**ABSTRACT**

**Objective:** The purpose of this study was to determine the effective concentration of indigenously prepared chitosan gel for smear layer removal.

**Materials and Methodology:** Thirty single-rooted premolars were decoronated to a standard length enlarged along with irrigation of 2 mL 3% NaOCl on each change of instrument. Specimens were then divided into three equal groups. In the first group, 0.1% chitosan gel was used as a lubricating agent and final rinsing was done with 2 ml saline. Similarly, in other two groups, 0.2% and 0.3% chitosan gel was used as a lubricating agent and finally rinsed with saline. Samples were longitudinally sectioned subjected to scanning electron microscope evaluation for smear score. Results were analyzed statistically by Kruskal–Wallis test and Mann–Whitney Post hoc analysis.

**Results:** No significant difference between 0.1%, 0.2%, and 0.3% chitosan gel on smear layer removal in the coronal third and in middle and apical third, and there was no significant difference between 0.2% and 0.3% chitosan gels on smear layer removal.

**Conclusion:** All the experimental gel concentrations of chitosan as lubricant showed smear layer removal at all the thirds of the root canal. Smear layer removal was better in the coronal and middle third than in apical third with all the gel concentrations of chitosan. No significant difference in smear layer removal between 0.2% and 0.3% chitosan gel at all the thirds of the canal. Considering the smear layer removal efficacy at minimal concentration, 0.2% chitosan gel can be effective.

**Keywords:** Chitosan gel, ethylenediamintetraacetic acid gel, sodium hypochlorite

**INTRODUCTION**

Effective removal of the smear layer from all the sections of root canal, i.e., coronal, middle, and especially in the apical third, has always been a displeasing and everlasting obstacle. It is well known that bacteria not only remain but also survive in smear layer.[1] Hence, the removal of smear layer is critical as the antimicrobial action of medicaments can be delayed or hindered, and the ability of sealer to penetrate the dentinal tubules and adaptation to the root canal fillings can be compromised.[2-3]

Several chelators have been introduced to aid in the negotiation of root canal and removal of the inorganic portion of smear layer as NaOCl can effectively remove only the organic components in it.[4] Ethylenediamintetraacetic acid (EDTA) at neutral Ph promotes chelation of calcium in dentine and is recommended for the removal of smear layer.[5] Other substances used for smear layer removal...
include EDTAC, REDTA, RC-Prep, Glyde file, MTAD, and apple vinegar.

Over decades, EDTA is increasingly used in endodontics because of its better smear layer removal ability. However, like all materials used in endodontics, EDTA has its own limitations such as canal wall erosion, excessive removal of intertubular and peritubular dentine, reduction in the microhardness of root dentine, and decreased adhesion of resin-based sealers to the canal walls.\(^{[6,7]}\) Biocompatibility of EDTA is also of concern as extrusion into periapical tissues causes irreversible decalcification of periapical bone.\(^{[8]}\)

Further, the role of type of EDTA whether paste/gel or solution to be used is unclear in the literature. While the literature reports predominantly on the mode of action of liquid chelator solutions for root canal irrigation, the chelators recommended for use during rotary root canal preparation should have a paste or gel consistency as endodontic instruments are subjected to different forces, including flexion, torsion, traction, and apical pressure.\(^{[9]}\) EDTA gel when used with various rotary instruments, dentinal crack formation was similar with all these instruments and use of EDTA as gel formulation did not reduce crack occurrence.\(^{[10]}\)

To address limitations of EDTA, chitosan, a deacetylated derivative of chitin, has attracted a great attention due to its nontoxicity, biodegradability, and biocompatibility.\(^{[11]}\) In addition, it has antibacterial, antioxidant, film-forming ability, gel enhancer, etc.\(^{[12]}\)

Remarkable smear layer removal of chitosan solution irrigant similar to EDTA with minimal canal wall erosion has been proved in the studies done by Silva \( et \ al.,\) Darrag, etc.\(^{[13,14]}\) Considering the advantages of gel formulations and trends in the usage of naturally available eco-friendly materials, indigenously prepared chitosan gel was used to determine its efficacy at various concentrations on smear layer.

**MATERIALS AND METHODOLOGY**

Thirty extracted single-rooted premolar teeth with single canal and complete root formation were collected. Teeth with caries or fracture, open apices, calcified canals, multiple canals, with anatomical variations, internal resorption, and previous restoration or endodontic treatment were excluded.

Samples were cleaned of debris and decoronated to a standard length of 15 mm with a diamond disc and 15 K-file inserted till tip was visible at the apical foramen, and the working length, 1 mm from this length, was established for shaping the canals. All canals were prepared using K3 rotary instrument till size 30, 0.06 taper. The specimens were grouped into three groups based on the concentration of gel used:
- Group 1: 0.1% chitosan gel was used as lubricant
- Group 2: 0.2% chitosan gel was used as lubricant
- Group 3: 0.3% chitosan gel was used as lubricant.

At the change of each instrument, canal was rinsed with 2 ml 3% NaOCl solution and finally rinsed with 2 ml saline solution in all the groups.

After cleaning and shaping, samples were split longitudinally dehydrated and sputter coated and observed under scanning electron microscope with a magnification of \(\times\) 10000. The presence of smear layer on the surface of root canal was observed at coronal, middle, and apical portion of each canal and scored as follows:
- Score 1: No smear layer, dentinal tubule open and free from debris
- Score 2: Moderate smear layer, outlines of dentinal tubules visible or partially filled with debris
- Score 3: Abundant smear layer preventing the visualization of dentinal tubule opening.

**Statistical test used**

Kruskal–Wallis test and Mann–Whitney post hoc analysis were used for statistical analysis.

**RESULTS**

The test results revealed that mean smear layer scores in the coronal third were highest in 0.1% chitosan group followed by 0.3% chitosan group and least with 0.2% chitosan gel group [Table 1]. This difference was statistically insignificant \((P > 0.01)\).

The test results revealed that mean smear layer scores in the middle third were highest in 0.1% chitosan gel followed by 0.3% chitosan gel and least with 0.2% chitosan gel [Table 2]. This difference was statistically significant \((P < 0.001)\). Multiple comparison test demonstrated significant difference in the mean scores between 0.1% chitosan gel

| Groups | \(n\) | Mean | SD | Minimum | Maximum | \(P\) |
|-------|-----|-----|----|--------|--------|-----|
| Group 1 | 10  | 2.2 | 0.4 | 2      | 3      | 0.06|
| Group 2 | 10  | 1.6 | 0.5 | 1      | 2      |     |
| Group 3 | 10  | 1.8 | 0.6 | 1      | 3      |     |

SD: Standard deviation
and 0.2% chitosan gel group and 0.1% and 0.3% chitosan gel group.

However, there was no statistically significant difference between 0.2% and 0.3% chitosan gel groups.

The test results revealed that mean smear layer scores in the apical third were highest in 0.1% chitosan gel group followed by 0.2% chitosan gel and least with 0.3% chitosan gel group [Table 3]. This difference was statistically significant ($P < 0.001$) [Figures 1-3].

Multiple comparison test demonstrated significant difference in the mean scores in 0.1% chitosan gel and 0.3% chitosan gel group. However, there was no statistically significant difference between 0.1% and 0.2% chitosan gel groups and 0.2% and 0.3% chitosan groups.

**DISCUSSION**

Access cavity preparation, cleaning, shaping, obturation of the root canals, and adequate coronal seal contribute to the overall success of endodontic treatment. During cleaning and shaping procedure, irrigants are used to remove the pulp tissue, debris, and smear layer which is formed by the action of instruments on the root canal wall.\[15\] The thickness of smear layer is determined by the type, sharpness of the cutting instrument, and its rotational or reciprocating movement.\[16\]

Haapasalo and Orstavik suggested that removal of smear layer allows better action of medicament and sealer penetration, thereby providing coronal and apical seal that is paramount for the success. Hence, various endodontic irrigants are used for effective removal of canal contents before, during, and after instrumentation.\[17\]

**Table 2: Smear score in the middle third**

Comparison of mean scores for smear layer removal in the middle one-third area between different study groups using Kruskal-Wallis test followed by Mann-Whitney post hoc analysis

| Groups | n  | Mean | SD  | Minimum | Maximum | $P$     | Mann-Whitney post hoc  |
|--------|----|------|-----|---------|---------|--------|------------------------|
| Group 1| 10 | 2.6  | 0.5 | 2       | 3       | 0.004*| Group 1 versus Group 2 0.002* |
| Group 2| 10 | 1.7  | 0.5 | 1       | 2       |       | Group 1 versus Group 3 0.01*  |
| Group 3| 10 | 1.9  | 0.6 | 1       | 3       |       | Group 2 versus Group 3 0.42 |

*Statistically significant. SD: Standard deviation

**Table 3: Smear score in the apical third**

Comparison of mean scores for smear layer removal in the apical one-third area between different study groups using Kruskal-Wallis test followed by Mann-Whitney post hoc analysis

| Groups | n  | Mean | SD  | Minimum | Maximum | $P$     | Mann-Whitney post hoc  |
|--------|----|------|-----|---------|---------|--------|------------------------|
| Group 1| 10 | 2.5  | 0.5 | 2       | 3       | 0.01* | Group 1 versus Group 2 0.06  |
| Group 2| 10 | 1.9  | 0.7 | 1       | 3       |       | Group 1 versus Group 3 0.005* |
| Group 3| 10 | 1.5  | 0.7 | 1       | 3       |       | Group 2 versus Group 3 0.20 |

*Statistically significant. SD: Standard deviation
Sodium hypochlorite is effective for dissolving the pulp tissue, biofilm, and the organic part of smear layer hence should be used throughout the instrumentation phase. Inorganic portion of smear layer is removed by chelators like EDTA which reacts with calcium in dentine and forms soluble calcium chelates.

To address the limitations associated with EDTA, chitosan has been introduced as an irrigant in endodontics. Chitin and chitosan, the naturally abundant and renewable polymers, have excellent properties such as biodegradability, biocompatibility, nontoxicity, and adsorption. Chitin is a major structural component of the exoskeleton of crustaceans such as crabs and shrimps because of its abundance it is referred as “animal cellulose.” In addition, it is bacteriostatic, accelerates the formation of osteoblasts, hemostatic, fungistatic, anti-inflammatory, immunoadjuvant, etc., Its application in diversified areas is due to the reactivity of primary amino group and primary and secondary hydroxyl group.

Interaction of chitosan depends on the involved ions, the chemical structure of chitosan, and the pH of the solution. Currently, there are two theories that explain the chelation process of chitosan. One says that two or more amino groups of one chitosan chain will bind to the same metallic ion. Whereas in another theory only one amino group of the structure is involved in binding.

A study done by Silva et al concluded that 15%EDTA, 0.2% chitosan and 10% citric acid effectively removed smear layer from apical and middle thirds of root canal. 0.2% chitosan solution, when used for 3 min, had minimal erosive effect and better smear layer removal.

Gels as lubricants are mainly used to prevent inadvertent extrusion into the periapical area and to maximize the localization of irrigant for sufficient duration in the root canal space. The usage of gel forms of irrigants was observed in literature not only for chelators but also for other routinely used irrigants such as hypochlorite and chlorhexidine.

Antimicrobial irrigant 2% chlorhexidine gluconate gel produced the cleanest dentin wall surface than with similar solution form. The mechanical properties of the gel seem to be the main factor for this difference because the same chemical agent, when used in liquid presentation, displayed a lower cleanliness efficiency. Due to its viscosity, the gel seems to compensate for chlorhexidine’s inability to dissolve pulp tissue by promoting a better mechanical cleansing of the root canal and removing dentin debris and remaining tissues.

Viscous chelators are used predominantly to aid the negotiation of calcified canals, decrease frictional stress during root canal preparation using rotary instruments. The viscosity prevents accidental extrusion from the irrigation needle and allows the clinician to control the volume easily. However, EDTA gel preparations may fail to reach the apical area of the root canal. Thus, the viscosity of any solution to be used as an irrigant for smear layer removal should be regulated carefully. Although the action of gel and solution forms of chelators are of same advantage, lubricant in gel form does not extrude from the root canal.

In the present study, chitosan gel was indigenously made by adding methylcellulose as the vehicle, as it is a group of nonionic, surface active, water-soluble polymers with a remarkable property of rheology control and biocompatibility. Methylcellulose is used as a carrier for medicinal substances in hydrogel formulations for administration to the skin, in transdermal systems, and on the oral mucosa in dental dressings. In a study done by Krishna and Parameswaran, indigenously prepared EDTA gel where methylcellulose was added to 19% EDTA solution showed less reduction in microhardness than File-Eze after 1 min; however, the difference was not significant after 5 min.

In the coronal third mean smear layer scores among the groups were statistically insignificant.

The test results revealed that mean smear layer scores in the middle thirds was highest in 0.1% chitosan gel followed by 0.3% chitosan gel and least with 0.2% chitosan gel. This difference was statistically significant. There was significant difference in the mean scores between 0.1% chitosan gel and 0.2% chitosan gel group and 0.1% and 0.3% chitosan gel group. However, there was no statistically significant difference between 0.2% and 0.3% chitosan gel groups.
The test results revealed that mean smear layer scores in the apical third were highest in 0.1% chitosan gel group followed by 0.2% chitosan gel and least with 0.3% chitosan gel group. There was significant difference in the mean smear scores in 0.1% chitosan gel and 0.3% chitosan gel group in the apical third. However, there was no statistically significant difference between 0.1% and 0.2% chitosan gel groups and 0.2% and 0.3% chitosan groups.

Use of chelator gel could remove smear layer, particularly well in the coronal and middle third of root canal. The images of three sections of root canal wall showed that the effects of chelators gels on smear layer removal were more obvious in coronal and middle thirds. This because more abundant and greater diameter in the coronal and middle third exposes dentine to higher volume of gel allowing better action and improving the efficacy of smear layer removal.

In coronal, middle, and apical third, between 0.2% chitosan gel and 0.3% chitosan, there was no statistically significant difference, but significant difference is present between 0.1% and 0.3% chitosan gel groups.

It is, therefore, necessary to choose an optimum chitosan concentration without affecting the permeate flux. Higher concentrations mean high viscosities, thereby causing concentration polarization and affecting membrane performance. The shear-thickening behavior at low chitosan concentrations suggests neutralization due to interaction of amine groups from multiple chitosan molecules. This behavior is not observed at high chitosan concentration.[30]

Rheological studies have shown that the process is nonlinear, and gels show thixotropic properties. Increasing the concentration of chitosan has an influence on increasing the viscosity, hardness, and cohesiveness of the tested gels. Depending on the composition of the substrate, gels are characterized by a variety of pharmaceutical availability. The addition of increasing concentrations of chitosan has an influence on the increased rate of the release process.[29]

The advantage of gel is its thixotropic nature, i.e., thick at room temperature and develops creamy consistency when agitated. In this way, EDTA preparation not only adheres well to working instrument but also disperses well inside the root canal. There is no pharmacological effect as such, as complex effect of EDTA is only seen after the removal of the EDTA softened surface dentine layer using mechanical instrumentation. Intermittent use in combination with a NaOCl solution is recommended.[31]

Under the experimental conditions, within the limitations of the study, it can be concluded that 0.2% chitosan gel could effectively remove the smear layer and can be considered as an alternative to EDTA-based gels. However, future studies are needed to evaluate the effect of chitosan gel on mineral content of root dentine and other physical properties like microhardness. For routine use of chitosan gel as a lubricant in clinical practice, further in vivo studies have to be considered.

CONCLUSION

Under the experimental conditions, the following conclusions can be drawn:

- All the experimental gel concentrations of chitosan as a lubricant showed smear layer removal at all the thirds of the root canal
- Smear layer removal was better in the coronal and middle third than in apical third with all the gel concentrations of chitosan
- There was no significant difference between 0.2% and 0.3% chitosan gels on smear layer removal. Considering the smear layer removal efficacy at minimal concentration, 0.2% chitosan gel can be recommended.

Further in vivo studies need to be done before chitosan gel lubricant to be used in routine clinical practice.

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Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod 1975;1:238-42.
2. Foster KH, Kulild JC, Weller RN. Effect of smear layer removal on the diffusion of calcium hydroxide through radicular dentin. J Endod 1993;19:136-40.
3. Gettleman BH, Messer HH, EIDDeeb ME. Adhesion of sealer cements to dentin with and without the smear layer. J Endod 1991;17:15-20.
4. Bystrom A, Sundqvist G. The antibacterial action of sodium hypochlorite and EDTA in 60 cases of endodontic therapy. Int Endod J 1985;18:35-40.
5. Vonder Fehr FR, Nygaard-Östby B. Effects of EDTAC and sulfuric acid on root canal dentine. Oral Surg Oral Med Oral Pathol 1963;16:199-205.
6. Barón M, Llena C, Forner L, Palomares M, González-García C, Salmerón-Sánchez M. Nanostructural changes in dentine caused by endodontic irrigants. Med Oral Patol Oral Cir Bucal 2013;18:e733-6.
7. Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-prep on bond strengths of resin cement to endodontic surfaces. J Endod 2001;27:753-7.
8. Segura JJ, Calvo JR, Guerrero JM, Sampedro C, Jimenez A, Llamas R. The disodium salt of EDTA inhibits the binding of vasoactive intestinal
peptide to macrophage membranes: Endodontic implications. J Endod 1996;22:337-40.
9. Hülsmann M, Heckendorff M, Lennon A. Chelating agents in root canal treatment: Mode of action and indications for their use. Int Endod J 2003;36:810-30.
10. Aydin U, Aksoy F, Karataslioglu E, Yildirim C. Effect of ethylenediaminetetraacetic acid gel on the incidence of dentinal cracks caused by three novel nickel-titanium systems. Aust Endod J 2015;41:104-10.
11. Hudson SM, Smith C. Polysaccharide: Chitin and chitosan: Chemistry and technology of their use as structural materials. In: Kaplan DL, editor. Biopolymers from Renewable Resources. New York: Springer-Verlag; 1998. p. 96-118.
12. Pradeep K, Joydeep D, Tripathi VS. Chitin and Chitosan: Chemistry properties and applications. J Sci Ind Res 2004;63:20-31.
13. Darrag AM. Effectiveness of different final irrigation solutions on smear layer removal in intraradicular dentine. Tanta Dent J 2014;11:93-9.
14. Silva PV, Guedes DF, Nakadi FV, Pécora JD, Cruz-Filho AM. Chitosan: A new solution for removal of smear layer after root canal instrumentation. Int Endod J 2013;46:332-8.
15. Silva PV, Guedes DF, Pécora JD, da Cruz-Filho AM. Time-dependent effects of chitosan on dentin structures. Braz Dent J 2012;23:357-61.
16. Zand V, Lotfi M, Rahimi S, Mokhtari H, Kazemi A, Sakhamanesh V, et al. A comparative scanning electron microscopic investigation of the smear layer after the use of sodium hypochlorite gel and solution forms as root canal irrigants. J Endod 2010;36:1234-7.
17. Haapasalo M, Orstavik D. In vitro infection and disinfection of dentinal tubules. J Dent Res 1987;66:1375-9.
18. Cheba BA. Chitin and chitosan: Marine biopolymers with unique properties and versatile applications. Global J Biotech Biochem 2011;6:149-53.
19. Guibal E, Milot C, Roussy J. Influence of hydrolysis mechanisms on molybdate sorption isotherms using chitosan. Sep Sci Technol 2000;35:1021-38.