Original Article

Effectiveness of silver–graphene oxide nanoparticle against *Enterococcus faecalis* biofilms in the root canal

Sadhana Majhi, Elkanti Soujanya, Mamta Kaushik, X. Nagamaheshwari, Yesha Yadav, Simran Kaur

Department of Conservative Dentistry and Endodontics, Army College of Dental Sciences, Secunderabad, Telangana, India

Abstract

Aim: This study aims to assess the effectiveness of silver–graphene oxide (AgGO) nanoparticles against *Enterococcus faecalis* biofilm and smear layer in comparison with 3% sodium hypochlorite (NaOCl) and 2% chlorhexidine (CHX) using passive ultrasonic irrigation (PUI) technique.

Subjects and Methods: Eighty single-rooted teeth were collected, sterilized, and prepared for this study. *E. faecalis* was inoculated into the root canals and incubated for 21 days. Teeth were divided into four groups for irrigation: Group I (0.25% AgGO), Group II (3% NaOCl), Group III (2% CHX), and Group IV (0.9% saline), followed by PUI. Microbiological evaluation was performed by collecting bacterial samples and quantifying colony-forming units/ml. Scanning electron microscope was used to assess biofilm and smear layer removal.

Statistical Analysis Used: Kruskal–Wallis test, Bonferroni correction, intraclass correlation test.

Results: Antibacterial activity and smear layer removal were highest in Group II followed by Group I and Group III which showed similar results. Group IV showed the least results.

Conclusions: 3% NaOCl was the most effective solution in terms of antibacterial activity and smear layer removal. 0.25% AgGO and 2% CHX showed moderate effectiveness in removing *E. faecalis* biofilms and smear layer which suggests that both have potential as alternative irrigants.

Keywords: Antibacterial activity; *Enterococcus faecalis* biofilm; passive ultrasonic irrigation; silver–graphene oxide nanoparticles

INTRODUCTION

Microbes are responsible for pulpal and periradicular diseases. Oral microbes can form biofilm on a variety of surfaces but dominantly on soft and hard tissues. The control of oral biofilms becomes imperative in the quest to preserve oral health and prevent gingivitis, dental caries, periodontitis, pulpal, and periapical diseases.[1]

Address for correspondence:
Dr. Elkanti Soujanya,
Department of Conservative Dentistry and Endodontics, Army College of Dental Sciences, Secunderabad, Telangana, India.
E-mail: soujanyae.17@gmail.com

Date of submission : 28.09.2024
Review completed : 24.10.2024
Date of acceptance : 11.12.2024
Published : 13.01.2025

Access this article online

Quick Response Code:

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

DOI: 10.4103/JCDE.JCDE_701_24

Biofilm exists in fins, isthmuses, apical ramifications, and auxiliary canals. These areas may further experience an accumulation of dentin debris from instrumentation that is hypothesized to act as protection for the biofilm.[2]

Enterococcus faecalis, an anaerobic Gram-positive coccus, is found more often in patients with unsuccessful endodontic treatment than in cases of primary infection.[3]

The smear layer is a nonhomogeneous substratum of coagulated proteins, organic and inorganic particles, blood cells, bacteria, and fungi that adheres weakly to the root canal walls.

How to cite this article: Majhi S, Soujanya E, Kaushik M, Nagamaheshwari X, Yadav Y, Kaur S. Effectiveness of silver–graphene oxide nanoparticle against *Enterococcus faecalis* biofilms in the root canal. J Conserv Dent Endod 2025;28:27-32.

Microorganisms in the smear layer may survive instrumentation and then reinfect the canal.[4]

The primary objectives of endodontic therapy are to thoroughly disinfect the root canal system and to prevent reinfection.[5]

Sodium hypochlorite (NaOCl) is the most frequently used root canal irrigant due to its excellent antibacterial action, especially against biofilm and its components and pulp tissue remnants. Its potent oxidant property and cytotoxicity toward periapical tissue can adversely affect dentin mechanical characteristics.[6]

Chlorhexidine (CHX) is an alternative irrigant to NaOCl due to its broad-spectrum antibacterial activity with considerably reduced toxicity than NaOCl. It has a substantivity property that eventually leads to persistent antimicrobial activity. The absence of tissue-dissolving ability of CHX is a huge drawback.[5]

Infection control strategies incorporating nanoparticles (NPs) have gained attention for their functional characteristics. It has been reported that silver NPs (AgNPs) enhance antibacterial activity in comparison with conventional materials based on Ag.[7]

Graphene oxide (GO) exhibits inhibition of growth against a number of bacterial species and is regarded as a quite potential material for biological purposes. It has increased binding ability and enhances synergistic antibacterial activity.[7]

Effective irrigant delivery and agitation are essential for successful endodontic treatment. Passive ultrasonic irrigation (PUI) enhances cleaning by removing more planktonic bacteria, organic tissue, and dentine debris than conventional syringe irrigation.[8]

Till date, there is no reported literature on the evaluation of the effectiveness of Ag–graphene oxide (AgGO) NP against *E. faecalis* biofilms and smear layers in the root canal. Therefore, this study aimed to assess the effectiveness of AgGO nanoparticles against *E. faecalis* biofilm and smear layer in comparison with 3% NaOCl and 2% CHX using PUI technique.

The null hypothesis of this study is that there is no significant difference in the efficacy of 3% NaOCl, 2% CHX, and 0.25% AgGO NPs.

SUBJECTS AND METHODS

The study was approved by the institutional ethical committee (ACDS/IEC/123/2022). Eighty single-rooted teeth that were extracted for orthodontic or periodontal reasons were used. Teeth with single canals and complete apices were included; those with cracks, caries, prior treatment, or anomalies were excluded. Teeth were collected, cleaned, and stored in 0.01% NaOCl (Prime Sodium Hypochlorite, Maharashtra, India).

Preoperative radiographs were taken to confirm single canals and the absence of pathology. Samples were autoclaved for 15 min at 121°C to ensure sterilization of the extracted teeth before initiation of the procedure. Teeth were decoronated and roots standardized to 15 mm. Size 10 K-files were used to access the root canals (Mani, Tochigi, Japan) and prepared using ProTaper Universal (Dentsply, Maillefer, Ballaigues, Switzerland) rotary files till F3, with 3% NaOCl (Prime Sodium Hypochlorite, Maharashtra, India) irrigation and 17% ethylenediaminetetraacetic acid (EDTA) (Anabond Desmear, Bengaluru, India) for smear layer removal. Canals were cleaned with distilled water, dried, and apex sealed with type II glass ionomer cement (GC Gold label 2 Universal Restorative, Loyang Way, Singapore). Roots were coated with nail varnish to prevent bacterial leakage and placed in Eppendorf tubes (Micro Centrifuge Tube 1.5 ml, Badli, New Delhi) with brain heart infusion (BHI) broth (TM Media-Rogosa SL Broth, Delhi, India), followed by autoclaving for 15 min at 121°C to ensure complete elimination of any contaminants or microorganisms present in the samples before inoculation with *E. faecalis*.

Cultivation and inoculation of bacteria

E. faecalis ATCC (51299) was suspended in 10 ml BHI broth and incubated for 24 h. The suspension was adjusted to 1.5×10^8 colony-forming units (CFUs)/ml (0.5 McFarland standards) and checked with a calibrated turbidity meter DensiChek (Biomerieux, France). A 20 µl aliquot of the *E. faecalis* suspension was pipetted into root canals within a biosafety cabinet (IGene Labserve Biosafety Cabinet, India). Parafilm was used to seal the coronal portion of the root canal. Samples were incubated at 37°C for 21 days, with the inoculum renewed every 3 days to maintain bacterial viability.

Preparation of 0.25% AgGO nanoparticles

Stock solution was made by adding 5 mg of GO (BITS Pilani, Hyderabad, India) to 1 ml of AgNps (BITS Pilani, Hyderabad, India). For dilution, 50 µl of the 5 mg/ml stock solution was added to 1.95 ml of AgNps, creating a 0.125 mg/ml solution, equivalent to 0.25% AgGO NPs. This mixture was stirred vigorously. The AgGO NPs were used within 24–48 h of preparation and preserved in a refrigerator.

Sample disinfection treatment

After incubation, eighty contaminated samples were divided into four groups ($n = 20$): Group I (0.25% AgGO),

Group II (3% NaOCl), Group III (2% CHX), and Group IV (0.9% saline). Irrigation was performed with 1.5 ml of the experimental irrigant for 1 min per sample using a sterile syringe and 26-G needle (DispoVan, India), followed by PUI (Aceton Satelec P5 Newtron XS, Gujrat, India) with an IrriSafe® ultrasonic tip (IRR 25/25) (Aceton Satelec, Gujrat, India) used with an ultrasonic equipment with a power set at 5. The irrigant was activated for three cycles of 20 s each, with the tip placed 1 mm from the apical foramen. All samples were flushed with distilled water to remove any residual irrigants.

Microbiological examination

Bacterial samples were collected using sterile F3 paper points (Waldent, New Delhi, India) soaked in root canals for 1 min. The points were placed in microcentrifuge tubes containing 1 ml of BHI broth and vortexed for 1 min in vortex (Tarsons, Kolkata, India). A 100 µl aliquot was streaked onto BHI agar plates and was incubated for 48 h. Colonies were quantified using digital colony counting and expressed as CFU/ml [Figure 1].

Scanning electron microscope examination

Samples were split in half in buccolingual direction. Biofilm and smear layer percentages were assessed using scanning electron microscope (SEM) (Apreo S Lovac, US). Samples were secured using carbon tape (Royal tapes Pvt. Ltd., Chennai, India) to aluminum stubs, ensuring visibility of the root canal. 20–30 nm gold was sputtered using a gold sputtering machine (Leica Microsystems, Germany). The samples were assessed with SEM and photomicrographs were taken at magnifications of ×3000, ×5000, and ×8000. Images were analyzed to assess the reduction of *E. faecalis* and stored for further analysis [Figure 2].

Photomicrograph analysis

The evaluation of photomicrographs involved two independent investigators who conducted a blinded assessment to analyze smear layer removal. The scoring criteria employed for this assessment were outlined by Hu¨lsmann in 1997.[9]

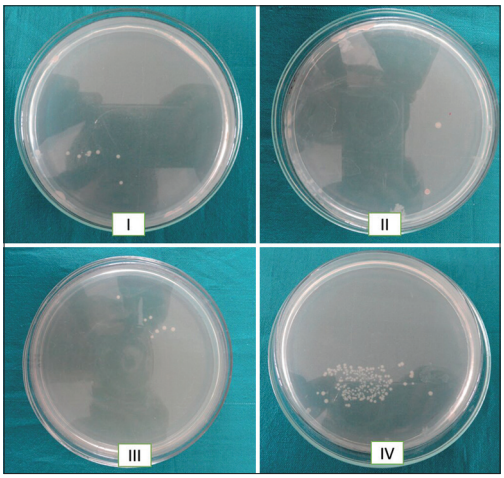

Figure 1: Antibacterial activity of Group I, II, III, and IV

A scoring index of 1–5 was used as described:

Score 1: No smear layer; dentinal tubules open.

Score 2: Small amount of smear layer; many dentinal tubules open.

Score 3: Homogeneous smear layer covering the root canal walls; only a few dentinal tubules open.

Score 4: Complete root canal wall covered by a homogeneous smear layer; no dentinal tubules open.

Score 5: Heavy, nonhomogeneous smear layer completely covering root canal walls.

The scores assigned were compared and tabulated to analyze smear layer removal.

Statistics

The data were gathered and analyzed using SPSS, Version 26, (IBM Corp., Armonk, NY, US). The significance level was maintained at 5%. Comparison of antibacterial activity and smear layer removal among the four groups was done using the Kruskal–Wallis test. Pairwise comparisons were done using Bonferroni correction. Interobserver reliability was tested using intraclass correlation test.

RESULTS

Antibacterial activity

Group II showed the highest results, followed by Groups III and I, with Group IV showing the least ($P \leq 0.05$) [Table 1].

On pairwise comparison, significant differences were found between Groups I and II, I and IV, II and III, II and IV, and III and IV ($P \leq 0.05$). There was no significant difference between Group I and III ($P > 0.05$).

Smear layer removal

Group II showed the best results followed by Groups I and III, with Group IV showing the least smear layer removal ($P \leq 0.05$) [Table 2].

On pairwise comparison, Group II showed significantly best results than the other three groups. Group IV showed the least results. Group I and Group III showed similar results.

The overall assessment of interobserver reliability using intraclass correlation coefficient test showed almost perfect agreement.

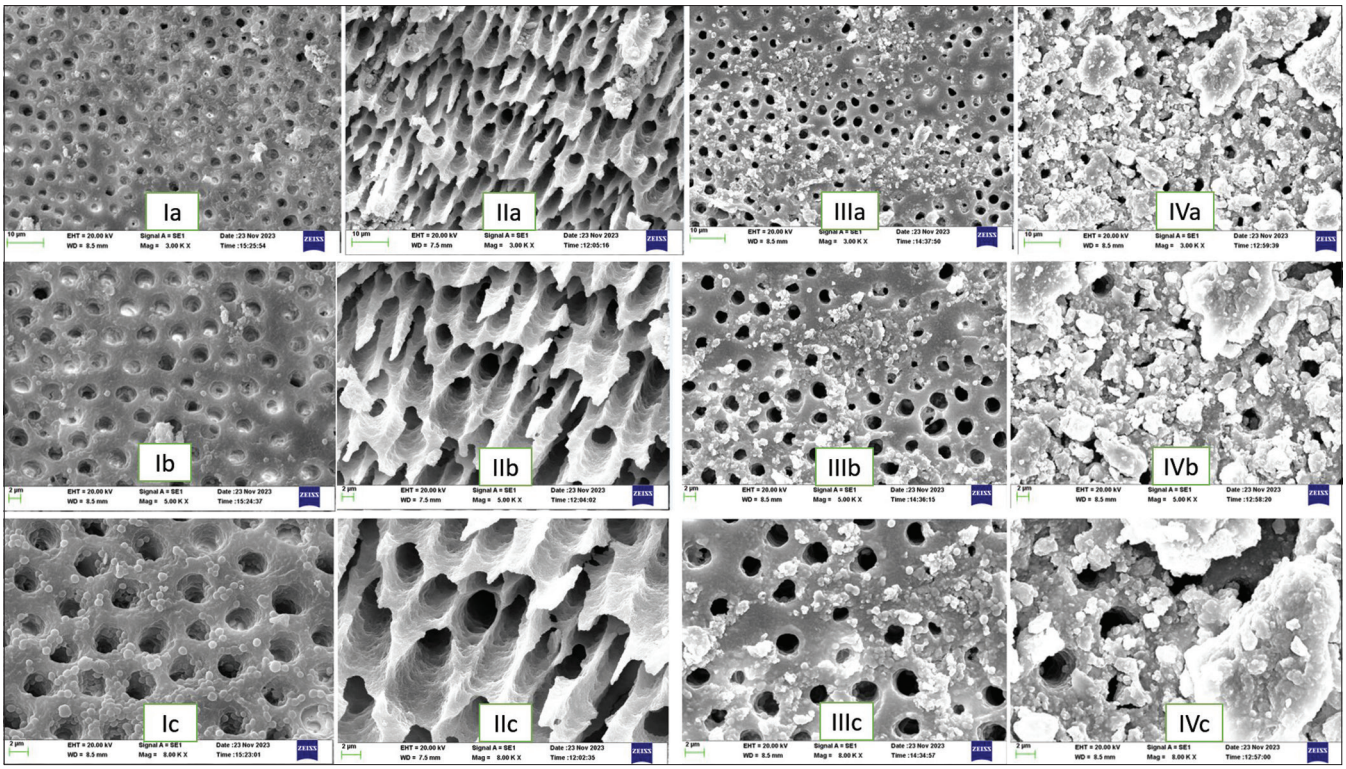

Figure 2: Scanning electron microscope images of Group Ia-c, IIa-c, IIIa-c, and IVa-c, respectively, at – I-IVa: ×3000, I-IVb: ×5000, I-IVc: ×8000

Table 1: Comparison of antibacterial activity among the four groups

Group	*n*	CFU/mL		Log CFU		*P*
		Mean	SD	Mean	SD	
Group I (0.25% AgGO)	20	295.00	170.06	1.92	1.05	<0.001*
Group II (3% NaOCl)	20	32.50	46.67	0.75	0.95	
Group III (2% CHX)	20	302.00	174.32	2.04	1.14	
Group IV (0.9% saline)	20	1605.00	263.53	3.20	0.07	

*A significant difference at $P \leq 0.05$. Kruskal–Wallis test. SD: Standard deviation, CFU: Colony-forming unit, AgGO: Silver–graphene oxide, NaOCl: Sodium hypochlorite, CHX: Chlorhexidine

DISCUSSION

The persistent infections within the root canal system are responsible for endodontic failure which is essentially a monoinfection with predominantly Gram-positive microorganisms that are less responsive to antimicrobial agents.[10]

E. faecalis (ATCC51299) monospecies biofilm model was established.[10] The effectiveness of 0.25% AgGO NPs, 3% NaOCl, 2% CHX, and 0.9% saline using PUI technique were assessed with respect to the 21-day mature *E. faecalis* biofilm as it is more resilient to disinfectant solutions than immature biofilms.[11]

3% NaOCl and 17% EDTA were used for standard irrigation to simulate clinical workflow and effectively eliminate the smear layer.[12] Contact time of 1 min was used as irrigant; contact time of less than 1 min was inadequate to completely eradicate *E. faecalis* when NaOCl or CHX was used.[13]

The use of PUI after hand or rotary instrumentation significantly reduces the number of bacteria than syringe needle irrigation. This is due to the following: (a) acoustic streaming, in which high-power ultrasound eliminates bacterial biofilms and (b) cavitation that temporarily weakens the cell membrane, making the bacteria more susceptible to irrigants.[14] Cameron observed that the smear layer was completely eliminated when PUI was utilized with 3% NaOCl.[15] Subsequent research by Alaçam using various NaOCl concentrations supported these findings.[16]

The CFUs procedure has been extensively employed for the microbiological examination of bacteria within the dentinal tubules.[11]

SEM has been the most common technique to assess the smear layer's removal. Owing to its excellent resolution, it has been widely utilized for qualitative observation of biofilm disruption and smear layer removal.[17]

In this study, 3% NaOCl demonstrated superior results. Its effectiveness is attributed to its ability to dissolve organic debris and its strong proteolytic effect, which aids in cleansing the root canal system. Its antibacterial action

Table 2: Comparison of smear layer removal among the four groups as evaluated by observer 1 and observer 2

Group	*n*	Observer 1, mean	Observer 1, SD	Observer 2, mean	Observer 2, SD	*P*
Group I (0.25% AgGO)	20	2.70	0.73	2.75	0.72	<0.001*
Group II (3% NaOCl)	20	1.40	0.50	1.40	0.50	
Group III (2% CHX)	20	2.70	0.73	2.75	0.72	
Group IV (0.9% saline)	20	4.65	0.49	4.35	0.49	

*Indicates a statistically significant difference at $p \leq 0.05$. AgGO: Silver–graphene oxide, NaOCl: Sodium hypochlorite, CHX: Chlorhexidine, SD: Standard deviation

is due to hypochlorous acid, which oxidizes bacterial enzymes and disrupts metabolism. These findings align with previous studies by Rajasekhar *et al*.[18] and Nanda *et al*.[19]

In this study, no statistically significant difference was seen between 0.25% AgGO and 2% CHX, indicating their potential as alternative irrigation solutions. AgGO's antibacterial properties and CHX's substantivity contribute to bacterial reduction within root canals. These findings contrast with previous studies; for instance, Sharma *et al*. found AgGO more effective against *E. faecalis* compared to NaOCl and saline,[20] while González-Luna *et al*. reported superior smear layer removal efficacy of AgNp over NaOCl.[21]

AgNPs release Ag ions in aqueous environments, known for their bactericidal potential through mechanisms like the production of reactive oxygen species (ROS), ATP disruption, and damage to cell membranes. However, AgNPs' effectiveness is limited by short irrigation periods and biofilm matrix protection.[18] GO exhibits antibacterial properties through cell wall disruption and ROS generation.[20]

CHX offers broad-spectrum antibacterial action and binds to dentine, gradually releasing to protect against microbial colonization.[13] Studies support 2% CHX's efficacy in reducing bacterial populations in infected canals.[22,23] The findings of this study are not in accordance with Charlie *et al*. who concluded that 2% CHX and NaOCl were ineffective in removing the smear layer.[24]

0.9% saline has demonstrated the least results. These findings were in accordance with previous studies.[13,19]

Limitations include the *in vitro* nature of the study and the use of single-rooted teeth with straight canals, which may not represent the complexity of curved canals. In addition, monospecies biofilm limits the replication of the mixed cultures found *in vivo*.

CONCLUSIONS

3% NaOCl was the most effective solution in terms of antibacterial activity and smear layer removal. 0.25% AgGO and 2% CHX showed moderate effectiveness in removing *E. faecalis* biofilms and smear layer which suggests that they have potential as alternative irrigants.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Kakehashi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1965;20:340-9.
2. Boutsioukis C, Arias-Moliz MT. Present status and future directions – Irrigants and irrigation methods. Int Endod J 2022;55 Suppl 3:588-612.
3. Elakanti S, Cherukuri G, Rao VG, Chandrasekhar V, Rao AS, Tummala M. Comparative evaluation of antimicrobial efficacy of QMix™ 2 in 1, sodium hypochlorite, and chlorhexidine against *Enterococcus faecalis* and *Candida albicans*. J Conserv Dent 2015;18:128-31.
4. Vemuri S, Kolanu SK, Varri S, Pabbati RK, Penumaka R, Bolla N. Effect of different final irrigating solutions on smear layer removal in apical third of root canal: A scanning electron microscope study. J Conserv Dent 2016;19:87-90.
5. Ruksakiet K, Hanák L, Farkas N, Hegyi P, Sadaeng W, Czumbel LM, *et al*. Antimicrobial efficacy of chlorhexidine and sodium hypochlorite in root canal disinfection: A systematic review and meta-analysis of randomized controlled trials. J Endod 2020;46:1032-41.e7.
6. Cai C, Chen X, Li Y, Jiang Q. Advances in the role of sodium hypochlorite irrigant in chemical preparation of root canal treatment. Biomed Res Int 2023;2023:8858283.
7. Ioannidis K, Niazi S, Mylonas P, Mannocci F, Deb S. The synthesis of nano silver-graphene oxide system and its efficacy against endodontic biofilms using a novel tooth model. Dent Mater 2019;35:1614-29.
8. van der Sluis LW, Versluis M, Wu MK, Wesselink PR. Passive ultrasonic irrigation of the root canal: A review of the literature. Int Endod J 2007;40:415-26.
9. Zhu X, Yin X, Chang JW, Wang Y, Cheung GS, Zhang C. Comparison of the antibacterial effect and smear layer removal using photon-initiated photoacoustic streaming aided irrigation versus a conventional irrigation in single-rooted canals: An *in vitro* study. Photomed Laser Surg 2013;31:371-7.
10. Pandey A, Bhushan J, Joshi RK, Uppal AS, Angrup A, Kansal S. Comparative evaluation of antimicrobial efficacy of chitosan nanoparticles and calcium hydroxide against endodontic biofilm of *Enterococcus faecalis*: An *in vitro* study. J Conserv Dent Endod 2024;27:750-4.
11. Parolia A, Kumar H, Ramamurthy S, Davamani F, Pau A. Effectiveness of chitosan-propolis nanoparticle against *Enterococcus faecalis* biofilms in the root canal. BMC Oral Health 2020;20:339.
12. Prasanthi P, Garlapati R, Nagesh B, Sujana V, Kiran Naik KM, Yamini B. Effect of 17% ethylenediaminetetraacetic acid and 0.2% chitosan on pushout bond strength of biodentine and ProRoot mineral trioxide aggregate: An *in vitro* study. J Conserv Dent 2019;22:387-90.
13. Goud S, Aravelli S, Dronamraju S, Cherukuri G, Morishetty P. Comparative evaluation of the antibacterial efficacy of *Aloe vera*, 3% sodium hypochlorite, and 2% chlorhexidine gluconate against *Enterococcus faecalis*: An *in vitro* study. Cureus 2018;10:e3480.
14. Nair RM, Jayasree S, Poornima ES, Ashique M. Comparative evaluation of antimicrobial efficacy on *Enterococcus faecalis* and smear layer removal in curved canals by different irrigation techniques: An *in vitro* study. J Conserv Dent 2022;25:409-14.
15. Cameron JA. The synergistic relationship between ultrasound and sodium hypochlorite: A scanning electron microscope evaluation. J Endod 1987;13:541-5.
16. Alaçam T. Scanning electron microscope study comparing the efficacy of endodontic irrigating systems. Int Endod J 1987;20:287-94.

17. Lui JN, Kuah HG, Chen NN. Effect of EDTA with and without surfactants or ultrasonics on removal of smear layer. J Endod 2007;33:472-5.
18. Rajasekhar R, James B, Devadathan A, Soman S, Sebastian V, Sathyan M. An *in vitro* evaluation of antibacterial and smear layer removal efficacy of silver nanoparticles as final irrigant against *Enterococcus faecalis*. World J Dent 2022;13:148-54.
19. Nanda Z, Singh R, Kamble PP, Deshmukh G, Patil N, Patil AB, *et al.* Efficacy of different root canal irrigating solutions in removing smear layer: A scanning electron microscopic study. Cureus 2023;15:e44618.
20. Sharma DK, Bhat M, Kumar V, Mazumder D, Singh SV, Bansal M. Evaluation of antimicrobial efficacy of graphene silver composite nanoparticles against *E. faecalis* as root canal irrigant: An *ex-vivo* study. Int J Pharm Med Res 2015;3:267-72.
21. González-Luna PI, Martínez-Castañón GA, Zavala-Alonso NV, Patiño-Marin N, Niño-Martínez N, Morán-Martínez J, *et al.* Bactericide effect of silver nanoparticles as a final irrigation agent in endodontics on *Enterococcus faecalis*: An *ex vivo* study. J Nanomater 2016;2016:7597295.
22. Neelakantan P, Herrera DR, Pecorari VG, Gomes BP. Endotoxin levels after chemomechanical preparation of root canals with sodium hypochlorite or chlorhexidine: A systematic review of clinical trials and meta-analysis. Int Endod J 2019;52:19-27.
23. Mohammadi Z. Chlorhexidine gluconate, its properties and applications in endodontics. Iran Endod J 2008;2:113-25.
24. Charlie KM, Kuttappa MA, George L, Manoj KV, Joseph B, John NK. A scanning electron microscope evaluation of smear layer removal and antimicrobial action of mixture of tetracycline, acid and detergent, sodium hypochlorite, ethylenediaminetetraacetic acid, and chlorhexidine gluconate: An *in vitro* study. J Int Soc Prev Community Dent 2018;8:62-9.