Journal of Dentistry and Oral Biology

Research Article
Published: 05 May, 2017

Antimicrobial Efficacy of Silver Nanoparticles with and Without Different Antimicrobial Agents against *Enterococcus Faecalis*: Ex Vivo Study

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Abstract

Aim: the aim of the present Ex- Vivo study was to check the antimicrobial efficacy of silver nanoparticles with and without different antimicrobials against *E. faecalis*.

Material and Methods: 126 recently extracted single rooted human teeth were contaminated with *E. faecalis*. The teeth were randomly divided into 5 experimental (n=21) and 1 control group (n=21). Each subgroup was then exposed to different antimicrobials namely Calcium hydroxide (group 1), 2% Chlorhexidine (CHX) (group 2), Silver nanoparticles (SNP) (group 3), SNP with $Ca(OH)_2$ (group4), SNP with 2% CHX (group 5) and saline as control group (group 6). Cultures were made from each group after 24 hours, 7 day and 14 day and colony forming units were counted. The Kruskal-Wallis test was used to compare the study parameters among the groups at 24 hours, 7 days and 14 days.

Results: Significant difference was found in the antimicrobial efficacy of different intracanal medicaments against *E. Faecalis* after 24 hours, 7 days and 14 days. 2% CHX was found to be most effective medicament at 24 hours, 7 days and 14 days against *E. faecalis*. Combination of SNP with 2% CHX and $Ca(OH)_2$ and SNP alone ranked second in their antimicrobial efficacy against *E. faecalis* at 24 hours, 7 days and 14 days respectively.

Conclusion: 2% CHX was more effective as intracanal medicament against *E. faecalis* biofilm in both short and long term duration i.e. at 24 hours, 7 days and 14 days.

Keywords: Antimicrobial agents; Calcium hydroxide; Chlorhexidine; Nanoparticles; *Enterococcus faecalis*

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Received Date: *21 Mar 2017*
Accepted Date: *28 Apr 2017*
Published Date: *05 May 2017*

Citation:
Chandra A, Yadav RK, Shakya VK, Luqman S, Yadav S. Antimicrobial Efficacy of Silver Nanoparticles with and Without Different Antimicrobial Agents against Enterococcus Faecalis: Ex Vivo Study. J Dent Oral Biol. 2017; 2(6): 1047.

Introduction

Successful endodontic treatment depends upon various factors such as chemo mechanical debridement, optimum irrigation, appropriate and specific intracanal medicaments and three dimensional obturation in order to completely seal the root canal system [1]. Mechanical debridement with the help of endodontic files has its own limitations. Past studies have suggested that biomechanical preparation to its maximum efficacy still leaves around 30-35% of the root canal untouched, and thereby the raising concerns about complete root canal debridement [2]. Chemical disinfection through various irrigants and intracanal medicaments such as sodium hypochlorite, chlorhexidine and calcium hydroxide, have also been reported to be insufficient as far as their action against the resistant microbes that cause secondary infection is concerned [2-4].

Most commonly isolated species from the secondary infection of the root canal system is *Enterococcus faecalis* and is reported to be present in more than 60% of the re infection cases [5]. Studies have suggested that resistance incorporated in *E. faecalis* against commonly used intracanal medicaments is because of its ability to form biofilm [6]. Biofilm is a complex extracellular polymeric matrix that protects the bacteria against nutrient deprived and other unfavourable conditions such as high alkaline and salt concentrations created by intracanal medicaments [6,7].

Culture studies have indicated the presence of multiple flora in secondary infection of the root canal system rather than single microorganism. Apart from *E. faecalis* other commonly isolated

Table 1: Comparison of mean change in *E. Faecalis* within the group from 24 hours to 7 and 14 days.

Groups	Mean change (Mean±SD)					
	24 hours to 7 days	p-value[1]	24 hours to 14 days	p-value[1]	7 days to 14 days	p-value[1]
Group 1	21527.27±3188.06	0.0001*	31854.55±4273.87	0.0001*	10327.27±3287.09	0.0001*
Group 2	1629.09±2855.19	0.0001*	4669.09±3013.88	0.0001*	3040.00±2387.01	0.0001*
Group 3	632.73±2577.31	0.0001*	17083.64±2031.03	0.0001*	17716.36±2636.10	0.0001*
Group 4	8530.91±3292.52	0.0001*	4603.64±2505.09	0.0001*	13134.55±3414.49	0.0001*
Group 5	2349.09±3326.05	0.0001*	858.18±3139.83	0.0001*	1490.91±2893.68	0.0001*
Group 6	4690.91±5055.64	0.0001*	1345.45±4813.61	0.0001*	3345.45±6525.61	0.0001*

[1]Wilcoxon rank sum test, *Significant

Table 1 shows the comparison of mean change in *E. Faecalis* within the group from 24 hours to 7 and 14 days. There was significant (p=0.0001) in *E. Faecalis* in all the groups from 24 hours to 7 and 14 days.

microorganism includes candida species [8,9]. Because of the increased evidence suggestive of biofilm mediated resistance of the microflora towards the commonly used intracanal medicaments, a greater emphasis is now being developed materials that can disrupt the biofilm and eliminate the microorganisms from the root canal system.

Nanoparticles are class of newer medicaments which are hypothesised to have antibacterial effect by causing disruption of the biofilm because of their smaller nano size structure. The nano size provides increased surface area which can absorb other medicaments and exert antimicrobial effect [10,11]. They have charged polycationic structure which provides greater interaction with the negatively charged bacterial cell wall and causes more and more of antibiotic interaction with the microorganism [11].

Various nanoparticles have been reported to have antibacterial effect such as Ag, Mg, Zn etc. Silver nanoparticles prevent adhesion of microorganism and biofilm formation and have antimicrobial effect against majority of organisms such as streptococci, fungi, viruses etc. [12]. Because of the promising antimicrobial effect of silver nanoparticles against various microorganisms, present study was conducted with the aim of checking the antimicrobial efficacy of silver nanoparticles with and without different antimicrobial agents such as calcium hydroxide and chlorhexidine against *E. faecalis*.

Material and Methods

Preparation of teeth specimen

The study was conducted after getting ethical clearance from the Research cell, King George's Medical University, Lucknow, India in collaboration with Council of Scientific and Industrial Research (CSIR) Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India.

The study was conducted on 126 single canal freshly extracted human teeth. The teeth were radiographically confirmed to have single canal. The teeth were decoronated at 14mm from the root apex using diamond disc. The working length was established at 1mm short of the apex (13mm) and it was confirmed by inserting 15K file (Dentsply Maillefer, Ballaigues, Switzerland) into the canal till the point it became visible from the apex and then withdrawing it 1mm short of apex. Biomechanical preparation was done using a series of Protaper universal files (Dentsply Maillefer, Ballaigues, Switzerland) to an F3 master apical file. Glyde™ (Dentsply Maillefer, Ballaigues, Switzerland) was used as a lubricant for assisting the file motion inside the canal and in between the mechanical preparation,

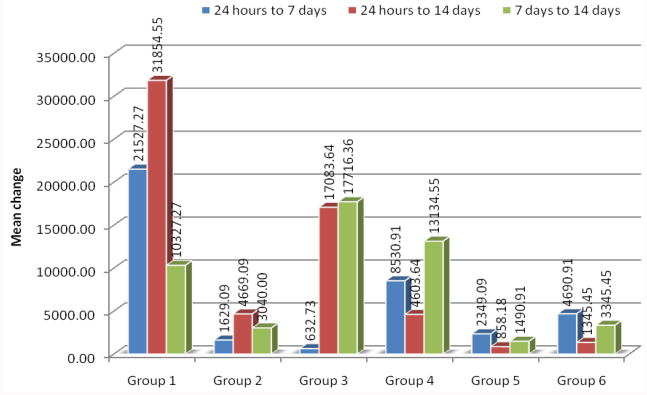

Figure 1: Comparison of mean change in *E. Faecalis* within the group from 24 hours to 7 and 14 days.
The comparison of mean change in *E. Faecalis* within the group from 24 hours to 7 and 14 days. There was significant (p=0.0001) in *E. Faecalis* in all the groups from 24 hours to 7 and 14 days.

3% NaOCl was used an irrigant. 3ml of 17% ethylene diamine tetra-acetic acid (EDTA) [Prevest Denpro Limited, Jammu, India] plus 3ml of NaOCl were used as an irrigant at the end of the biomechanical preparation for 3minutes to remove the smear layer. Final irrigation was performed with 3ml of physiological saline solution. Outer surface of the teeth specimen was coated with 2 layer of nail polish and apex was sealed with self-cure Glass Ionomer cement. The teeth were then transferred into 2-mL microtubes and autoclaved at 121°C for 15 minutes.

Microbiological Procedures

Clinical isolates of *Enterococcus faecalis* were used as the test microorganism. Bacterial colonies isolated for 24 hours were suspended in 5 ml of brain/heart infusion broth (BHI) and then incubated at 37°C for 4 hours.

The 126 root specimens were transferred into sterile 11 cell culture well plates (each plate having 24 wells) under sterile conditions. Roots were mounted in the well plates containing 2% sterile agar media which was allowed to solidify so that root specimens can be stabilized.

10µl of 0.5 McFarland solution of bacterial suspension was transferred into each canal under laminar flow hood under sterile conditions using micro pipettes. After injection, each specimen was entirely submerged in BHI broth and the tubes were incubated an aerobically at 37°C for 24hrs. Then the cell culture plates were recapped and sealed using three layers of parafilm, and were incubated at 37°C

for 21 days. Every alternate day, 10µl of fresh BHI broth was added to ensure viability of the bacteria.

Antimicrobial Activity of the medicaments

At the end of the incubation period, the medium in the well plates was aspirated aseptically and the canals were dried using sterile paper points. Then the teeth were randomly divided into 5 experimental group (n = 21) and one control group (n = 21) and subjected to the following intracanal dressings: Group 1 – Ca(OH)2, Group 2 – 2% CHX, Group 3 ppm–100 ppm Silver Nanoparticles of 10 nm size (SNP), Group 4 – 100 ppm of 10 nm SNP + $Ca(OH)_2$ in 1:1 ratio, Group 5 ppm–100 ppm of 10 nm SNP + 2% CHX in 1:1 ratio and Group 6 (Control i.e. only sterile saline).

The medicaments were carried into the canal using lentulo spirals (Dentsply Maillefer, Ballaigues, Switzerland). The canal orifice was covered with sterile aluminium foil. The teeth then were put in sterile well plates and they were sealed with several layers of parafilm. After the loading of the various medicaments, all the groups were subdivided randomly into three equal subgroups and then incubated for different time periods of 24 hours, 7 days and 14 days at 37 °C temperature and 100% humidity.

Microbiological sampling

Each root canal specimen was flushed with 5mL of sterile saline, irrigated with 1mL of 0.5% citric acid to neutralise $Ca(OH)_2$. The specimen medicated by 2% chlorhexidine solution were neutralized with 0.5% Tween 80 in 0.07% lecithin, and again irrigated with 5 ml sterile saline.

Microbiological samples were obtained by inserting a sterile #30 paper point and placing it into the canal for 60 seconds and then transferring this paper point to the micro test tube containing 1ml of physiological saline solution and shaking it for 30 seconds on a vortex. Then, a 0.1 ml aliquot of the microbial suspension was seeded on a BHI agar plate. All the samplings were carried out two times for each group and the average was analysed. After 48 hours of incubation, the number of colony-forming units (CFUs) was recorded. Bacterial purity was verified using gram staining and colony morphology.

Data analysis

The results are presented in Mean±SD. The Kruskal-Wallis test was used to compare the study parameters among the groups at 24 hours, 7 days and 14 days. The Wilcoxon rank sum test was used to compare the mean change in the study parameters from 24 hours to 7 days and 14 days within the group. The p-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results

Comparison of medicaments after 24 hours

Significant difference was found in the antimicrobial efficacy of different intracanal medicaments against *E. faecalis* after 24 hours. Significantly reduced number of microbiological colonies was recovered on the experimental plates when compared with the control plate. Minimum number of CFU's was found in 2% CHX followed by SNP + 2% CHX group (Table 1 and Figure 1).

Comparison of medicaments after 7 days

Significant difference was found in the antimicrobial efficacy of different intracanal medicaments against *E. faecalis* after 7 days. Significantly reduced number of microbiological colonies was recovered on the experimental plates when compared with the control plate. In the *E. faecalis* group, minimum number of CFU's were found in 2% CHX followed by SNP + $Ca(OH)_2$ group (Table 1 and Figure 1).

Comparison of medicaments after 14 days

Significant difference was found in the antimicrobial efficacy of different intracanal medicaments against *E. faecalis* after 14 days. Significantly reduced number of microbiological colonies was recovered on the experimental plates when compared with the control plate. Minimum number of CFU's were found in 2% CHX followed by SNP group (Table 1 and Figure 1).

Discussion

Complete root canal disinfection is the mainstay of successful endodontic treatment [13]. Major challenges involved in achieving the above mentioned goal are the microorganism induced biofilms and the limitations associated with the currently used intracanal medicaments and irrigants in eliminating the same. Especially microorganisms recovered from the persistent periradicular infection or the secondary infection are found to have biofilm that have high resistance against the commonly used intracanal medicaments [14,15]. Studies have suggested that most common bacteria isolated from the secondary root canal infection is the *E. faecalis* [5]. Hence the present study was conducted with theobjective of checking the antimicrobial efficacy of silver nanoparticles with and without different antimicrobials against *E. Faecalis.*

$Ca(OH)_2$ has been used historically because of its proven antimicrobial property. But its action against *E. faecalis* is very limited. $Ca(OH)_2$ acts by releasing hydroxyl ions and creating alkaline environment [16]. *E. faecalis* because of its resistant nature is able to survive in alkaline environment and easily skip the action of $Ca(OH)_2$ [17]. Hence, the search for a novel intracanal medicament propelled researchers to introduce nanoparticles in the field of endodontics.

Chlorhexidine has a unique property of substantively [18] and has been proven to have efficient antimicrobial action against *E. faecalis* either alone or in combination with other antimicrobials [18-20]. But recently, biofilm formation by the microorganisms is a major obstacle for the action of chlorhexidine [21].

Silver nanoparticles of 10 nm size were used in the current study because smaller size provides more surface area and hence more amount of antimicrobial activity can be anticipated [22,23]. In earlier studies, it was found that nanoparticles of 10nm or lesser size are likely to have more antimicrobial effect [23]. Although various other nanoparticles are also reported to have antimicrobial activity, in the present study specifically silver nanoparticles were used because of its ability to react with thiol group of DNA molecule of bacteria, causing its condensation and cell death [24].

In the present study, Silver nanoparticles were used as vehicle for Calcium hydroxide and chlorhexidine and the antimicrobial efficacy of the combinations was compared with respect to the individual antimicrobials after 24 hours, 7 day and 14 days. 24 hours' evaluation showed chlorhexidine alone group to be significantly better in comparison with the other medicaments against *E. faecalis* followed by combination of SNP and 2% CHX. Above result can be explained by the fact that antimicrobial efficacy of CHX is reduced when combined with the other antimicrobials [25]. To the best of the knowledge, when the present study was conducted no other available

study had evaluated the effect of combination of silvernano particles with CHX against *E. faecalis*. Combination of SNP with $Ca(OH)_2$ was found to better in comparison to SNP and $Ca(OH)_2$ alone. This finding was in agreement with the findings of other studies which suggested combination of SNP and $Ca(OH)_2$ to be better in comparison to SNP and $Ca(OH)_2$ alone [26]. Possible reason for this can be (i) Inherent capacity of *E. faecalis* to tolerate alkaline environment created by $Ca(OH)_2$ and (ii) 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.

In the present study, evaluation after 7 days showed, 2% CHX to be more effective followed by combination of SNP and $Ca(OH)_2$ against *E. faecalis*. Results agree with the findings of the similar studies reported earlier [26,27]. In those study, authors found significantly less CFU's in the group having combination of SNP and $Ca(OH)_2$ in comparison to $Ca(OH)_2$ alone group.

In the present study, 14 days' evaluation showed 2% CHX and $Ca(OH)_2$ to be better against *E. faecalis* rather than their combination with SNP. Similar results were reported in an earlier study where the authors noticed combination of CHX and $Ca(OH)_2$ to be less effective in comparison to the individual medicaments [28]. Very limited studies exist which have evaluated the effect of combination of medicaments against *Candida albicans*. Results of the present study can be attributed to the lesser availability of free $Ca(OH)_2$ and CHX when combined with SNP over 14 days of time.

Present study is possibly one of the first studies which have evaluated the antimicrobial efficacy of silver nanoparticles in combination with chlorhexidine against *E. faecalis* and *Candida albicans*. Size and concentration of silver nanoparticles used in the present study was very small i.e. 10 nm and 100 ppm respectively which has not been reported in dentistry till now. Although concerns regarding the cytotoxicity of silver nanoparticles have been raised, recently in a study by Gomes Filhalo. it was seen that 47 ppm of silver nanoparticles were biocompatible in comparison to 2.5% NaOCl against fibrous connective tissue of rat.

Conclusion

The results from the current study indicate the potential antimicrobial efficacy achieved when silver nanoparticles were combined with 2% CHX and $Ca(OH)_2$ against *E. faecalis* over different time periods. There was significant reduction of microbial colonies achieved with the nanoparticles combination indicating at potential to be used as inter appointment medicament. Although 2% CHX was found to have significantly better results in comparison to the silver nanoparticles combination in the present Ex Vivo setup, more studies with In Vivo conditions are required to validate the findings of present study.

Acknowledgement

No funding was provided what so ever for conducting the present study. Authors would like to thank Dhananjay Kumar Singh, PhD scholar, Molecular Bioprospection Department, Council of Scientific and Industrial Research- Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India for his contribution towards the microbiological aspect of the study.

Conflict of Interest

Authors deny any financial support or relationships that may pose conflict of interest by disclosing any financial arrangements with a company whose product figures have been mentioned in the submitted manuscript or with a company making a competing product, or any conflict relating to technology or methodology.

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(9):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(3):221-30.
3. Salzgeber RM, Brilliant JD. An in vivo evaluation of the penetration of an irrigating solution in root canals. J Endod. 1977;3(10):394-8.
4. Vassiliadis LP, Sklavounos SA, Stavrianos CK. Depth of penetration and appearance of Grossman sealer in the dentinal tubules: an in vivo study. J Endod. 1994;20(8):373-6.
5. Neelakantan P, Cheng CQ, Mohanraj R, Sriraman P, Subbarao C, Sharma S. Antibiofilm activity of three irrigation protocols activated by ultrasonic, diode laser or Er:YAG laser *in vitro*. Int Endod J. 2015;48(6):602-10.
6. 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(4):509-14.
7. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod. 2010;36(6):1030-5.
8. Sen BH, Safavi KE, Spångberg LS. Colonization of Candida albicans on cleaned human dental hard tissues. Arch Oral Biol. 1997;42(7):513-20.
9. Sen BH, Piskin B, Demirci T. Observation of bacteria and fungi in infected root canals and dentinal tubules by SEM. Endod Dent Traumatol. 1995;11(1):6-9.
10. 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(6109):903-10.
11. Rai MK1, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol. 2012;112(5):841-52.
12. Ping Gong, Huimin Li, Xiaoxiao He, Kemin Wang, Jianbing Hu, Weihong Tan, et al. Preparation and antibacterial activity of Fe3O4@Ag nanoparticles. Nanotechnology. 2007;18(28):604-11.
13. Siqueira JF Jr, Araújo MC, Garcia PF, Fraga RC, Dantas CJ. Histological evaluation of the effectiveness of five instrumentation techniques for cleaning the apical third of root canals. J Endod. 1997;23(8):499-502.
14. Kishen A. Advanced therapeutic options for endodontic biofilms. Endod Topics. 2010;22:99-123.
15. Siqueira JF Jr, Rocas IN, Ricucci D. Biofilm in endodontic infection. Endod Topics. 2010;22:33-49.
16. Siqueira JF Jr, Lopes HP. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. Int Endod J. 1999;32(5):361-9.
17. Stuart C, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32(2):93-8.
18. Basrani B, Tjäderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, et al. Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against Enterococcus faecalis *in vitro*. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96(5):618-24.
19. Gomes BP, Souza SF, Ferraz CC, Teixeira FB, Zaia AA, Valdrighi L, et

al. Effectiveness of 2% chlorhexidine gel and calcium hydroxide against Enterococcus faecalis in bovine root dentine *in vitro*. Int Endod J. 2003;36(4):267-75.

20. Shen Y, Qian W, Chung C, Olsen I, Haapasalo M. Evaluation of the effect of two chlorhexidine preparations on biofilm bacteria *in vitro*: a three-dimensional quantitative analysis. J Endod. 2009;35(7):981-5.
21. Shen Y, Stojicic S, Haapasalo M. Antimicrobial efficacy of chlorhexidine against bacteria in biofilms at different stages of development. J Endod. 2011;37(5):657-61.
22. Guzman M, Dille J, Godet S. Synthesis and antibacterial activity of silver nanoparticles against gram-positive and gram-negative bacteria. Nanomedicine. 2012;8(1):37-45.
23. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. The bactericidal effect of silver nanoparticles. Nanotechnology. 2005;16(10):2346-53.
24. Castellano JJ, Shafii SM, Ko F, Donate G, Wright TE, Mannari RJ, et al. Comparative evaluation of silver-containing antimicrobial dressings and drugs. Int Wound. 2007;4(2):14-22.
25. 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(1):28-33.
26. 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.
27. Afkhami F, Pourhashemi SJ, Sadegh M, Salehi Y, Fard MJ. Antibiofilm efficacy of silver nanoparticles as a vehicle for calcium hydroxide medicament against Enterococcus faecalis. J Dent. 2015;43(12):1573-9.
28. Ballal V, Kundabala M, Acharya S, Ballal M. Antimicrobial action of calcium hydroxide, chlorhexidine and their combination on endodontic pathogens. Aust Dent J. 2007;52(2):118-21.