Content available at: https://www.ipinnovative.com/open-access-journals

IP Annals of Prosthodontics and Restorative Dentistry

Journal homepage: https://www.aprd.in/

Original Research Article

Smear layer removal potential of a naturally occurring antioxidant: An in-vitro scanning electron microscopic study

Suresh Shenvi[1]*, **Sonal B Joshi**[1]

[1] *Dept. of Conservative Dentistry and Endodontics, KLE VK Institute of Dental Sciences, KLE Academy of Higher Education and Research (KLE University), Belgaum, Karnataka, India*

ARTICLE INFO

Article history:
Received 10-11-2024
Accepted 19-12-2024
Available online 19-02-2025

Keywords:
Decalcification
Ethylenediaminetetraacetic acid
Irrigation
Maleic acid
Root dentine
Smear layer
N-acetyl-cysteine

ABSTRACT

Introduction: The primary objective of endodontic therapy is to thoroughly disinfect the root canal system. The smear layer formed during instrumentation presents a substantial impediment to optimal root canal disinfection, as it provides a medium for bacterial proliferation, hinders the penetration of disinfecting agents, and creates a physical barrier that undermines the efficacy of root canal sealers by obstructing the interface between the obturating core material and the dentinal tubules. Consequently, the removal of the smear layer is of utmost importance.

Aims and Objectives: This in vitro study assessed the efficacy of 20% N-acetyl-cysteine (NAC) and 17% ethylenediaminetetraacetic acid (EDTA) in removing the smear layer from root canal dentine.

Materials and Methods: Ninety-two single-rooted teeth were instrumented and divided into two groups: 20% NAC for 1 minute, and 17% EDTA for 1 minute. Following irrigation, the samples were analyzed using scanning electron microscopy (SEM) to evaluate smear layer removal in the coronal, middle, and apical thirds of the root canals.

Results: No statistically significant difference was observed between 20% NAC and 17% EDTA in their ability to remove the smear layer across all sections of the root canal.

Conclusion: 20% NAC demonstrates smear layer removal capability comparable to 17% EDTA as a final irrigant.

1. Introduction

Endodontics is a clinical field primarily focused on preventing and treating root canal infections. The intricate anatomy of the root canal system presents significant challenges in this field. While recent advancements in rotary instrumentation have improved mechanical preparation, these techniques alone are insufficient for eliminating bacteria from the root canal system. Consequently, the utilization of appropriate irrigants during biomechanical preparation is essential for achieving comprehensive debridement and ensuring the effective adhesion of obturating materials to the root canal walls.[1,2]

In endodontics, forming a smear layer during instrumentation is a well-known issue because it offers a favorable environment that harbors and protects microbial life and hinders the entry of irrigating solutions as well as intracanal medicaments into the dentinal tubules. Therefore, a successful course of treatment requires total elimination of the smear layer in the root canal. Chelating agents are essential to this process since they are chemical substances that make it easier to remove the smear layer.[3,4]

EDTA (Ethylenediaminetetraacetic acid) is a widely employed chelating agent used as a final irrigating solution in endodontics. It primarily targets the inorganic

* *Corresponding author.*
E-mail address: sureshshenvi123@gmail.com (S. Shenvi).

https://doi.org/10.18231/j.aprd.2025.008

components of dentin, including the hydroxyapatite within the smear layer. Sodium hypochlorite must be used before the last EDTA rinse in order to eliminate the smear layer entirely. In five minutes, the dentin is decalcified to a depth of around 20 to 30 μm due to EDTA's reaction with the calcium ions in the dentin, which causes calcium chelation. Ongoing efforts are focused on identifying more biocompatible solutions to minimize the potential adverse effects on periapical tissues.[5]

An antioxidant that occurs naturally, N-acetyl cysteine (NAC) is the acetylated version of the amino acid cysteine. It has been well-established in clinical medicine for its efficacy in detoxifying heavy metals like mercury and lead. While NAC's antibacterial and antioxidant qualities have been thoroughly investigated, its possibility as a type of Chelation agent in dentistry, particularly those that remove the smear layer, received little attention.[6] Therefore, this investigation has aimed to assess how well NAC & EDTA removed the root dentin's smear layer.

2. Materials and Methods

2.1. Sample preparation

For this investigation, A total of 92 extracted human mandibular premolar teeth with single-root canals and straight roots were selected. All of the samples had radiographs made in order to verify that there was just one canal with a closed apex. After using a curette to remove the superficial soft tissues, the teeth were kept in 0.1 percent thymol. The teeth's crowns were cut off utilizing a diamond disc to bring the root length uniformly down to 14 millimeters. The working length was then determined by subtracting one millimeter from the reported length after a size 10K file (Mani Inc., Tochigi Ken, Japan) was inserted into each root canal until it was barely visible at the apical foramen as observed with magnifying loupes. For the chemomechanical preparation, ProTaper nickel-titanium rotary tools (Dentsply Maillefer, Switzerland) were utilized, as well as the canals were instrumented up to size F3. Following each instrument change, 2mL of a 2.5% NaOCL solution was irrigated.

The final irrigating protocol was then used to randomly split the samples into two groups. Group I received 5 milliliters of 17 percent EDTA for one minute and Group II received 5 mL of 20% NAC for one minute. During irrigation, in every root canal, a 30-gauge needle tip has been inserted 1 millimeter short of the working length. To remove any possible precipitate, 5 mL of deionized water was used to rinse each root canal after these irrigation techniques. The canals were then dried using sterile paper points. On the buccal as well as lingual surfaces of every root, longitudinal grooves have been formed utilizing a slow-moving diamond bur, taking care not to puncture the canal. The roots were then cut in half using a chisel & stored in deionized water at 37 degrees Celsius to prepare them for examination under a scanning electron microscope.

2.2. Estimation of smear layer removal

The samples were put on metal stubs and subjected to an ion-sputtering procedure to cover them with gold or palladium after being dehydrated using ethanol at progressively higher concentrations—25%, 50%, 75%, and 100%. A JSM-6010 SEM (JEOL, Tokyo, Japan) was used to inspect them to determine whether the smear layer was there or not. At 20 kV and 1000X magnification, to examine the surface morphology of the canal wall, images were captured, with particular attention paid to the middle (6 to 7mm from the apex), apical (1 to 2mm from the apex), and coronal (10–12mm from the apex) 3rd of each specimen (Figure 1). Two impartial observers who have been unaware of the group assignments assessed these areas. The following standards, generated by Torabinejad et al., were used to score the images:

1. No smear layer (the surface of the canal wall is free of smear layers, & every dentinal tubule is clear & open)
2. Moderate smear layer (debris is present in the tubules; the canal wall surface lacks a smear layer)
3. Heavy smear layer (layer of smears that covers the tubules as well as the canal wall).

2.3. Statistical snalysis

The results were statistically evaluated using the Mann-Whitney U test for inter-group comparisons, as the scores obtained were ordinal. A significance level of $P < 0.05$ was set for all analyses.

3. Results

Overall, there were no statistically significant differences in smear layer removal between 17% EDTA and 20% NAC across all sections. Notably, both groups showed an increase in smear layer scores from coronal to apical sections. There was a significant difference in smear layer scores across the sections, with coronal scores significantly differing from the middle ($P < 0.0001$) and apical sections ($P < 0.0001$) respectively. However, there was no significant difference between the middle and apical sections ($P = 0.99$) for both groups. Table 1 and Figure 2 shows the distribution of smear scores at all cross-sectional levels for both study groups.

4. Discussion

Residual necrotic material and debris within the root canal system can serve as a nutrient source for persistent microbial growth and proliferation. Therefore, complete removal of these germs from the root canal system prior to the final obturation operation is critical to the effectiveness of endodontic treatment. Effective irrigation during and

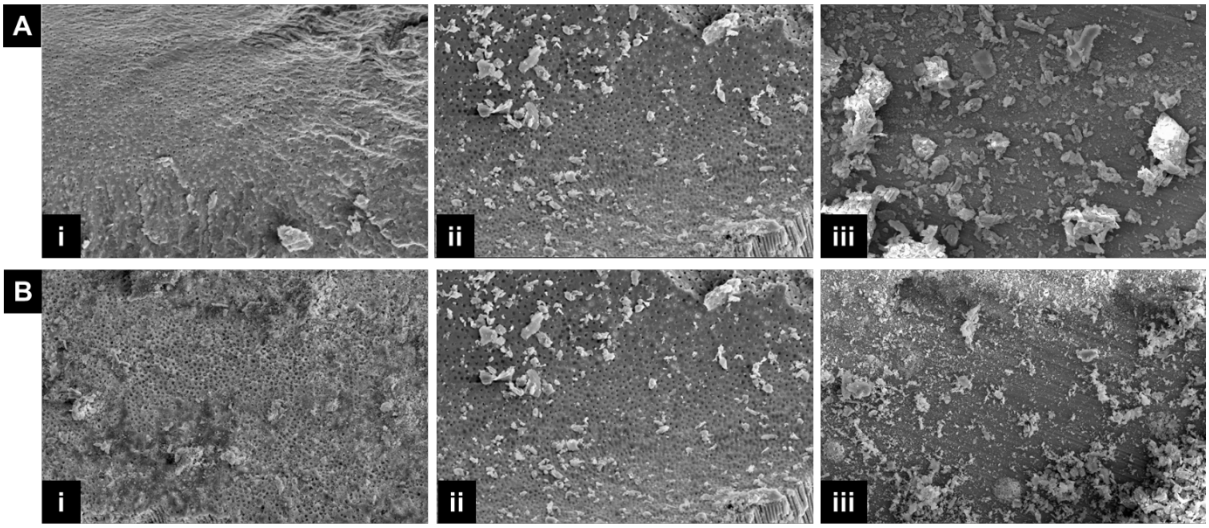

Figure 1: Photomicrographs demonstrating smear layer removal in root canal thirds at 1,000× magnification. **A:** Treated with 20% NAC; **B:** Treated with 17% EDTA in, **i)** Coronal third, **ii)** Middle third, and, **iii)** Apical third.

Table 1: Descriptive statistics (median and interquartile range) and Intergroup comparisons for the smear scores after irrigating root dentine with the respective irrigating solutions

Groups/ Cross-sections	**17% EDTA(n =46)**	**N-Acetyl Cysteine(n =46)**	**P value †**
Coronal	2(2-2.5) ^{a}	2(1.75-2)^{a}	0.07^{NS}
Middle	3(2-3)^{b}	3(2-3)^{b}	0.81^{NS}
Apical	3(3-3)^{b}	3(3-3)^{b}	0.99^{NS}
P value§	<0.0001*	<0.0001*	

n: number of samples per group
†: analyzed by the Mann-Whitney's test
§: analyzed by Friedman's ANOVA test
Different superscript letters indicate significant differences between the pairs of cross-sectional levels for each study group
*: statistically significant (P≤0.05); NS: not statistically significant (P>0.05)

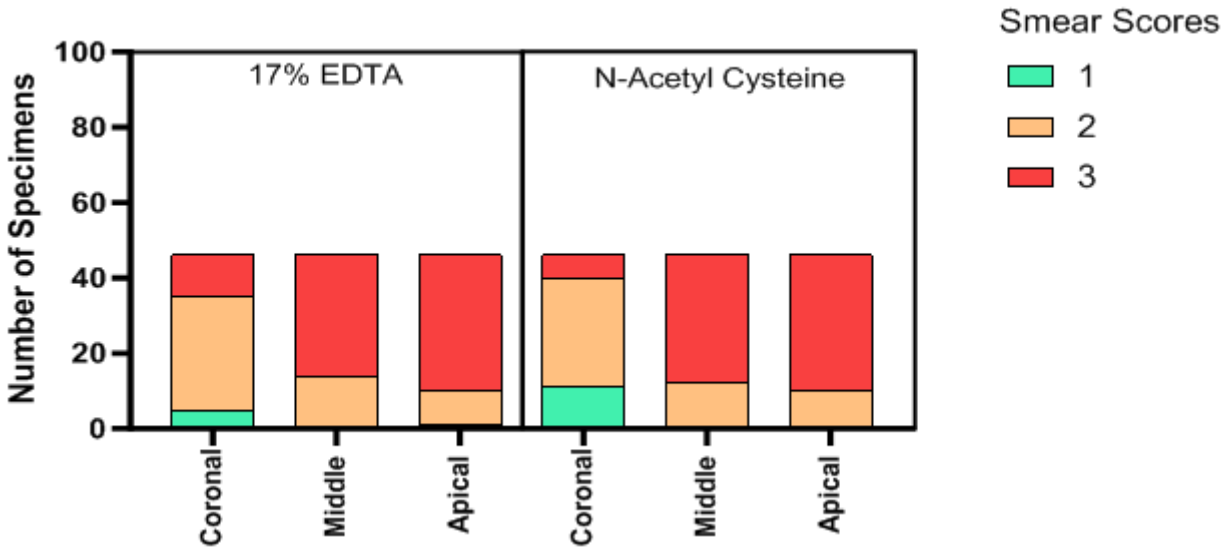

Figure 2: Distribution of smear scores at all cross-sectional levels for both the study groups

after instrumentation is essential because it lubricates the root canal system, which makes the smear layer removal easier, as well as ensures thorough debridement, thereby minimizing the risk of treatment failure and improving the overall outcome of the endodontic treatment.[7]

EDTA is the most widely utilized chelating agent in endodontics, well known for its capacity to integrate with the calcium in the smear layer to create stable compounds, thereby enabling its effective removal. However, EDTA's potent demineralizing action can lead to the widening of dentinal tubules, dentin softening, and denaturation of collagen fibers, potentially resulting in excessive dentin erosion. This undesirable effect on dentin structure has prompted recent research to focus on identifying more efficient and biocompatible alternative chelating agents that can efficiently eliminate the smear layer and reduce the with limited demineralisation.[8,9]

An antioxidant that contains thiols, N-acetylcysteine, exhibits antibacterial properties against endodontic pathogens such as Enterococcus faecalis as well as Streptococcus mutans. Its active thiol group neutralizes free radicals and disrupts disulfide bonds, and studies have indicated that its efficacy in removing the smear layer remains unaffected by dentin.[10] Clinically, NAC is used for its anti-inflammatory and chelating properties, particularly in the treatment of chronic respiratory diseases and the removal of methylmercury toxins. Hence the study aims to further evaluate NAC's chelating ability by comparing its effectiveness in removing the smear layer from the root canal system to that of the commonly used chelating agent, EDTA.

In this investigation, the root canals were chemomechanically prepared using 2.5% sodium hypochlorite in between each instrumentation step. In this concentration, NaOCl worked well to eliminate the smear layer's organic components. This approach is supported by previous research indicating that diluted NaOCl solutions, such as the 2.5% concentration used here, are not inferior to higher concentrations in removing the organic portion of the smear layer. The utilization of 2.5% NaOCl, a commonly recommended concentration, was intentionally chosen to balance the need for effective smear layer removal while minimizing the potential risk of dentin erosion or other adverse effects that may be linked to the application of larger NaOCl concentrations.[11,12]

Prior research has shown that applying 17% EDTA for just one minute can effectively remove the smear layer without having the negative effects of extended exposure, which include increased erosion, degradation of the dentinal surface and expansion of the dentinal tubule apertures. Therefore, a 1-minute application of EDTA was chosen for this study. Scanning electron microscopy was selected as the evaluation method, as it is a widely available and often employed instrument to evaluate the smear layer removal process. Additionally, prior literature has indicated that utilizing a side-vented, 30-gauge needle for irrigation can result in fewer extrusions compared to other needle types, which was an important consideration in our experimental design.[13–15]

The current investigation discovered that the layer of smears in the middle, along with coronal sections of the root canal, could be better eliminated by both irrigants—17% EDTA and 20% NAC. This result is consistent with other research showing that EDTA works best to eliminate the middle & coronal thirds' smear layers but less effectively from the apical 3rd. This could be a result of sclerotic dentin in the apical 3rd, which could impair effectiveness of 17 percent EDTA.[16–18] The results of this investigation indicate that N-acetylcysteine might be a more useful as well as desirable chelating agent compared to EDTA as NAC demonstrated similar, if not superior, efficacy in eliminating the root canal system's smear layer. Other studies have also shown that NAC causes a significantly smaller reduction in the microhardness of root dentin compared to EDTA. Additionally, NAC exhibits comparable antibacterial properties to other irrigants, such as chlorhexidine, which is less efficient in smear layer removal.[19,20]

Recent research has also revealed that NAC has achieved higher bond strength than chlorhexidine, which may be attributed to its ability to reduce extracellular polysaccharide production, therefore improving the obturating materials' adherence to the dentin walls of the roots. Furthermore, studies have indicated that chlorhexidine has less soluble substances, which can leave residues on the canal walls, potentially affecting the overall sealing quality.[21–23]

5. Conclusion

The study concludes by reiterating how crucial efficient smear layer removal is to the outcome of endodontic therapy. The smear layer may be effectively removed by both 17% EDTA and 20% NAC, especially in the intermediate and coronal regions of the root canal system. However, NAC emerged as a promising alternative to EDTA, not only due to its comparable chelating and antibacterial properties but also because of its potential advantages in enhancing the adhesion of obturating materials. These findings suggest that NAC could offer a more biocompatible and efficient solution for root canal irrigation, minimizing the risks of excessive dentin erosion while maintaining the structural integrity of the dentin. Further research is recommended to explore NAC's long-term effects on dentin and its potential role in improving clinical outcomes in endodontic treatment.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Choudhury P, Raghu R, Shetty A, Santhosh L, Subhashini R, Nikhitha KL, et al. J Conserv Dent Endod. *J Conserv Dent Endod*. 2024;27(7):724–9.
2. Abidin T, Susilo D, Gani BA. The effectiveness of nano-chitosan high molecular 0.2% as irrigant agent against Enterococcus faecalis with passive ultrasonic irrigant. *J Conserv Dent*. 2022;25(1):37–41.
3. Hussein ER, Shukri BM, Ibrahim RH. The effect of chitosan nanoparticle, citric acid, and ethylenediaminetetraacetic acid on dentin smear layer using two different irrigation needles: A scanning electron microscope study. *J Conserv Dent*. 2022;25(4):431–5.
4. Mahesh M, Pillai R, Varghese NO, Salim AA, Murali N, Nair SS, et al. An in vitro study of comparative evaluation of efficacy of electrochemically activated water as a root canal irrigant in smear layer removal. *J Conserv Dent*. 2020;23(5):447–50.
5. Karthikeyan HR, Rajakumaran A, Rajendran MR, Balaji L. Evaluation of Effect of Natural Extract Sodium Gluconate on Smear Layer and Dentine Decalcification Compared with EDTA - An In-vitro Study. *Eur Endod J*. 2023;8(4):274–9.
6. Rajakumaran A, Ramesh H, Ashok R, Balaji L, Ganesh A. Smear Layer Removal and Microhardness Alteration Potential of a Naturally Occurring Antioxidant - An In Vitro Study. *Cureus*. 2019;11(7):5241. doi:10.7759/cureus.5241.
7. Kuruvilla A, Jaganath BM, Krishnegowda SC, Ramachandra PK, Johns DA, Abraham A, et al. 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. *J Conserv Dent*. 2015;18(3):247–51.
8. Veeraiyan M, Kumar YP, Chandhar CY, Priyanka Y, Jaiswal M, Kemasaram D. Evaluation of Smear Layer Removal and Micro Hardness Alteration of Radicular Dentin after Using Various Chelating Agents - An Atomic Force Microscopic Study. *J Pharm Bioallied Sci*. 2023;15(1):582–7.
9. Widbiller M, Rosendahl A, Schlichting R, Schuller C, Lingl B, Hiller KA, et al. Impact of Endodontic Irrigant Activation on Smear Layer Removal and Surface Disintegration of Root Canal Dentine In Vitro. *Healthcare (Basel)*. 2023;11(3):376. doi:10.3390/healthcare11030376.
10. Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, et al. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. *Free Radic Res*. 2018;52(7):751–62.
11. Torabinejad M, Cho Y, Khademi AA, Bakland LK, Shabahang S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. *J Endod*. 2003;29:233–9.
12. Darrag AM. Effectiveness of different final irrigation solutions on smear layer removal in intraradicular dentin. *Tanta Dent J*. 2014;11(2):93–9.
13. Silva PB, Krolow AM, Pilownic KJ, Casarin RP, Lima RK, Leonardo RT, et al. Apical Extrusion of Debris and Irrigants Using Different Irrigation Needles. *Braz Dent J*. 2016;27(2):192–5.
14. Teja KV, Ramesh S, Choudhari S, Janani K, Jose J, Vasundhara KA, et al. A questionnaire-based cross-sectional survey of Indian postgraduates and endodontists on awareness, attitude, and practice of using conventional syringe needle irrigation during root canal treatment. *Saudi Endod J*. 2022;12(3):302–8.
15. Orlowski NB, Schimdt TF, Teixeira CDS, Garcia L, Savaris JM, Tay FR, et al. Smear Layer Removal Using Passive Ultrasonic Irrigation and Different Concentrations of Sodium Hypochlorite. *J Endod*. 2020;46(11):1738–44.
16. Kuah HG, Lui JN, Tseng PS, Chen NN. The effect of EDTA with and without ultrasonics on removal of the smear layer. *J Endod*. 2009;35(3):393–6.
17. Yoshida T, Shibata T, Shinohara T, Gomyo S, Sekine I. Clinical evaluation of the efficacy of EDTA solution as an endodontic irrigant. *J Endod*. 1995;21(12):592–3.
18. Kaushal R, Bansal R, Malhan S. A comparative evaluation of smear layer removal by using ethylenediamine tetraacetic acid, citric acid, and maleic acid as root canal irrigants: An in vitro scanning electron microscopic study. *J Conserv Dent*. 2020;23(1):71–8.
19. Palaniswamy U, Lakkam SR, Arya S, Aravelli S. Effectiveness of N-acetyl cysteine, 2% chlorhexidine, and their combination as intracanal medicaments on Enterococcus faecalis biofilm. *J Conserv Dent*. 2016;19(1):17–20.
20. Kuruvilla A, Jaganath BM, Krishnegowda SC, Ramachandra PK, Johns A, Abraham A, et al. 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. *J Conserv Dent*. 2015;18(3):247–51.
21. Kanisavaran ZM. Chlorhexidine gluconate in endodontics: an update review. *Int Dent J*. 2008;58(5):247–57.
22. Choi YS, Kim C, Moon JH, Lee JY. Removal and killing of multispecies endodontic biofilms by N-acetylcysteine. *Braz J Microbiol*. 2018;49(1):184–8.
23. Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. *Int Endod J*. 2009;42(4):288–302.

Author's biography

Suresh Shenvi, Reader https://orcid.org/0000-0003-4466-774X

Sonal B Joshi, Professor and Head https://orcid.org/0000-0002-9660-2705

Cite this article: Shenvi S, Joshi SB. Smear layer removal potential of a naturally occurring antioxidant: An in-vitro scanning electron microscopic study. *IP Ann Prosthodont Restor Dent* 2025;11(1):35-39.