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ORIGINAL ARTICLE

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The Effect of 17 % EDTA, 10 % Citric Acid, and 0.2 % Chitosan on Smear Layer Removal and Microhardness of the Root Canal Dentin: An in Vitro Study

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Abstract

Introduction: The endodontic procedure involves adequate mechanical preparation and irrigation of the root canal, followed by three-dimensional filling. However, mechanical instrumentation results in an irregular and amorphous smear layer within the root canals. This may impact endodontic success, thus requiring the complete removal of debris from the root canal. Furthermore, the application of irrigant reduces the hardness of dentin. Therefore, the role of the irrigant solution is crucial in the removal of debris. The present study evaluated smear layer removal and microhardness reduction in the middle and apical thirds of root canals irrigated with 17 % ethylenediaminetetraacetic acid (EDTA), 10 % citric acid, and 0.2 % chitosan, using a scanning electron microscope and a vickers hardness tester. **Methods:** Sixty extracted single-rooted mandibular premolars were utilized. Access opening, cleaning, and shaping were done with intermittent irrigation using 3% sodium hypochlorite after each file. Depending on the final irrigating solvent, they were divided randomly into three groups (n = 15): group 1 (17 % EDTA), group 2 (10 % citric acid), and group 3-(0.2 % chitosan). They were longitudinally sectioned after decoronation. To assess the smear layer at various levels, a scanning electron microscope examination was performed. Using a Vickers indenter, dentin microhardness was determined. One-way analysis of variance and Kruskal-Wallis test was conducted for statistical analysis. **Results:** Studies revealed no statistically significant variance in smear layer removal between 0.2 % chitosan and 17 % EDTA in the middle and apical third, but there was a statistically significant difference with 10% citric acid. The microhardness reductions of 0.2 % chitosan, 10 % citric acid, and 17 % EDTA did not differ statistically significantly. **Conclusion:** The utilization of 0.2 % chitosan as a final rinse irrigant shows promising potential as an alternative to EDTA.

KEYWORDS

– Root canal irrigants; smear layer; EDTA; citric acid; chitosan

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1 | INTRODUCTION

To ensure the thorough cleaning and disinfection of the root canal is main goal of an endodontic therapy. The success relies on adequate canal preparation, effective irrigation and complete three-dimensional canal filling. These objectives can be achieved through a combination of mechanical instrumentation and chemical methods.[1] Mechanical instrumentation creates a thin layer called the "smear layer" on the canal walls made up of inorganic along with organic particles include odontoblastic processes, necrotic debris, and dentin particles. The smear layer is amorphous and irregular, covering the prepared root canal walls and blocking the dentinal tubules orifice. Consequently, it limits the penetration of irrigants, sealers and intracanal medicaments, through dentinal tubules.[2] An ideal irrigant serves multiple purposes. It should physically flush out debris, act as a tissue solvent, exhibit bactericidal properties, and provide lubrication. Root canal irrigants, by removing both organic and inorganic matter from the dentin surface, can induce changes in the mineral composition of dentin and alter calcium (Ca) to phosphorus (P) ratio present in hydroxyapatite. The aforementioned changes may affect the dentin's microhardness, permeability, solubility, and the way resin-based materials adhere to the root dentin surface.[3] Several compounds have been employed as root canal irrigants. These include reducing agents (e.g., sodium hypochlorite [NaOCl], chlorine dioxide [ClO_2]), oxidizing agents (e.g., hydrogen peroxide), bactericidal agents (e.g., chlorhexidine [CHX]), bacteriostatic agents (e.g., MTAD), chelating agents (e.g., ethylenediaminetetraacetic acid [EDTA], MTAD, HEPB), and acids (e.g., maleic acid, citric acid, polyacrylic acid).[4] EDTA is the most commonly used decalcifying agent. At pH 7, this synthetic version of amino acid is biocompatible and functions like a root canal irrigant. One of its main properties is the ability to chelate metallic ions necessary for microbial growth, effectively killing them, although it does not possess direct antibacterial effects. Within 5 minutes, Calcium can be removed from dentin at depths of about 20–30 µm when EDTA doses of 15–17% are used.[5] Citric acid, a weak organic acid, is another irrigating agent used to eliminate the root canal's smear layer. Concentrations ranging from 1% to 50% have been employed, with 10% citric acid proving to be more efficient.[6] Chitosan, an organic polymer derived from the chitin found in crab exoskeletons, has drawn interest in dentistry research, as it is biocompatible to the tissues, biodegradable, bio adhesion, and low systemic toxicity. Under acidic conditions, it has a high chelating ability for various metal ions. Consequently, chitosan is used as a chelating agent and has ecological interest due to its low cost and abundance in nature.[7] This study compared the efficiency of 17 % EDTA, 10 % citric acid, and 0.2 % chitosan in removing smear layers after root canal instrumentation using scanning electron microscopy (SEM) and their impact on the microhardness of the root canal dentin using the Vickers hardness test.

2 | METHOD

After obtaining approval from the Institutional Ethical Committee, the collection of teeth samples was determined. Sixty extracted human permanent mandibular premolars were selected for the study. They were extracted for orthodontic purposes and periodontal reasons. The presence of a single patent canal was verified on radiographs. To eliminate organic debris the teeth were stored in 1 % NaOCl. They were then removed, washed under the tap water and stored in 10 % formalin solution for disinfection. The initial step is utilizing a BR 41 round bur to create a coronal access cavity, which provided direct pathway to access all the canals. The canals were then identified using a DG-16 endodontic probe. Establishing canal patency required a size 10 K-file. The working length was determined with a 10K file 1 mm short of this measurement and was confirmed by radiograph. Modeling wax was carefully adapted to the apical foramen of the teeth within a transparent plastic container made of soft poly-vinyl siloxane impression material. This method attempted to prevent irrigant outflow through the apex while mimicking realistic conditions. Starting with hand files up to size 15 k-files were placed in the canal. The proglider (16/0.02) rotary file were used till it reaches the working length. One flare (25/0.04) was used for coronal enlargement. The root canal was prepared by the same operator using rotary Protaper Next consisting of 3 files X1(17/0.04), X2(25/0.06), X3(30/0.07). The canals were irrigated using 2 ml of 3% sodium hypochlorite, which

was administered with a syringe and a 30-gauge needle, inserted to a depth of 2 mm short of the entire working length. Additionally, a 27-gauge needle was employed to ensure a higher volume of irrigant, particularly for effectively removing the coronal and middle one-third debris. Finally, with 5 ml of saline the canals were rinsed and randomly divided into three groups (n-15) according to the final irrigating solution used for smear layer removal

- Group 1: Canals irrigated with 5 ml of 17 % EDTA for 5 mins.
- Group 2: Canals irrigated with 5 ml of 10 % citric acid for 5 mins.
- Group 3: Canals irrigated with 5 ml of 0.2 % Chitosan for 5 mins

All groups were activated ultrasonically with (25/04) EndoActivator tip used in "up and down" short vertical movements with an oscillation of 2-3 mm for 30 seconds and operated at a speed of 10 kHz for the 30 seconds. Following an extensive flushing of the root canals with 5 ml of distilled water to offset detrimental effects of irrigants, the canals were completely dried out and sterilized cotton pellets were placed at the orifices of the canals.

Preparation of samples for SEM analysis

Decoronation was performed with diamond disc, resulting in standardized root length of 16 mm for each case. Using a diamond bur, two longitudinal grooves were made on the buccal and palatal/lingual surfaces of each root. Care was taken to avoid any penetration into the root canal. Final separation was done by splitting the root with chisel and mallet. The sections were meticulously cleansed to remove any grinding residue and subsequently dried using a combination of water and an air blast for duration of 3 seconds. Till the SEM was carried out the specimens were stored in 2 % glutaraldehyde aqueous solution and then dehydrated in alcohol (70-90 %) and dried.

SEM examination

Each group's coded samples were firmly taped to aluminium stubs using carbon tape (Royal Tapes Pvt Ltd, Chennai, India). Using a gold sputter coating device (Quorum, UK) the samples were covered in a layer of gold that was 20–30 nm thick. A field emission scanning electron microscope (SIGMA 0336 FESEM, ZIESS and MUNCHEN, GERMANY) was then used to conduct a complete analysis of the materials.

Analysis of photomicrographs

The SEM images were scored using qualitative evaluation of the canal cleanliness which was suggested by Torabinejad et al.[8]:

- Score 0 = smear layer and debris removed totally with opened dentinal tubules.
- Score 1 = smear layer exists only in the apertures of the dentinal tubules.
- Score 2 = the root canal surface and dentinal tubular apertures covered with a thin smear layer

Microhardness measurement

The surface hardness of the root dentin was evaluated using a Vickers Hardness Tester (Highwood micro Vickers hardness tester) under a 300 g load for a duration of 15 seconds. In each sample, indentations were made in middle and apical third. Representative hardness value was obtained for each sample.

2.1 | Statistical analysis

Smear layer removal score frequency between three groups was analyzed using Kruskal-Wallis test whereas mean microhardness reduction between three groups was analyzed by one-way analysis. P values < 0.05 were considered as statistically significant.

3 | RESULTS

The study compared the effects of three different irrigants (2 % chitosan, 17 % EDTA, and 10 % citric acid) on the smear layer removal and microhardness reduction of root canal dentin. The results showed that both 2 % chitosan (Fig. 1, 2) and 17 % EDTA (Fig. 3, 4) had a significant effect on the smear layer removal at the middle and apical thirds of the root canal, while 10 % citric acid did not (Fig. 5, 6, (Table 1). On the other hand, none of the irrigants had a significant effect on the microhardness reduction at either the middle or the apical third of the root canal (Table 1). The microhardness scores were higher for

17 % EDTA than for 10 % citric acid and 0.2 % chitosan, but the difference was not statistically significant.

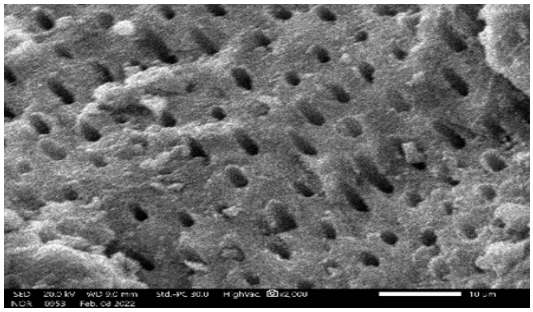

FIGURE 1 SEM image showing smear layer removal in middle third of root canal wall after treating with chitosan

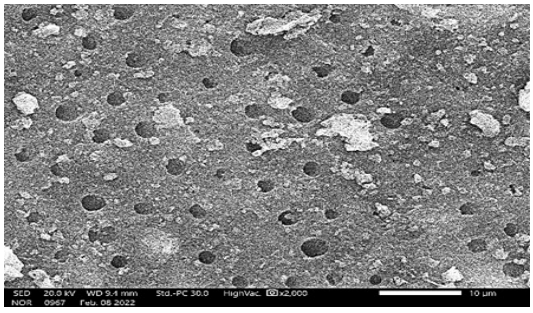

FIGURE 2 SEM image showing smear layer removal in apical third of root canal dentin after treating with chitosan

4 | DISCUSSION

Irrigation of the root canal system is a pivotal factor in achieving successful endodontic treatment, as it plays a crucial role in eliminating microorganisms and facilitating proper instrumentation and obturation by employing a combination of appropriate instrumentation, effective irrigation, and obturation. Among these fundamental steps, irrigation process stands out as the primary determinant of treatment success.[9] According to Calt et al.[10], the degree of material penetration, root length, canal diameter, application time, pH, and material concentration all affect how efficient chelating agents are. In the present research, it emerged that Chitosan, even at lower concentrations, showed identical effectiveness to EDTA in eradicating smear layers from various root canal locations. These findings align with earlier research conducted by Silva et al.[11], Darrag et al.[12], Sarkees et al.[5], and Del et al.[13].

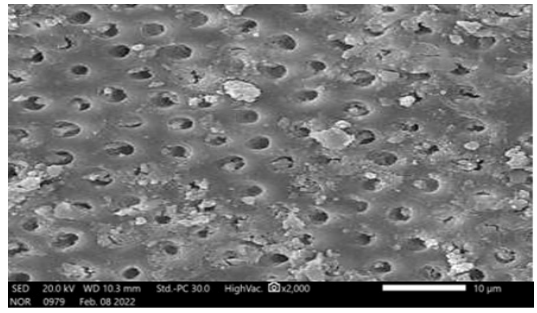

FIGURE 3 SEM image showing smear layer removal in middle third of root canal wall after treating with EDTA

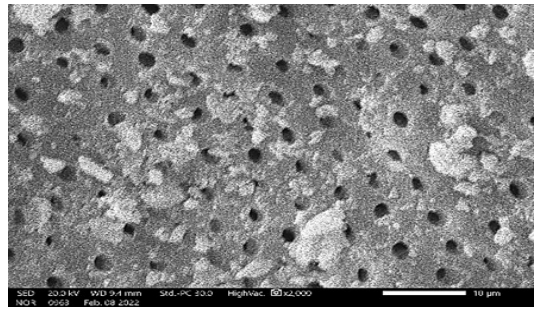

FIGURE 4 SEM image showing smear layer removal in apical third of root canal wall after treating with EDTA

TABLE 1 Mean values of microleakage for various groups of restorative materials used in the study

Root	EDTA	Citric Acid	Chitosan
Smear Layer Removal			
Middle	1.60 ± 0.50	2.53 ± 0.51	1.67 ± 0.48
Apical	2.40 ± 0.50	2.73 ± 0.45	2.20 ± 0.41
Microhardness			
Middle	42.7 ± 2.18	42.6 ± 1.81	42.6 ± 1.79
Apical	42.7 ± 2.19	42.6 ± 1.56	42.6 ± 1.81

Two possible answers can explain how chitosan works to chelate substances. According to the bridge model, which is the original idea, several amino acid sequences from a Chitosan chain link to a single metal ion.

Conversely, the secondary hypothesis proposes that only one amino group from the Chitosan structure is involved in binding process, acting as an anchor for the metal ion. The chain of chitin dimers that jointly make up the chitosan polymer is made up of many of them. Each chitin dimer, like the EDTA molecule, has two nitrogen atoms with pairs of free electrons, enabling ionic interactions with the metal and the chelating agent. The bipolymer produces a net positive charge (-NH3+) when subjected to an acidic environment because the amino groups become protonated. This charged form facilitates attraction towards other molecules and allows adsorption to take place. Adsorption, ion exchange, and chelation are just some of the potential routes that might result to complexes between Chitosan and metal ions.[14]

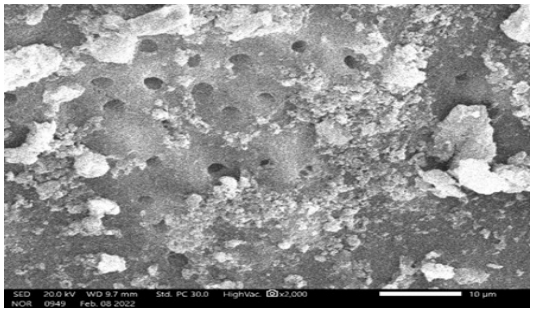

FIGURE 5 SEM image showing smear layer removal in apical third of root canal wall after treating with citric acid

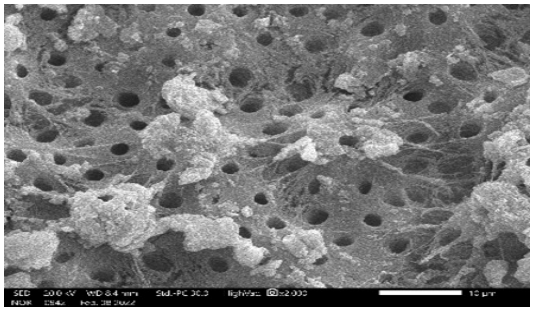

FIGURE 6 SEM image showing smear layer removal in middle third of root canal wall after treating with citric acid

The present study revealed that 10 % citric acid solution resulted in lower smear layer removal compared to 0.2 % Chitosan and 17 % EDTA. This can be attributed to the fact that citric acid being a weak acid, with a pH of 1.8, whereas 17 % EDTA and Chitosan have pH values of 7 and 7.4, respectively. It is likely that when concentration rises, citric acid's chelating effect becomes more apparent. According to Sayin et al.[15], the alteration of the Ca/P ratio resulting from the use of irrigating solutions can modify the composition of organic and inorganic components of dentin leading to decrease in microhardness. Saliva was the only irrigation solution found in Saleh et al.[16] study that did not result in a decrease in dentin microhardness. Under the condition of the present study, Chitosan, EDTA and Citric acid exhibits similar microhardness reduction in various spots of the root canal. 17 % EDTA showed greater reduction when compared to chitosan and citric acid but it was not statistically significant. Many explanations have been put forth to explain why the chelating activity of EDTA causes a decrease in dentin microhardness. One such theory is crystalline field theory, which suggests that the central metal's and ligands' attraction force is purely electrostatic. Bonding strength of the metallic ion surpasses the repulsive force exerted by EDTA atoms, resulting in the formation of a stable complex between EDTA and the calcium ions found in dentin. During this process, the carboxyl groups of the EDTA molecule become ionized, leading to the release of competing hydrogen atoms with the calcium ions. Similarly, citric acid exhibits statistically similar effect to EDTA. Calcium citrate is formed when citric acid reacts quickly with calcium. When used at similar concentration, citric acid should remove more calcium ions, resulting in a greater reduction in dentin microhardness.[17] Pimeta et al. said that when compared to the other tested solutions, the chelating effect of 0.2 % chitosan allied to its favorable characteristics and low concentration.[18]

5 | CONCLUSION

Chitosan presents itself as a viable alternative to EDTA and citric acid as a final irrigant. However, further rsearch is warranted to implement it in dental practice.

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Nil

Conflict of interest

The authors have no conflicts of interest to declare.

Supporting Information

Additional supporting information may be found at the journal's website.

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