony-forming units.

Conclusion: The present study is an *in vitro* study, in which we concluded that the combination of all the intracanal medicaments is the best for the elimination of *E. faecalis* biofilm from the root canal. The above findings need to be tested *in vivo* also.

Keywords: Aveu-Cal gel; Cavisept gel; colony-forming units; medicaments; silver nanocure gel

Address for correspondence:
Dr. Swaty Jhamb,
H. No. 2402/G.F, Sec. 38 C, Chandigarh or Room No. 201, Department of Conservative and Endodontics, Dr. H. S. J. I. D. S, Panjab University, Chandigarh, India.
E-mail: swatyjhambparmar@gmail.com

Date of submission : 10.09.2023
Review completed : 13.10.2023
Date of acceptance : 25.10.2023
Published : 13.01.2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jcde

DOI: 10.4103/JCDE.JCDE_180_23

How to cite this article: Jhamb S, Singla R, Kaur A, Sharma J, Bhushan J. *In vitro* comparison to study the antimicrobial effect of silver nanoparticles gel and its various combinants as an intracanal medicament against *Enterococcus faecalis*. J Conserv Dent Endod 2024;27:42-5.

INTRODUCTION

Endodontic treatment poses a challenge for the dentist as the root canals have a complex anatomy. Multiple factors as excellent biomechanical preparation along with debridement, copious irrigation, use of specific intracanal medicaments, and three-dimensional obturation to completely seal the root canal system govern the overall success of endodontic treatment.[1] In spite of good mechanical debridement and cleaning and shaping with the best file system, a lot of problems do exist. Complete root canal cleaning is still a cause of concern as the previous studies have highlighted that biomechanical preparation to its maximum efficacy still leaves around 30%–35% of the root canal untouched.[2] The mixed flora in the endodontic system leads to a number of problems. *Enterococci faecalis* (*E. faecalis*) form the pioneers of root canal infection, which are biofilm mediated.[3] Biofilm is a complex extracellular polymeric matrix that protects the bacteria against nutrient deprived and other unfavorable conditions, such as high alkaline and salt concentrations, created by intracanal medicaments.[4,5]

Calcium hydroxide and chlorhexidine (CHX) have been used for a long time for the removal of *E. faecalis*. Recently, nanoparticles have come into the limelight. Nanoparticles are a class of newer medicaments that are hypothesized to have antibacterial effect. They cause disruption of the biofilm due to their nano size and structure. The nano size provides an increased surface area which can absorb other medicaments and exert an antimicrobial effect.[6,7] Silver nanoparticles are commonly used as they show strong bactericidal potential against Gram-positive, Gram-negative, and multidrug-resistant bacteria.[8,9] Silver has an ability to interact with bacterial cell wall leading to structural changes and then damaging the tissue protein. In this study, we compared and evaluated the antibacterial effect of silver nanoparticles alone and the combination of silver nanoparticles with calcium hydroxide and CHX against *E. faecalis*. The study will help us evaluate and find the medicament that is highly efficacious in the removal of *E. faecalis* and hence will lessen the number of endodontic failures.

METHODOLOGY

Teeth preparation

After getting ethical clearance from the Institutional Research Cell, the present study was conducted. The power of the study was calculated, and 140 intact single-rooted teeth with straight canals and mature apices were collected from the department of oral and maxillofacial surgery (freshly extracted due to orthodontic or periodontal reasons). Teeth that had cracks or defects on the external surface, deep carious lesions, with previous restorations, immature apices, multiple roots, curved canals, severe anatomic variations, calcified canals, and teeth with multiple canals were excluded from the study. Intraoral periapical radiographs were taken, and any canal aberration was examined, and teeth that did not comply with the inclusion criteria were excluded from the study. The teeth were cleaned to remove any calculus deposits or soft tissue debris. A safe-sided diamond disc (NMD, India) was used to decoronate the teeth below the cementoenamel junction perpendicular to the long axis. The root length was standardized to 15 mm, and teeth were examined under magnification to assure the absence of defects or cracks. The working length was recorded 1 mm short of the apex (14 mm). This was confirmed by inserting a 15K file (Mani, Inc, Japan) into the canal till the point it became visible from the apex and then withdrawing it 1 mm short of the root apex. Cleaning and shaping were done using a series of ProTaper files (Dentsply Maillefer, Ballaigues, Switzerland) to the F3 master apical file. Seventeen percent ethylenediaminetetraacetic acid (EDTA) in a quantity of 3 ml was used as a lubricant for assisting the file motion inside the canal, and in between the mechanical preparation, 3 ml of 5.25% NaOCl was used as an irrigant. Three milliliters of 17% EDTA followed by 3 ml of NaOCl was used as an irrigant at the end of the biomechanical preparation for 3 min to remove the smear layer. Three milliliters of physiological saline solution was used to complete the final irrigation step. Two layers of nail polish were coated on the outer surface of tooth specimens, and the apex was sealed with self-cure glass ionomer cement. Two milliliter microtubes were taken, and teeth specimens were transferred into them. They were then autoclaved at 121°C for 30 min. Samples were randomly selected, and cultures were obtained to verify sterilization.

Microbiological procedures

Inoculation of specimens with bacteria

E. faecalis (ATCC 29212) as a pure culture was used as the test microorganism. Isolates of bacterial colonies were obtained on blood agar after 24 h of incubation at 37°C and then suspended in 5 ml of brain heart infusion broth (BHI). Colonies were then incubated at 37°C for 4 h. 20 µl of 0.5 McFarland solution of bacterial suspension was transferred into each canal under a laminar flow hood. Then, the microtubes were recapped and sealed using three layers of parafilm and incubated at 37°C for 21 days. 1 ml of freshly prepared BHI broth was placed into the root canals every 3 days. At the end of the incubation period, the medium in the microtube was aspirated aseptically, and the canals were dried using sterile paper points. Bacterial viability and purity were checked in a few randomly picked tubes. The teeth were randomly divided into four experimental groups ($n = 30$) and two control groups ($n = 10$) and subjected to the following intracanal dressings:

- Group 1 – Silver nanocure gel
- Group 2 – Silver nanocure gel + Cavisept gel (1:1)
- Group 3 - Silver nanocure gel + Aveu-Cal gel (1:1)
- Group 4 – Silver nanocure gel + Cavisept gel + Aveu-Cal gel (1:1:1)
- Group 5 - Positive control (specimens were inoculated with *E. faecalis* and left untreated to confirm the presence of infection)
- Group 6 - Negative control (no bacterial contamination of specimens).

Lentulo spirals were used to carry the above-mentioned intracanal medicaments in the root canals of prepared teeth. The orifice of the root canal was covered with sterile aluminum foil. Sterile microtubes were used to keep the teeth specimens, which were then sealed with several layers of parafilm. After the loading of the various medicaments, all the teeth were incubated at 37°C temperature and 100% humidity for 14 days.

Microbiological sampling

All root canal specimens were flushed with 5 ml of sterile saline. 1 mL of 0.5% citric acid as an irrigant was used to neutralize $Ca(OH)_2$ in tooth specimens medicated using calcium hydroxide (Aveu-Cal). 0.5% Tween 80 in 0.07% lecithin along with 5 ml sterile saline was used as an irrigant to neutralize specimens medicated using 2% CHX gel (Cavisept). A sterile #30 paper point was placed into the canal for 60 s, and a microbial sample was taken. The paper point was transferred to the micro test tube containing 1 ml of physiological saline solution and shaken for 30 s on a vortex. A 0.1 ml aliquot of the microbial suspension was seeded on a BHI agar plate. This was then incubated at 37°C for 48 h. Sampling was carried out two times for each group, and the average was analyzed. The number of colony-forming units (CFUs) was recorded using colony counter after 48 h of incubation. Gram staining and colony morphology were used to verify bacterial purity. The results were recorded [Table 1] and subjected to statistical analysis using the Kruskal–Wallis tests.

RESULTS

The statistical analysis of the colony-forming units was done using the Kruskal–Wallis tests using SPSS version 11 Inc (Chicago,Illinois, USA). The test results came out to be significant at $P < 0.05$ ($P = 0.00012$), which indicates that the CFU values we got using different combinations of medicaments are not the same, rather their difference is statistically significant [Figure 1].

Table 1: Bacterial readings

Groups	Mean of CFUs
1 - Silver nanocure gel	76,598
2 - Silver nanocure gel+Cavisept gel (1:1)	71,009
3 - Silver nanocure gel+Aveu-Cal gel (1:1)	52,080
4 - Silver nanocure gel+Cavisept gel+Aveu-Cal gel (1:1:1)	40,263
5 - Positive control (specimens were inoculated with *Enterococcus faecalis* and left untreated to confirm the presence of infection)	105,550
6 - Negative control (no bacterial contamination of specimens)	0

CFUs: Colony-forming units

DISCUSSION

The main purpose of the current study was to evaluate the effectiveness of silver nanoparticles and its various combinations against *E. faecalis* persistent in root canals. *E. faecalis* was used as a test organism as it plays an important role in endodontic retreatment and failure cases. *Enterococcus* has the ability to survive harsh conditions by the formation of a biofilm. Furthermore, this biofilm formation is responsible for the ineffectiveness of various intracanal medicaments.[10-12] The fact that *E. faecalis* is proven to be resistant to variable intracanal medications and is frequently associated with persistent disease after endodontic treatment led to its use for the inoculums in this study.[13,14]

When Silver nanocure gel (Group 1) was used alone, it showed a slight antibacterial effect. This is in contrast with numerous studies where a strong antibacterial effect of Silver nanoparticles was observed against different bacterial strains.[15-17] The variation may be explained due to the differences in the mode of application, concentration, and particle diameter used.

When Group 1 was compared with Group 2, no significant reduction was observed in the colony-forming units. CHX is the most effective antimicrobial agent; its effectiveness can be attributed to its substantivity within the oral cavity; however, the above result can be explained by the fact that the antimicrobial efficacy of CHX is reduced when combined with the other antimicrobials.[18,19]

When Group 1 was compared with Group 3, a significant reduction in colony-forming units was observed. A combination of SNP with $Ca(OH)_2$ was found to be better in comparison to SNP and $Ca(OH)_2$ alone. This finding was in agreement with the results of the previous study.[20]

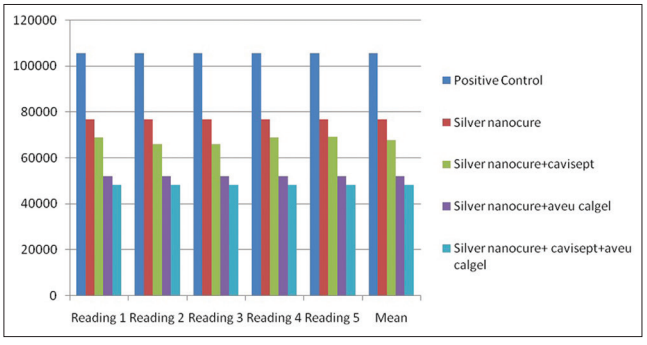

Figure 1: Colony forming units with different medicaments

Possible reasons for this can be (i) the inherent capacity of *E. faecalis* to tolerate an alkaline environment created by $Ca(OH)_2$ and (ii) the creation of pits by SNP in the cell wall of the microorganism leading to disruption of biofilm and increased amount of $Ca(OH)_2$ delivery.

When Group 1 was compared with Group 4, there was a significant reduction in the bacterial colony-forming units. This could be due to the fact that the medicaments when combined together exerted a synergistic effect.[21] This increased the efficiency of the intracanal medicaments, and hence, this led to the maximum eradication of *E. faecalis* from the microbial flora of root canals. This was observed in our study. As per our knowledge, this is the second study done so far in the literature that has tested all three medicaments in combination.

Limitations

The study has an *in vitro* design which is its major drawback. The efficacy of the combinations suggested above needs to be tested *in vivo* to arrive at a concrete finding. The study has its major advantage in the fact that all the medicaments were tested with the latest scientific approach in combination with nanotechnology. The bright future of these small particles is truly wished.

CONCLUSION

The *in vitro* study led to the conclusion that Silver nanocure gel, Cavisept gel, and Aveu-Cal gel when combined in a 1:1:1 ratio yielded the highest antibacterial efficacy. The combination seems to be a perfect choice to defeat most resistant microorganisms from the root canals.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Paredes-Vieyra J, Enriquez FJ. Success rate of single- versus two-visit root canal treatment of teeth with apical periodontitis: A randomized controlled trial. J Endod 2012;38:1164-9.
2. Peters OA, Schönenberger K, Laib A. Effects of four Ni-Ti preparation techniques on root canal geometry assessed by micro computed tomography. Int Endod J 2001;34:221-30.
3. Madhubala MM, Srinivasan N, Ahamed S. Comparative evaluation of propolis and triantibiotic mixture as an intracanal medicament against *Enterococcus faecalis*. J Endod 2011;37:1287-9.
4. Du T, Wang Z, Shen Y, Ma J, Cao Y, Haapasalo M. Effect of long-term exposure to endodontic disinfecting solutions on young and old *Enterococcus faecalis* biofilms in dentin canals. J Endod 2014;40:509-14.
5. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod 2010;36:1030-5.
6. Cheng Z, Al Zaki A, Hui JZ, Muzykantov VR, Tsourkas A. Multifunctional nanoparticles: Cost versus benefit of adding targeting and imaging capabilities. Science 2012;338:903-10.
7. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 2012;112:841-52.
8. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, *et al*. The bactericidal effect of silver nanoparticles. Nanotechnology 2005;16:2346-53.
9. Ayala-Nunez NV, Villegas HH, Turrent LC, Padilla CR. Silver nanoparticles toxicity and bactericidal effect against methicillin resistant *Staphylococcus aureus*: Nanoscale does matter. J Nanobiotechnol 2009;5:2-9.
10. Kishen A. Advanced therapeutic options for endodontic biofilms. Endod Top 2010;22:99-123.
11. Siqueira JF Jr., Rocas IN, Ricucci D. Biofilm in endodontic infection. Endod Top 2010;22:33-49.
12. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. J Endod 2006;32:93-8.
13. Hasselgren G, Olsson B, Cvek M. Effects of calcium hydroxide and sodium hypochlorite on the dissolution of necrotic porcine muscle tissue. J Endod 1988;14:125-7.
14. Orstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. Endod Dent Traumatol 1990;6:142-9.
15. Wu D, Fan W, Kishen A, Gutmann JL, Fan B. Evaluation of the antibacterial efficacy of silver nanoparticles against *Enterococcus faecalis* biofilm. J Endod 2014;40:285-90.
16. Bo D, Kayombo CM. Effect of nanosilver gel, chlorhexidine gluconate, and camphorated phenol on *Enterococcus faecalis* biofilm. Int Sch Res Notices 2014;2014:380278.
17. Bruniera JF, Silva-Sousa YT, Lara MG, Pitondo-Silva A, Marcaccini AM, Miranda CE. Development of intracanal formulation containing silver nanoparticles. Braz Dent J 2014;25:302-6.
18. Halkai KR, Halkai R, Mudda JA, Shivanna V, Rathod V. Antibiofilm efficacy of biosynthesized silver nanoparticles against endodontic-periodontal pathogens: An *in vitro* study. J Conserv Dent 2018;21:662-6.
19. de Souza-Filho FJ, Soares Ade J, Vianna ME, Zaia AA, Ferraz CC, Gomes BP. Antimicrobial effect and pH of chlorhexidine gel and calcium hydroxide alone and associated with other materials. Braz Dent J 2008;19:28-33.
20. 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;40:61-5.
21. Jhamb S, Singla R, Kaur A, Sharma J, Bhushan J. An *in vitro* determination of antibacterial effect of silver nanoparticles gel as an intracanal medicament in combination with other medicaments against *Enterococcus fecalis*. J Conserv Dent 2019;22:479-82.