|| ISSN(online): 2589-8698 || ISSN(print): 2589-868X ||
International Journal of Medical and Biomedical Studies
Available Online at www.ijmbs.info
PubMed (National Library of Medicine ID: 101738825)
Index Copernicus Value 2018: 75.71
Original Research Article
Volume 3, Issue 12; December: 2019; Page No. 23-28

NANO-ENGINEERED IRRIGATING SOLUTIONS AND LASERS – AN ANTIBACTERIAL STUDY AGAINST *ENTEROCOCCUS FAECALIS*

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Article Info: Received 10 November 2019; Accepted 8 December. 2019
DOI: https://doi.org/10.32553/ijmbs.v3i12.771
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Conflict of interest: No conflict of interest.

Abstract

Background: *Enterococcus faecalis (E. faecalis)* is the most commonly detected micro-organism in asymptomatic and persistent endodontic infections. Thorough disinfection of the root canal is more important than proper shaping for a successful endodontic treatment. This study is an attempt to implement nanoparticles which have proven anti-bacterial efficacy as irrigating solutions against *E. faecalis*.

Aim: To compare and evaluate the anti-bacterial efficacy of 5% sodium hypochlorite (NaOCl), 0.2% chitosan nanoparticles solution (ChNP) and 0.01% silver nanoparticles solution (AgNP) against *E.faecalis* with and without diode laser activation.

Materials and Methods: 70 single rooted mandibular premolars were included in this study. Access opening, working length determination and biomechanical preparation were standardized. Samples were embedded in putty material inside an eppendorf tube to simulate periodontal ligament and autoclaved followed by inoculation of E.faecalis (MTCC 439) and incubated at 37℃ for 7 days. The samples were divided into 7 groups (10 samples in each group). Group 1- No treatment (Negative control), group 2 - 5% NaOCl without activation (Positive control), group 3 - 5% NaOCl with diode laser activation, group 4- 0.01% AgNP without activation, group 5- 0.01% AgNP with diode laser activation, group 6- 0.2% ChNP without activation and group 7- 0.2% ChNP with diode laser activation. A final rinse of the respective irrigants and activation were performed, followed by counting the colony forming units. Statistical analysis used were one-way analysis of variance (ANOVA) followed by Tukey's post-hoc tests. Results showed that diode laser in combination with 5% NaOCl and 0.2% ChNP had significant effects in the reduction of microbial colony counts of *E. faecalis* in comparison to other experimental groups.

Keywords: Nanoparticles, Irrigation, LASER.

Introduction

Biofilm is a microbial sessile community which contains cells attached to a substratum, within a matrix of extracellular polysaccharides and it shows alterations in phenotype, growth and gene expression.(1) E. *faecalis* present in the biofilms plays a significant role in the primary and persistent pathologies in the root canal system.(2) This was attributed to multiple factors, especially its capacity to survive in environments of nutritional deprivation and when commensality with other bacteria is meagre.(3) Ultimately, the success of root canal treatment relies on the elimination of the microbial film, which can be challenging due to the invasion of the microorganism into the dentinal tubules and the anatomic complexities of the root canal. *E. faecalis,* a gram positive facultative anaerobe, is reported to be present in approximately 20-30% of primary infections and 67-77% of secondary infections.(4) Hence, thorough disinfection of the root canals is highly essential to overcome the ill-effects of these microbes. Root canal disinfection includes mechanical cleaning and irrigation using solutions with antimicrobial potential.

Sodium hypochlorite is considered as the gold standard root canal irrigant, because of its antimicrobial efficacy and tissue dissolution capacity.(5)

Antimicrobial delivery system through nanoparticles is one of the innovations to improve the characteristics of antibacterial agents used in root canal treatment.(6) Nanomaterials are particles with external dimensions of 1–100 nm, large surface/area mass ratio and increased chemical reactivity, which enable them to interact to a greater extent with the

negatively charged surface of bacterial cells, resulting in enhanced antimicrobial activity.(7,8) Nanoparticles have also been studied in the endodontic field in an attempt to reduce E. faecalis adherence to dentine, eliminate biofilms and enhance root canal disinfection of dentinal tubules.(6)

Silver nanoparticles are capable of attaching to and penetrating into the cell walls of both Gram-positive and Gram-negative bacteria, disturbing cell function by releasing silver ions; thus, they are used for the treatment and prevention of drug-resistant microorganisms and inhibition of the biofilm formation.(7)

Chitosan, a primary component of crustacean exoskeletons procured by alkaline deacetylation from chitin is a cationic biopolymer,(9) which is known to remineralize the demineralized structure with its functional phosphate groups and combines with calcium ions for crystal nucleation, leading to the formation of a calcium phosphate layer. It improves the resistance of the dentinal surface to degradation by collagenase.(10) Furthermore, chitosan presents with biocompatibility, chelating capacity and also antimicrobial effects against a broad range of gram-positive and gram-negative bacteria as well as fungi.(11)

Lasers being another phenomenal field, have excellent bactericidal effect.(12) Diode lasers emit radiation within the visible (mostly 660 nm) and infrared (810 to 980 nm) range of the electromagnetic spectrum. Because of the higher absorption coefficient in water (0.68 cm-1), diode lasers have lower penetration depth into the dentine (up to 750 μm) compared to Nd:YAG laser.(13) There are only few data about the antimicrobial effectiveness of diode lasers in root canal treatment available in the literature so far.

Thus, the aim of this study is to compare and evaluate the anti-bacterial efficacy of 5% NaOCl, 0.01% silver nanoparticles (AgNP)(14) and 0.2% chitosan nanoparticles (ChNP)(15) solutions against *E.faecalis*, with and without laser activation.

Materials and Methods

Specimen preparation

Seventy non-carious, extracted single-rooted premolars were used. The teeth presented straight root canals, fully formed apices and root canal length was standardized to 12mm by decoronating with a diamond disc. Apical patency was established with a #10-K file (Mani Inc., Japan). The orifice enlargement was done using #6, 5, 4 Gates Glidden drills (Dentsply Maillefer, USA), followed by biomechanical preparation upto F3 (Protaper gold, Dentsply Maillefer, USA), 1 mm short of the apex i.e.,working length [WL]. Intermittent 5% NaOCl (Prime Dental Products Pvt., Ltd., India), 0.9% saline (Fresenius Kabi Private Limited, India), 17% EDTA (RC Help, Prime Dental Products Pvt. Ltd., India) irrigation were carried out between each instrumentattion. The apex of all specimens were made impermeable with two layers of epoxy adhesive (LOCTITE Pvt., Ltd., India).(16) To obtain a closed root canal system that mimicked clinical *in vivo* scenarios and to ensure easy handling, a customized model was fabricated for each tooth by mounting into a 1.5-mL polypropylene Eppendorf tube (Eppendorf Private Limited, India) filled with silicone rubber impression material.(17) These specimens were then autoclaved at 121 °C, 15 lbs for 30 minutes.

Biofilm formation

Pure standard strain of MTCC 439 Enterococcus faecalis (CSIR Institute of Microbial Technology, Chandigarh, India) was inoculated in 5ml of brain heart infusion (BHI) broth (HI Media, India) for 24 hrs at 37℃. The cell suspension was adjusted spectrophotometrically to match the turbidity of 3.0 × 10^8 CFU/mL (equivalent to 1.0 McFarland standard). Under laminar air flow chamber ensuring asepsis, 0.1-mL aliquots of *E. faecalis* culture was inoculated into each canal with a sterile needle. The medium was replaced every 24 h, for seven days, with fresh BHI broth (100μl). The entire set-up was incubated at 37°C during the experimental period.

Groups

Seventy root segments were randomly divided in seven groups (*n = 10*).

Group1- No treatment (Negative control)

Group 2- 5% NaOCl (Prime dental products Pvt. Ltd., India) without activation (Positive control)

Group 3- 5% NaOCl with LASER activation (IMDSL Automatic Dental Diode Laser - 10W, Macromed, India)

Group 4- 0.01% AgNP solution (Nano Research, Bihar) without activation

Group 5- 0.01% AgNP solution with LASER activation

Group 6- 0.2% ChNP solution (Everest Biotech, Bangalore) without activation

Group 7- 0.2% ChNP solution with LASER activation

For all the non-laser activation groups, the respective irrigating solutions were used. A sterile needle (26 gauge) was introduced into the root canal until 1 mm short of WL and 5 mL of the specific solution was irrigated, for 5 min. Thereafter, the root canals were washed with 2 mL of sterile saline solution.

For the laser activation groups, a sterile needle was introduced into the root canal until 1 mm short of the WL and 2.5 mL of the specific solution was injected, for 2 min. LASER activation by diode laser, delivered by 400 µm fiber tip kept 1-2 mm away from the apex was activated at 25 uJ Energy; 1 W power; Pulse mode; 20 sec- 3 cycles in sweeping motion from apical to coronal third for 1 min. Then, irrigation was done with respective irrigating solutions (2.5 ml) again for 2 min. Finally the canals were flushed with 2 ml of sterile saline solution.

Colony counting

Immediately after each irrigation protocol, three #35 sterile absorbent paper points (META BIOMED INC., USA) were consecutively inserted into the root canals until the WL. After 1 min, they were transferred to sterile test tubes containing 1 mL of sterile BHI broth. All tubes were vortex mixed for 1 min to dislodge bacteria from paper points. The bacterial suspension was serially diluted. Aliquot portions (100 µL) of 1000-fold and 10,000-fold dilution were taken from each sample and pipetted in triplicate onto the surface of BHI agar plates, which were incubated at 37°C for 24 h. Then, the number of Colony Forming Units (CFU) per plate was determined.

Statistical Analysis

Data was tabulated and the results were presented in mean±SD. One way analysis of variance followed by Tukey's post-hoc tests were used to compare CFU among the groups. The *p*-value<0.05 was considered significant. All the analysis was carried out on SPSS 16.0 version (Chicago, Inc., USA).

Results

Figures 1- 7 show the CFU of various groups. There was significant (p=0.01) difference in CFU among the groups (p<0.05). Table 1 and graph 1 show the inter group comparison on the basis of colony forming units. The lowest CFU were noted in Groups 3 and 7 (NaOCl + Laser and ChNP + Laser) and highest CFU were noted in Group 4 (AgNP) among experimental groups. Intergroup comparison between Group 4 and 5 (AgNPs without and with laser) and other groups were also found to be significant. Although significant differences were noted among all the groups (p<0.05) but inter group comparison between group 3 (NaOCl + laser) and group 7 (ChNPs + laser) was found to be insignificant (p>0.05).

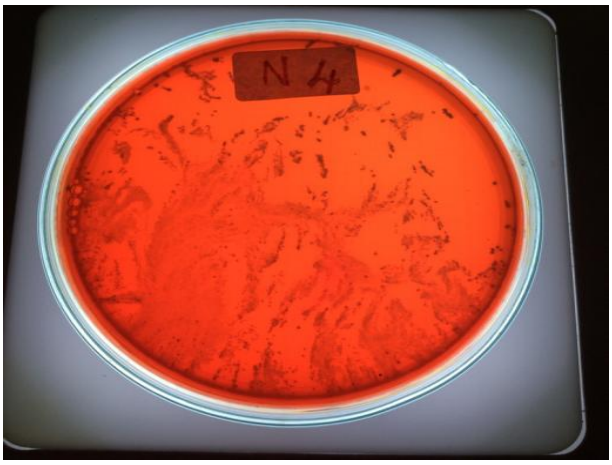


Fig 1: No treatment

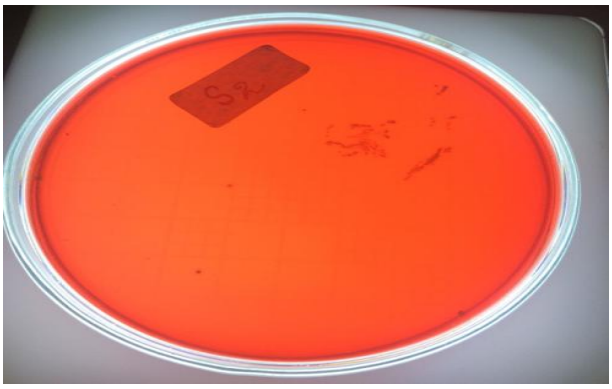


Fig 2: NaOCl,

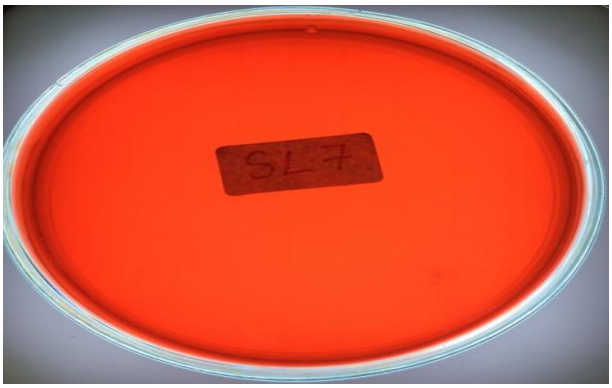


Fig 3: NaOCl + Laser

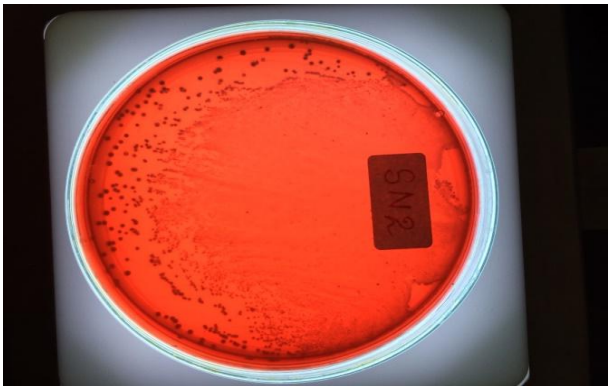


Fig 4: AgNP,

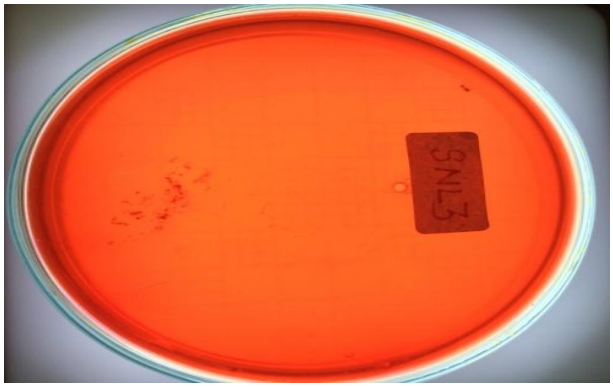


Fig 5: AgNP + Laser

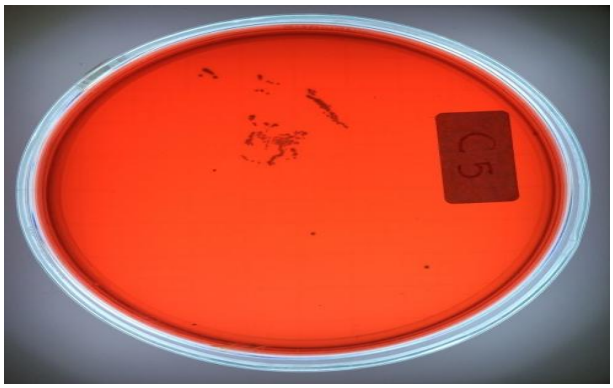


Fig 6: ChNP.

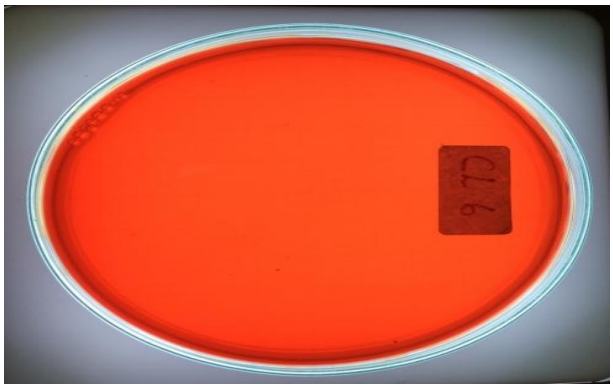
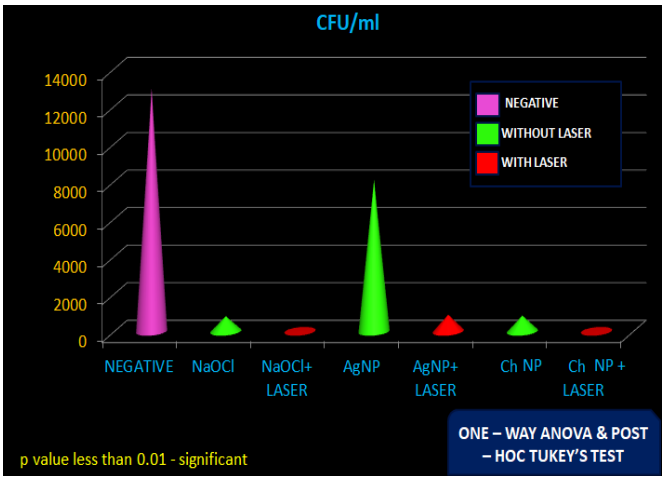


Fig 7: ChNP + Laser

Table 1: CFU of various groups

GROUPS	COLONY FORMING UNITS (CFU)
1 – No treatment	12306
2- NaOCl	694
3- NaOCl + Laser	23
4- AgNP	7146
5- AgNP + Laser	836
6- ChNP	721
7- ChNP + Laser	17



Graph 1: CFU of *E.faecalis* after treatment with various irrigants

Discussion

The major challenges faced by endodontists in routine practice are attributed to the complexities of the root canal anatomy, polymicrobial nature of infection and biofilm formation by microbes. *E. faecalis*, a gram-positive facultative anaerobe can be encountered even in a perfectly obturated root canal and produce the most resistant biofilm which is substantiated in any study that had evaluated the antimicrobial efficacy of root canal irrigants.(18) Hence in the present study *E. faecalis* was used as the test microorganism against which the antimicrobial efficacy of irrigants was evaluated.

NaOCl, especially with high concentrations has a great amount of undissociated hypochlorous acid (HClO) and chloride ions in solution, which is directly related to its disinfecting efficiency.(19) 5% NaOCl was used because of its high efficacy.

Nanotechnology created a great revolution and impact on all aspects of health including the dentistry by producing functional materials which exert their antimicrobial effect by interacting with the negatively charged bacterial cell wall.(8)

AgNP interact with the cell membrane, increase permeability and prevent DNA replication of bacteria.(6,7) Though they have proven antibacterial properties, higher concentrations may be toxic to the host cells due to their small size, chemical composition, surface properties and nonspecific oxidative damages.(20) In this study, there was inadequate interaction period between positively charged AgNP and negatively charged bacterial cells and this might be the reason for their inability to reduce the microbes effectively.(21) When AgNPs were used as medicament for 7 days, adequate interaction occurred between the positively charged nanoparticles and resident biofilm bacteria/structure, resulting in marked destruction of biofilm structure and killing of biofilm bacteria.(6,21)

Chitosan nanoparticles are good antibacterial agents aiding in destruction of biofilms of root canal.(8) The pronounced antibacterial efficacy of cationic nanoparticles might be due to the fact that positively charged nanoparticles electrostatically interact with the negatively charged bacterial cells, resulting in altered cell permeability, leakage of intracellular components and killing microbes.(22) Cationic antibacterial nanoparticulates such as Zinc oxide(ZnO)-NP, Ch/ZnO-NP, or Ch-layer-ZnO-NP significantly inhibited bacterial adherence to dentin, which, in turn, would prevent bacterial recolonization and biofilm formation.(8)

Diode lasers have lower thermal risks and penetration depth into the dentine upto 750 µm.(13) Looking back at the literature, there are only few data about the antimicrobial effectiveness of diode lasers in endodontics. A 97.56% reduction in the amount of bacteria using a 830-nm diode laser with output power of 1.5 W and claimed that diode laser can be considered as an alternative technique for root canal disinfection.(23) Diode laser application after smear layer removal could help in occluding the dentinal tubules particularly in the apical third area which will help in decreasing the risk of reinfection.(24) The results of a study by Mehrvarzfar et al is in accordance with this study stating that diode laser has the potential to significantly reduce the microbes when compared with the non-laser groups.(25)

The limitations of this study were:

Live and dead microbes or penetration depth of microbes are unknown, since advanced technologies or instrumentations were not used. Further in-vivo studies are necessary to evaluate the efficacy of irrigants and diode lasers against polymicrobial scenario. Further studies are necessary to know the effect of nanoparticles on viable tissues.

Conclusion

Diode laser in combination with 5% NaOCl and 0.2% ChNP solution had significant effects in the reduction of microbial colonies of *E. faecalis* in comparison to other experimental groups. It was also evident that all the laser activated groups, irrespective of the solutions, had statistically significant results when compared to their non- laser counterparts.

References

1. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002 Apr;15(2):167-93. Review.
2. Neelakantan P, Romero M, Vera J, Daood U, Khan A, Yan A, et al. Biofilms in Endodontics—Current Status and Future Directions. Int J Mol Sci. 2017 Aug 11;18(8):1748.
3. Stuart C, Schwartz S, Beeson T, Owatz C. Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment. J Endod. 2006 Feb;32(2):93–8.
4. Rocas I, Siqueirajr J, Santos K. Association of Enterococcus faecalis With Different Forms of Periradicular Diseases. J Endod. 2004 May;30(5):315–20.
5. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in Endodontics. Dent Clin North Am. 2010 Apr;54(2):291–312.
6. Samiei M, Farjami A, Dizaj SM, Lotfipour F. Nanoparticles for antimicrobial purposes in Endodontics: A systematic review of in vitro studies. Mater Sci Eng C. 2016 Jan;58:1269–78.
7. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria: Activity of silver nanoparticles against MDR bacteria. J Appl Microbiol. 2012 May;112(5):841–52.
8. 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. 2008 Dec;34(12):1515–20.
9. Sinha VR, Singla AK, Wadhawan S, Kaushik R, Kumria R, Bansal K, et al. Chitosan microspheres as a potential carrier for drugs. Int J Pharm. 2004 Apr;274(1–2):1–33.
10. Shrestha A, Friedman S, Kishen A. Photodynamically Crosslinked and Chitosan-incorporated Dentin Collagen. J Dent Res. 2011 Nov;90(11):1346–51.
11. No H. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int J Food Microbiol. 2002 Mar 25;74(1–2):65–72.

12. Moritz A, Schoop U, Goharkhay K, Jakolitsch S, Kluger W, Wernisch J, et al. The Bactericidal Effect of Nd:YAG, Ho:YAG, and Er:YAG Laser Irradiation in the Root Canal: An *in Vitro* Comparison. J Clin Laser Med Surg. 1999 Jan;17(4):161–4.

13. De Moura-Netto C, de Freitas Carvalho C, de Moura AAM, Davidowicz H, Antoniazzi JH. Influence of Nd:YAG and Diode Laser Irradiation on Apical Sealing When Associated with AH Plus and EndoREZ Endodontic Cements. Photomed Laser Surg. 2007 Oct;25(5):413–7.

14. Kushwaha V, Yadav R, Tikku A, Chandra A, Verma P, Gupta P, et al. Comparative evaluation of antibacterial effect of nanoparticles and lasers against Endodontic Microbiota: An in vitro study. J Clin Exp Dent. 2018;e1155–60.

15. Silva PV, Guedes DFC, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: a new solution for removal of smear layer after root canal instrumentation. Int Endod J. 2013 Apr;46(4):332–8.

16. De Almeida J, Cechella B, Bernardi A, de Lima Pimenta A, Felippe W. Effectiveness of nanoparticles solutions and conventional endodontic irrigants against Enterococcus faecalis biofilm. Indian J Dent Res. 2018;29(3):347.

17. Liu Y, Guo L, Li Y, Guo X, Wang B, Wu L. In vitro comparison of antimicrobial effectiveness of QMix and other final irrigants in human root canals. Sci Rep. 2016 Nov;5(1):17823.

18. Siqueira JF, Rôças IN, Ricucci D. Biofilms in endodontic infection: Biofilms and apical periodontitis. Endod Top. 2010 Mar;22(1):33–49.

19. Vianna ME, Gomes BPFA, Berber VB, Zaia AA, Ferraz CCR, de Souza-Filho FJ. In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endodontology. 2004 Jan;97(1):79–84.

20. Shrestha A, Kishen A. Antibacterial Nanoparticles in Endodontics: A Review. J Endod. 2016 Oct;42(10):1417–26.

21. 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 Feb;40(2):285–90.

22. Rabea EI, Badawy ME-T, Stevens CV, Smagghe G, Steurbaut W. Chitosan as Antimicrobial Agent: Applications and Mode of Action. Biomacromolecules. 2003 Nov;4(6):1457–65.

23. Ashofteh K, Sohrabi K, Iranparvar K, Chiniforush N. In vitro comparison of the antibacterial effect of three intracanal irrigants and diode laser on root canals infected with Enterococcus faecalis. 2014;6(1):5.

24. Parirokh M, Eghbal MJ, Asgary S, Ghoddusi J, Forghani F, Shahravan A. Effect of 808nm diode laser irradiation on root canal walls after smear layer removal: A scanning electron microscope study. 2007;2(2):6.

25. Mehrvarzfar P, Saghiri MA, Asatourian A, Fekrazad R, Karamifar K, Eslami G, et al. Additive effect of a diode laser on the antibacterial activity of 2.5% NaOCl, 2% CHX and MTAD against Enterococcus faecalis contaminating root canals: an in vitro study. J Oral Sci. 2011;53(3):355–60.