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To evaluate the effect of three different irrigating solutions on removal of smear layer – An SEM study

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Abstract

Background/Purpose: Successful endodontic therapy depends on effective chemo mechanical debridement and elimination of the smear layer, which may hinder sealer penetration and compromise long-term sealing. This study aimed to compare the smear layer removal efficacy of sodium hypochlorite (NaOCl) with etidronic acid (HEBP), 17% EDTA, and silver nanoparticles (AgNPs) using scanning electron microscopy (SEM).

Materials and Methods: Eighty extracted mandibular premolars were decoronated to 12 ± 1 mm and instrumented with rotary NiTi files. Samples were divided into four groups (n = 20) according to the final irrigant: Group 1 – saline (control), Group 2 – NaOCl + HEBP, Group 3 – 17% EDTA, and Group 4 – AgNPs. Roots were longitudinally split, gold sputter-coated, and examined under SEM (×1000). Smear layer removal was scored using the Torabinejad criteria. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test ($p < 0.05$).

Results: All irrigants were significantly more effective than saline ($p < 0.05$). AgNPs exhibited the lowest mean smear layer score [1.43 ± 0.08], indicating superior overall efficacy, particularly in the coronal third (mean = 1.00). EDTA showed the greatest smear layer removal in the apical third [0.55 ± 0.129]. NaOCl + HEBP improved cleanliness compared with saline but was less effective than EDTA or AgNPs in removing inorganic debris.

Conclusion: AgNPs demonstrated the highest overall efficacy in smear layer removal, while EDTA performed best in the apical region. NaOCl + HEBP showed moderate effectiveness. Sequential or combined use of irrigants may yield optimal outcomes. *In-`* validation is warranted.

Keywords: Smear layer, EDTA, sodium hypochlorite, etidronic acid, silver nanoparticles, endodontics

Introduction

The primary objective of root canal therapy is to thoroughly clean and shape the root canal system, followed by three-dimensional obturation to prevent reinfection [1]. During mechanical instrumentation, a smear layer—comprising both organic and inorganic debris—forms on the canal walls and occludes the dentinal tubules [2]. Irrigation plays a pivotal role in root canal disinfection by eliminating microorganisms, removing debris, dissolving smear layer components, and flushing out necrotic tissue and contaminants, thereby minimizing apical extrusion [3].

Various methods such as chemical irrigants, ultrasonic activation, and laser-assisted techniques have been investigated for smear layer removal, but none are universally effective [4]. This study focuses on comparing the action of different irrigants—Sodium hypochlorite (NaOCl) combined with HEBP (Etidronic acid), 17% EDTA (Ethylenediaminetetraacetic acid), and silver nanoparticles (AgNPs)—against normal saline.

NaOCl is widely used for its antimicrobial activity, tissue-dissolving property, and ability to inactivate endotoxins and disrupt biofilms, typically at 0.5–6% concentrations. However, it has limited ability to remove the inorganic component of the smear layer [6]. HEBP, a biocompatible weak chelator, can be combined with NaOCl without reducing its activity, thereby offering both antibacterial and smear layer–removing potential [7].

EDTA is an established chelating agent that binds calcium ions, removing the inorganic component of the smear layer. At 17%, it demineralizes dentin to a depth of 20–30 µm within 5–15 minutes, exposing collagen fibrils for subsequent NaOCl dissolution of organic residues [8].

Recently, AgNPs have emerged as promising irrigants due to their simultaneous antibacterial and smear layer–removing efficacy [9]. They release silver ions with broad-spectrum antimicrobial activity while maintaining relatively low toxicity [10].

The smear layer was first described by Violich *et al*. in 1970 [11] using scanning electron microscopy (SEM), which remains a standard tool for its evaluation owing to its high magnification capacity and ability to visualize dentinal tubules [12, 13]. The smear layer produced during root canal instrumentation is composed of dentine, remnants of pulp tissue, odontoblastic processes, and bacteria in an infected canal [14].

Accordingly, this study aims to evaluate and compare smear

layer removal in coronal, middle, and apical thirds of root canals using 17% EDTA, NaOCl+HEBP, and AgNPs, compared with normal saline, assessed under SEM.

Materials and Methods

Sample selection and preparation

Eighty freshly extracted, non-carious, single-rooted mandibular premolars with straight canals were selected. Teeth were sterilized per OSHA guidelines and stored in 10% formaldehyde, then kept hydrated in saline until use. Root length was standardized to 12 ± 1 mm after crown sectioning. Working length was determined by inserting a #10 K-file until visible at the apex and subtracting 1 mm.

Biomechanical preparation was performed with hand files to size 20, followed by Neoendo NiTi rotary files to size 30/04 using the crown-down technique. The apical third was enlarged to the same size for better irrigant penetration. Canals were irrigated with 1 mL of 3% NaOCl for 1 minute after each file.

Grouping

Samples were randomly divided into four groups (n = 20) based on the final irrigant:

- **Group 1 (Control):** 5 mL normal saline
- **Group 2:** 5 mL NaOCl + HEBP solution
- **Group 3:** 5 mL 17% EDTA
- **Group 4:** 5 mL silver nanoparticles solution

SEM preparation

Roots were grooved longitudinally and split. One half was selected for SEM. Samples were sputter-coated with gold, mounted, and observed at 1000× magnification. Coronal, middle, and apical thirds were imaged.

Evaluation criteria

Smear layer was scored as per Torabinejad *et al* [1].

- **Score 1:** No smear layer; tubules clean and open.
- **Score 2:** Moderate smear layer; debris within tubules.
- **Score 3:** Heavy smear layer; surface and tubules covered.

Statistical analysis

Data were analysed using SPSS v19. One-way ANOVA followed by Tukey's post hoc test was applied, with significance at $p < 0.05$.

Results

- All experimental groups performed significantly better than saline in smear layer removal ($p < 0.001$). AgNPs showed the best overall performance, particularly in the coronal third.
- **Overall:** AgNPs showed the lowest mean smear layer score (1.43 ± 0.08), followed by EDTA and NaOCl + HEBP.
- **Coronal third:** AgNPs were most effective (mean = 1.00).
- **Apical third:** EDTA showed the best cleaning ability (0.55 ± 0.129).
- **NaOCl + HEBP:** Superior to saline but less effective than EDTA or AgNPs.

Tables

Table 1: Overall comparison of smear layer removal among irrigants

Group	Mean (Std. Error)	Critical Value	P
Group 1- Normal Saline (Control Group)	2.88 (0.042)	74.61	<0.001*
Group 2- NAOCL + HEBP	1.52(0.090)		
Group 3 - EDTA	1.57(0.096)		
Group 4- Silver Nano particles	1.43(0.080)		

ANOVA test; *Significant.

Table 2: Pairwise mean comparison of irrigants (Tukey's post hoc)

Group I	Group J	Mean Difference	Std. Error	95% CI (Lower–Upper)	p-value
Saline	NaOCl+HEBP	1.367*	0.113	1.07–1.61	0.001*
Saline	EDTA	1.317*	0.113	1.02–1.61	0.001*
Saline	AgNPs	1.450*	0.113	1.16–1.74	0.001*
NaOCl+HEBP	EDTA	-0.050	0.113	-0.34–0.24	0.971
NaOCl+HEBP	AgNPs	0.083	0.113	-0.21–0.38	0.882
EDTA	AgNPs	0.133	0.113	-0.16–0.43	0.641

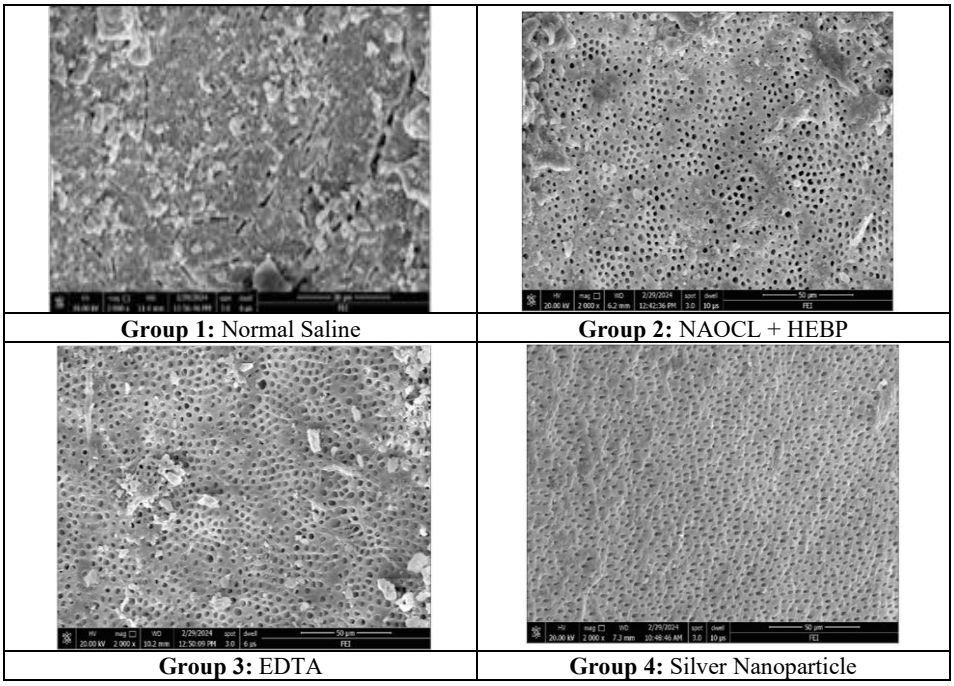
*Tukey's post hoc; significant at $p < 0.05$.

Table 3: Comparison of smear layer removal at coronal, middle, and apical thirds (irrespective of irrigant)

Root level	Mean	Std. Error	Critical Value	p-value
Coronal	1.49 (0.091)	48.01	<0.001*	
Middle	1.56 (0.092)			
Apical	2.50 (0.056)			

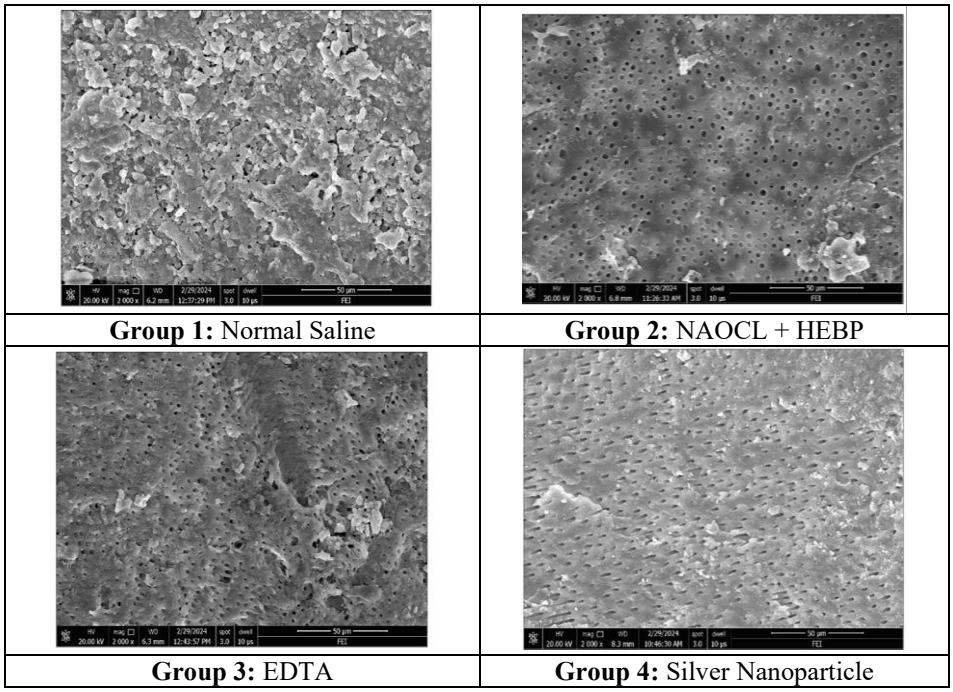
ANOVA test; *Significant.

Figures



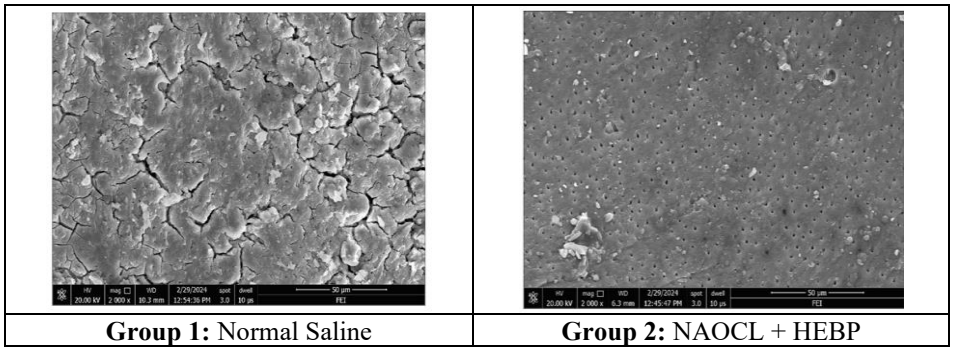
Group 1: Normal Saline	**Group 2:** NAOCL + HEBP
Group 3: EDTA	**Group 4:** Silver Nanoparticle

Fig 1: Scanning Electron microscopic images of the four sample groups Group 1: Normal Saline, Group 2 (NAOCL + HEBP), Group 3(EDTA), Group 4(Silver Nanoparticle) at the coronal third of the root canal (mag: 1000)



Group 1: Normal Saline	**Group 2:** NAOCL + HEBP
Group 3: EDTA	**Group 4:** Silver Nanoparticle

Fig 2: Scanning Electron microscopic images of the four sample groups Group 1: Normal Saline, Group 2 (NAOCL + HEBP), Group 3(EDTA), Group 4(Silver Nanoparticle) at the middle third of the root canal (mag: 2000)



Group 1: Normal Saline	**Group 2:** NAOCL + HEBP

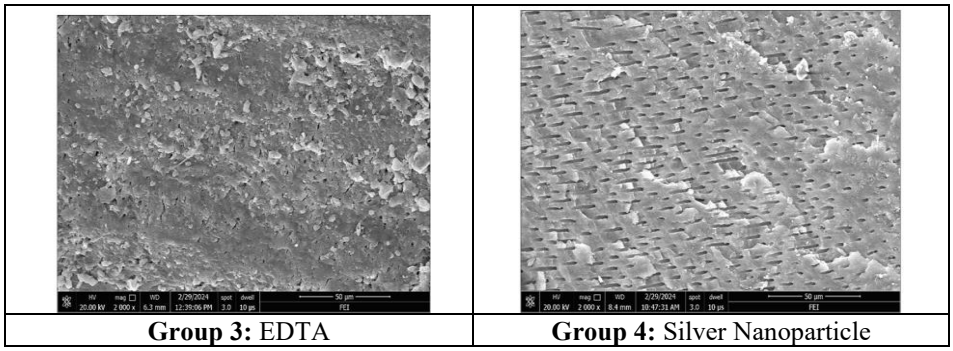


Fig 3: Scanning Electron microscopic images of the four sample groups Group 1: Normal Saline, Group 2 (NAOCL + HEBP), Group 3(EDTA), Group 4(Silver Nanoparticle) at the apical third of the root canal (mag: 2000)

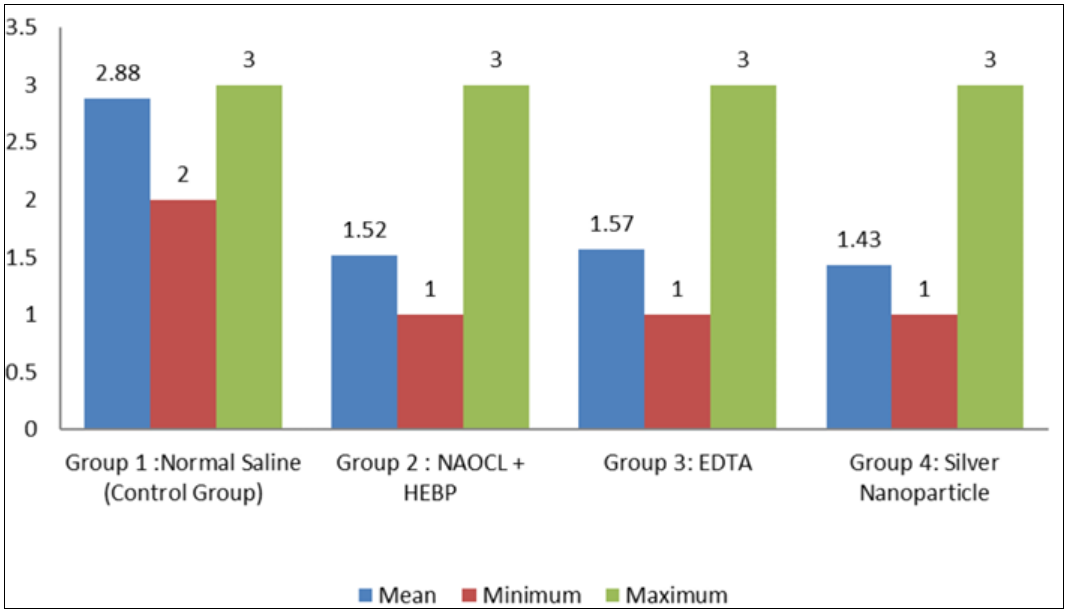


Fig 4: Bar graph comparing mean smear layer scores of irrigants across root thirds.

Discussion

This study utilized SEM to evaluate the smear layer on instrumented radicular dentinal surfaces, as SEM can detect particles of 0.5–1.5 µm [15]. The smear layer comprises dentinal debris, odontoblastic processes, pulpal remnants, and microorganisms [15, 16] and is more voluminous following motorized canal preparation than hand instrumentation [17]. Although first described decades ago, debate continues whether to remove or retain it. Techniques such as hydrodynamic and non-instrumental approaches have been proposed to retain the smear layer, which may seal dentinal tubules and limit microbial penetration [18, 20]. Conversely, removal is recommended to prevent debris accumulation, improve disinfection, and enhance obturation sealing [20].

The present in-*vitro* study compared three final irrigants—NaOCl with HEBP, 17% EDTA, and AgNPs—against normal saline. Irrigation is essential for lubrication and smear layer removal [21, 23]. A meta-analysis confirmed that removing the smear layer significantly enhances the seal of root canal obturation, irrespective of sealer type or technique [24]. However, conventional irrigants may leave up to 35% of canal surfaces untreated [25, 26] necessitating combined irrigant protocols.

All experimental irrigants significantly outperformed saline, with AgNPs showing superior removal in the coronal third, consistent with prior reports [9, 27, 33]. Their nanoscale size and high surface reactivity facilitate both mechanical debris removal and antimicrobial activity via reactive oxygen species generation, ATP disruption, and cell membrane injury [30, 31]. AgNPs reduce the risk of persistent infection, including against Enterococcus faecalis [8, 32, 33].

EDTA effectively chelates calcium in the apical third, removing the inorganic smear layer [8, 11, 35] although prolonged exposure may alter dentin microhardness and mechanical properties [22, 37]. NaOCl, despite excellent tissue dissolution, is insufficient alone for smear layer removal [9] reinforcing the need for combined irrigants. Smear layer removal is generally easier in coronal and middle thirds, while the apical third presents anatomical challenges [34, 36]. AgNPs and EDTA both improved smear layer removal in the apical third relative to saline [8, 34].

Overall, all experimental groups efficiently removed smear layer in coronal and middle thirds, with significant differences between saline and experimental groups at all levels. No statistically significant differences were noted among NaOCl+HEBP, EDTA, and AgNPs at coronal, middle, or apical thirds. These findings highlight the dual benefit of AgNPs, providing both smear layer removal and antimicrobial effects, making them a valuable addition to endodontic irrigation protocols [9, 27, 33].

Conclusion

Within the limitations of this in-*vitro* study, all experimental irrigants (NaOCl+HEBP, EDTA, AgNPs) demonstrated greater smear layer removal compared with saline. AgNPs exhibited the highest efficacy, particularly in the coronal third, whereas EDTA was more effective in the apical third. NaOCl+HEBP showed superior results to saline but was less effective than EDTA and AgNPs. Further *in-vivo* studies with larger sample sizes are warranted to assess long-term outcomes, biocompatibility, and clinical applicability.

References

1. Torabinejad M, Khademi A, Babagoli J, Cho Y, Johnson W, Bozhilov K, *et al*. A new solution for the removal of the smear layer. Journal of Endodontics,2003:29(3):170–175.
2. Shrivastav V, Ali N, Singh AR, Chauhan R. Comparative evaluation and efficacy of ethylenediaminetetracetic acid, carbonated water, and chloroquik as final irrigant in smear layer removal using scanning electron microscope. Endodontology,2020:32(2):209–215.
3. Cruz-Filho AM, Sousa-Neto MD, Saquy PC, Pécora JD. Evaluation of the effect of EDTAC, CDTA, and EGTA on radicular dentin microhardness. Journal of Endodontics,2001:27(3):183–184.
4. Sarkees M, Al-Maarrawi K. Chitosan: A natural substitute of EDTA solution for final irrigation in endodontics treatment. Nigerian Journal of Clinical Practice,2020:23(5):697–703.
5. Kamble AB, Abraham S, Kakde DD, Shashidhar C, Mehta DL. Scanning electron microscopic evaluation of efficacy of 17% ethylenediaminetetraacetic acid and chitosan for smear layer removal with ultrasonics: An in *vitro* study. Contemporary Clinical Dentistry,2017:8:621–626.
6. Hegde V, Thakkar P. Effect of continuous soft chelating irrigation protocol on removal of smear layer. Endodontology,2019:31(1):63–67.
7. Kaur S, Pradeep, Kumari V, Chaudhury T, Syam A. Effect of final irrigating solutions on smear layer removal and penetrability of an epoxy resin-based sealer into dentinal tubules. International Journal of Contemporary Medical Research,2019:6(2):B6–B10.
8. Kuruvilla A, Jaganath BM, Krishnegowda SC, Ramachandra PK, Johns DA, Abraham A. A comparative evaluation of smear layer removal by using EDTA, etidronic acid, and maleic acid as root canal irrigants: An in *vitro* scanning electron microscopic study. Journal of Conservative Dentistry,2015:18(3):247–251.
9. Soman S, Devadathan A, James B, Sathyan M, Rajasekhar R, Sebastian VM. An in *vitro* evaluation of antibacterial and smear layer removal efficacy of silver nanoparticles as final irrigant against Enterococcus faecalis. World Journal of Dentistry,2022:13(2):148–154.
10. Raffi M, Hussain F, Bhatti TM, Akhter JI, Hameed A, Hasan MM. Antibacterial characterization of silver nanoparticles against E. coli ATCC-15224. Journal of Materials Science and Technology,2008:24(2):192–196.
11. Violich DR, Chandler NP. The smear layer in endodontics – a review. International Endodontic Journal,2010:43(1):2–15.
12. Stojicic S, Zivkovic S, Qian W, Zhang H, Haapasalo M. Tissue dissolution by sodium hypochlorite. Effect of concentration, temperature, agitation, and surfactant. Journal of Endodontics,2010:36(9):1558–1562.
13. Paradella TC, Bottino MA. Scanning electron microscopy in modern dentistry research. Brazilian Dental Science,2012:15(2):43–48.
14. Torabinejad M, Handysides R, Khademi AA, Bakland LK. Clinical implications of the smear layer in endodontics. A review. Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontology,2002:94:658–666.
15. McComb D, Smith D. A preliminary scanning electron microscopic study of root canals after endodontic procedures. Journal of Endodontics,1975:1:238–242.
16. Mader CL, Baumgartner JC, Peters DD. Scanning electron microscopic investigation of the smeared layer on root canal walls. Journal of Endodontics,1984:10(10):477–483.
17. Czonstkowsky M, Wilson EG, Holstein FA. The smear layer in endodontics. Dental Clinics of North America,1990:34:13–25.
18. Ruddle C. Endodontic disinfection: Tsunami irrigation. Saudi Endodontic Journal,2015:5(1):1.
19. Lussi A, Nussbächer U, Grosrey J. A novel non-instrumented technique for cleansing the root canal system. Journal of Endodontics,1993:19(11):549–553.
20. Balto H, Salama F, Al-Mofareh S, Al-Yahya F. Evaluation of different irrigating solutions on smear layer removal of primary root dentin. Journal of Contemporary Dental Practice,2015:16(3):187–191.
21. Karunakaran JV, Kumar SS, Kumar M, Chandrasekhar S, Namitha D. The effects of various irrigating solutions on intra-radicular dentinal surface. An SEM analysis. Journal of Pharmacy and Bioallied Sciences,2012:4(2):125–S130.
22. Paul ML, Mazumdar D, Niyogi A, Baranwal AK. Comparative evaluation of the efficacy of different irrigants including MTAD under SEM. Journal of Conservative Dentistry,2013:16(4):336–341.
23. Elbahary S, Haj-Yahya S, Khawalid M, Tsesis I, Rosen E, Habashi W, *et al*. Effects of different irrigation protocols on dentin surfaces as revealed through quantitative 3D surface texture analysis. Scientific Reports,2020:10(1):22073.
24. Shravan A, Haghdoost AA, Adl A, Rahimi H, Shadifar F. Effect of smear layer on sealing ability of canal obturation: A systematic review and meta-analysis. Journal of Endodontics,2007:33(2):96–105.
25. Kazemi RB, Sen BH, Spångberg LS. Permeability changes of dentine treated with titanium tetrafluoride. Journal of Dentistry,1999:27(7):531–538.
26. Topbas C, Adiguzel O. Endodontic irrigation solutions: A review. International Dental Research,2017:7:54–61.
27. Lok CN, Ho CM, Chen R, He QY, Yu WY, Sun H, *et al*. Silver nanoparticles: Partial oxidation and antibacterial activities. Journal of Biological Inorganic Chemistry,2007:12(4):527–534.
28. 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.
29. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, *et al*. The bactericidal effect of silver nanoparticles. Nanotechnology,2005:16(10):2346–2353.
30. Marambio-Jones C, Hoek EMV. A review of the antibacterial effects of silver nanomaterials and potential implications for human health and the environment. Journal of Nanoparticle Research,2010:12(5):1531–1551.
31. AshaRani PV, Low Kah Mun G, Hande MP, Valiyaveettil S. Cytotoxicity and genotoxicity of silver nanoparticles in human cells. ACS Nano,2009:3(2):279–290.
32. 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. Journal of Nanomaterials, 2016, (1).
33. Tonini R, Giovarruscio M, Gorni F, Ionescu A, Brambilla E, Mikhailovna IM, *et al*. In *vitro* evaluation of antibacterial properties and smear layer removal/sealer penetration of a novel silver-citrate root canal irrigant. Materials,2020:13(1):194.
34. Ribeiro EM, Silva-Sousa YTC, Souza-Gabriel AE, Sousa-Neto MD, Lorencetti KT, Silva SRC. Debris and smear removal in flattened root canals after use of different irrigant agitation protocols. Microscopy Research and Technique,2012:75(6):781–790.
35. Zhou H, Li Q, Wei L, Huang S, Zhao S. A comparative scanning electron microscopy evaluation of smear layer removal with chitosan and MTAD. Nigerian Journal of Clinical Practice,2018:21(1):76–80.
36. Eick JD, Wilko RA, Anderson CH, Sorensen SE. Scanning electron microscopy of cut tooth surfaces and identification of debris by use of the electron microprobe. Journal of Dental Research, 1970, 59–68.