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Comparative evaluation of three different irrigants on smear layer removal in instrumented root canals: An in-vitro scanning electron microscopic study

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Abstract

Aim: The present study compares and assesses the effectiveness of 0.2% Chitosan nanoparticle solution, 18% Etidronic acid and 17% EDTA in removing smear layer using SEM image analysis.

Materials and Methods: Twenty freshly extracted mandibular premolars were used. Following biomechanical preparation, samples were divided into Group A(0.2% chitosan nanoparticle solution), Group B(18% Etidronic acid), and Group C(17% EDTA), and Group D(0.9% Saline) containing 5 samples each. After irrigation the samples were sectioned longitudinally and were subjected for SEM analysis at apical, middle, and coronal levels. Hullsman's criteria were used to assign scores to the images. The statistical analysis was conducted using Kruskal Wallis test, Mann Whitney – U test, with a significance level of $p< 0.05$.

Results: When 0.2% chitosan solution and 18% Etidronic acid were used as the final irrigation, smear layer removal in the coronal, middle, and apical thirds was more successful. All of the irrigants had weak smear layer removal properties at the apical third, whereas 0.2% chitosan was significantly better followed by 18% Etidronic acid.

Conclusions: Compared to 17% EDTA and saline irrigants, 0.2% chitosan nanoparticle solution was more successful in eliminating the smear layer followed by 18% Etidronic acid.

Keywords: 0.2% chitosan, ethylenediaminetetraacetic acid, Etidronic acid, smear layer.

Introduction

Success of endodontic treatment relies on complete cleaning and debridement of root canal space followed by complete obturation with biocompatible material. For complete eradication of bacteria from the canal space, instrumentation alone is not sufficient.[1] Endodontic smear layer which is formed due to instrumentation consists of tooth structure and some non-specific

inorganic and organic content. The organic constituents include reacted coagulated proteins, necrotic or viable pulp tissue, odontoblastic process, saliva, blood cells and microorganisms.[2] Smear plugs, created by pushing smear into dentinal tubules up to 40 microns deep, can harbour bacteria and hinder root canal cleaning. The decision to remove the endodontic smear layer is debated, but studies show it increases dentin permeability and may affect the quality of the root canal seal.[3]

The smear layer, which contains both inorganic and organic debris, requires sodium hypochlorite for organic tissue and a chelating agent to dissolve inorganic tissue. Ethylenediaminetetraacetic acid (17% EDTA), dissolves inorganic components, softens dentin, demineralizes, widens dentinal tubules, and denaturates collagen fibers. However, EDTA is considered a pollutant as this material is not found in the nature, so researchers are seeking biocompatible alternatives to minimize its harmful impact on periapical tissues.[4]

Chitosan, a natural derivative and polysaccharide, obtained from deacetylation of chitin, which is obtained from the shells of crabs and shrimps is now a day widely being used in endodontics for its bio-adhesion, biodegradability, broad spectrum of antimicrobial properties, low toxic profile and high chelating characteristics with respect to metal ions in extreme acidic condition.[5]

HEBP (1- Hydroxyethylidene – 1, 1- bisphosphonate), commonly known as Etidronate or Etidronic acid in pharmacology, is an osteoporotic drug. It is most commonly used in metal industry for anticorrosive effect and it also prevent rancidification and oxidation of fatty acids. Its ability to chelate metallic ions makes it a potential alternative to EDTA.[6]

The aim of the present study is to evaluate and compare the efficacy of Chitosan, Etidronic acid, and EDTA as irrigating solutions in removal of smear layer from the root canal system.

Materials and Methods

Twenty freshly extracted human mandibular premolars were collected, meeting the criteria of straight roots, fully formed apices, and no caries. Teeth were excluded if they were endodontically treated, fractured, had curved roots, or prior restorations. The teeth were cleaned with ultrasonic scaling, sterilized, and stored in distilled water until use.

Sample size calculation

Based on Mankeliya et al. (2021), with an effect size of 1.315, an alpha of 0.05, and 95% power, the sample size was calculated to be 16. This was rounded to 20 to account for potential loss, resulting in 5 samples per group.[7]

Procedure

The study used an open-end model to decoronate teeth to a uniform working length of 13mm. Glide path was established for all teeth using a 10k file (Mani Inc., Japan). Root canals were prepared up to apical size of 30, 0.09 taper, using rotary instruments Protaper (F3) size nickel-titanium files (Dentsply Sirona). A total of 5ml of irrigant was used in each canal, with instrumentation time of 15-20 minutes. Twenty teeth were divided into four groups: A - 0.2% Chitosan, B - 18% Etidronic Acid, C - 17% EDTA, and D - 0.9% Saline (control group). The specimens were irrigated with 10ml of distilled water to remove any precipitate. This procedure was identical for all groups.

Teeth Preparation for SEM analysis

The canal orifice of each sample was protected with a cotton pellet. Using diamond disc longitudinal deep grooves was made on the buccal and lingual surfaces, with precaution not to perforate the root canals. Following this, the roots were further split into two halves with chisel and mallet. One half among each tooth, containing greater part of the apex was selected as the representative sample and was scheduled for SEM Examination.

SEM Analysis

The specimens were then analyzed using a SEM (JEOL JSM-5510, Tokyo, Japan). The dentinal surface was observed at cervical, middle and apical thirds and visualized with a magnification of 2000x for the presence and absence of smear layer. Photomicrographs 2000x of these areas were taken on each coronal, middle and apical thirds. SEM images were then analyzed for the amount of smear layer present using a four-score system by other colleagues who were blinded to the irrigation regimens employed for each group.

Scoring index

To measure the quantity of smear layer that has been removed, a scoring system with a range of 1–4 established on the scores by Hulsmann was used.[4] (Figure 1)

- Score 1: dentinal tubules are fully opened
- Score 2: more than 50% of dentinal tubules are opened

- Score 3: less than 50% of the dentinal tubules are opened
- Score 4: almost all dentinal tubules are coated with a smear layer.

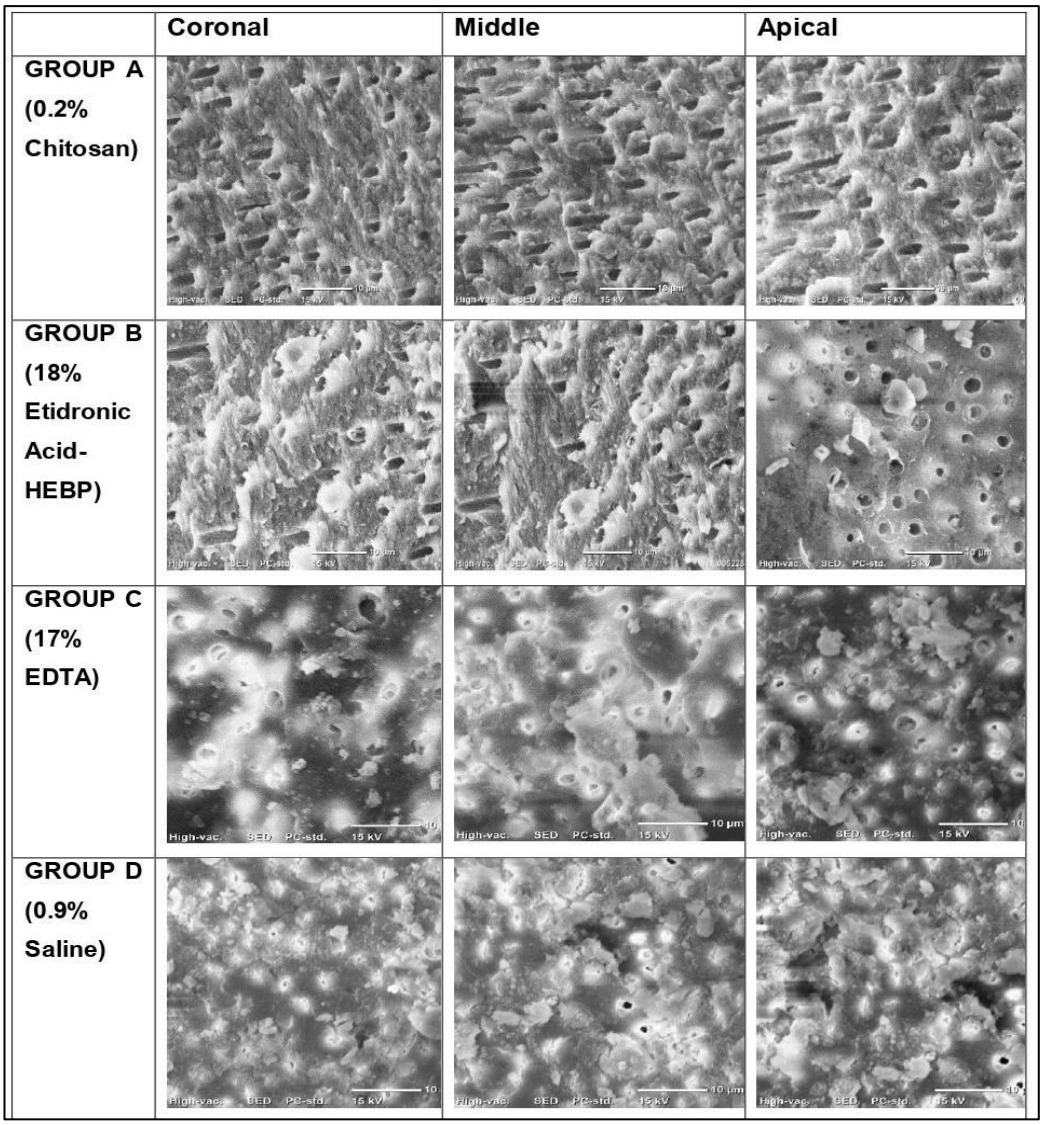

Figure 1: SEM images of specimens irrigated with different irrigants used in the study at coronal, middle and apical third respectively.

Statistical Analysis

All the data was collected and subjected to analysis using the Statistical Package for the Social Sciences (SPSS) version 23. Intergroup and Intragroup comparison of Smear layer removal was made using Kruskal Wallis test. Pairwise comparison was done using Mann Whitney – U test. The level of statistical significance was set at 0.05 ($p<0.05$).

Results

The study found that all tested irrigants effectively removed the smear layer from the root surface, with mean scores calculated at coronal, middle, and apical thirds. Intergroup comparisons were made using Kruskal Wallis Test (Table 1) at coronal, middle, and apical thirds, and Mann Whitney U Test (Table 2) for pairwise comparisons.

Table 1: Mean smear layer removal scores of the four experimental groups at the coronal, middle, and apical thirds

	GROUPS	N	Mean	Std. Deviation	Minimum	Maximum	Kruskal Wallis H	P value
Coronal third	Chitosan	5	.20	.447	0	1	13.789	0.003*
	Etidronic Acid	5	.80	.837	0	2		
	EDTA	5	1.60	.548	1	2		
	Saline	5	2.60	.548	2	3		
	Total	20	1.30	1.081	0	3		
Middle third	Chitosan	5	.60	.548	0	1	13.541	0.004*
	Etidronic Acid	5	1.20	.447	1	2		
	EDTA	5	2.00	.707	1	3		
	Saline	5	2.60	.548	2	3		
	Total	20	1.60	.940	0	3		
Apical third	Chitosan	5	1.00	.000	1	1	16.808	0.001*
	Etidronic Acid	5	2.00	.000	2	2		
	EDTA	5	2.60	.548	2	3		
	Saline	5	3.00	.000	3	3		
	Total	20	2.15	.813	1	3		

*Significant difference, SD=standard deviation

The study found significant differences in smear layer removal scores among four experimental groups at the coronal, middle, and apical thirds($p<0.01$) (Table 1). Chitosan showed the highest efficacy, followed by etidronic acid and EDTA. The lowest efficacy was found in the saline group, acting as a control (Figure 2). A pairwise comparison revealed a significant difference between chitosan and EDTA and saline (control) and all other groups ($p<0.05$) at the coronal thirds. In the middle thirds, a significant difference was found between the chitosan and EDTA group; chitosan and saline group; and etidronic acid and saline group ($p<0.05$). In the apical thirds, a significant difference was found between the chitosan and all other groups; etidronic acid and EDTA group; etidronic acid and saline group ($p<0.05$); but no difference was found between EDTA and saline group ($p>0.05$) (Table 2).

Table 2: Pairwise comparison of the mean smear layer removal scores of the four experimental groups at the coronal, middle, and apical thirds

Groups	Comparison between	Mann Whitney U	Z score	P value
Coronal Third	CHITOSAN Versus ETIDRONIC ACID	7.000	-1.315	0.189
	CHITOSAN Versus EDTA	1.000	-2.545	0.011*
	CHITOSAN Versus SALINE	0.000	-2.739	0.006*
	ETIDRONIC ACID Versus EDTA	5.500	-1.565	0.118
	ETIDRONIC ACID Versus SALINE	1.000	-2.479	0.013*
	EDTA Versus SALINE	3.000	-2.154	0.031*
Middle Third	CHITOSAN Versus ETIDRONIC ACID	6.000	-1.678	0.093
	CHITOSAN Versus EDTA	1.500	-2.410	0.016*
	CHITOSAN Versus SALINE	0.000	-2.694	0.007*
	ETIDRONIC ACID Versus EDTA	4.500	-1.848	0.065
	ETIDRONIC ACID Versus SALINE	1.000	-2.545	0.011*
	EDTA Versus SALINE	6.500	-1.386	0.166
Apical Third	CHITOSAN Versus ETIDRONIC ACID	0.000	-3.000	0.003*
	CHITOSAN Versus EDTA	0.000	-2.835	0.005*
	CHITOSAN Versus SALINE	0.000	-3.000	0.003*
	ETIDRONIC ACID Versus EDTA	5.000	-1.964	0.050*
	ETIDRONIC ACID Versus SALINE	0.000	-3.000	0.003*
	EDTA Versus SALINE	7.500	-1.500	0.134

*Significant difference

Additionally, comparisons were made for the mean smear layer removal scores between the coronal, middle, and apical thirds within each experimental group (intragroup comparison) using the Kruskal Wallis Test (Table 3) (Figure 3). Pairwise comparisons between the different sections of the tooth were assessed using Mann Whitney U Test (Table 4).

The study found a significant difference in smear layer removal efficacy between the coronal, middle, and apical thirds in the chitosan and etidronic acid groups ($p<0.05$), but no difference was observed in the EDTA and saline groups ($p>0.05$). The highest efficacy was found in the coronal thirds, followed by the middle third, and the least efficacy was found in the apical thirds, except for the saline group (Figure 3).

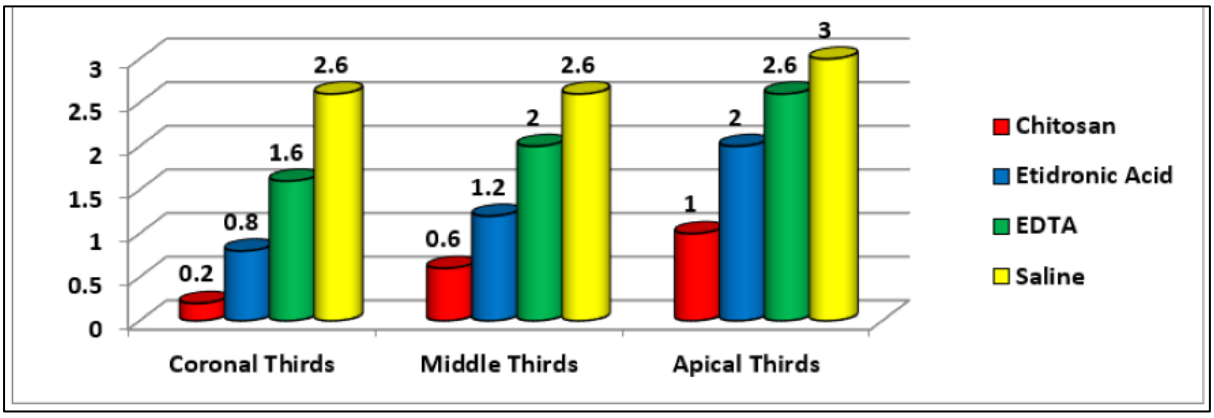

Figure 2: Mean Smear Layer Removal Scores of the four experimental groups at the coronal, middle and apical thirds

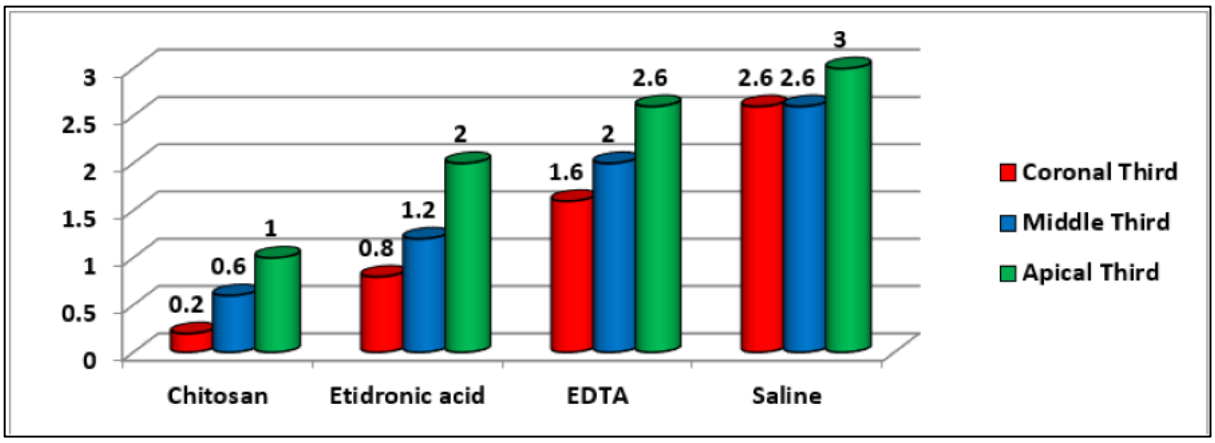

Figure 3: Mean Smear Layer Removal Scores at the coronal, middle and apical thirds within each of the experimental groups

Table 3: Mean smear layer removal scores at the coronal, middle, and apical thirds within each of the experimental groups

GROUPS		N	Mean	SD	Min.	Max.	Kruskal Wallis H	P value
CHITOSAN	coronal third	5	0.20	0.447	0	1	6.222	0.045*
	middle third	5	0.60	0.548	0	1		
	apical third	5	1.00	0.000	1	1		
	Total	15	0.60	0.507	0	1		
ETIDRONIC ACID	coronal third	5	0.80	0.837	0	2	7.562	0.023*
	middle third	5	1.20	0.447	1	2		
	apical third	5	2.00	0.000	2	2		
	Total	15	1.33	0.724	0	2		
EDTA	coronal third	5	1.60	0.548	1	2	5.170	0.075
	middle third	5	2.00	0.707	1	3		
	apical third	5	2.60	0.548	2	3		
	Total	15	2.07	0.704	1	3		
SALINE	coronal third	5	2.60	0.548	2	3	2.545	0.280
	middle third	5	2.60	0.548	2	3		
	apical third	5	3.00	0.000	3	3		
	Total	15	2.73	0.458	2	3		

*Significant difference, SD=standard deviation

Table 4: Pairwise comparison of the mean smear layer removal scores at the coronal, middle, and apical thirds within each of the experimental groups

GROUPS	Comparison between	Mann Whitney U	Z score	P value
CHITOSAN	Coronal versus Middle	7.500	-1.225	0.221
	Middle versus Apical	7.500	-1.500	0.134
	Coronal versus Apical	2.500	-2.449	0.014*
ETIDRONIC ACID	Coronal versus Middle	8.500	-0.949	0.343
	Middle versus Apical	2.500	-2.449	0.014*
	Coronal versus Apical	2.500	-2.372	0.018*
EDTA	Coronal versus Middle	8.500	-0.956	0.339
	Middle versus Apical	6.500	-1.386	0.166
	Coronal versus Apical	3.000	-2.154	0.031*
SALINE	Coronal versus Middle	12.500	0.000	1.000
	Middle versus Apical	7.500	-1.500	0.134
	Coronal versus Apical	7.500	-1.500	0.134

*Significant difference

Discussion

Endodontic therapy aims to remove diseased pulpal tissue, eliminate microorganisms in canals and dentinal tubules, and prevent recontamination. The root canal system is meticulously

cleaned, shaped, and disinfected. To ensure consistency in size, shape, and canal anatomy, human mandibular premolars decoronated from CEJ were employed in this study (7). Irrigation is crucial in every part of the root canal system, especially in the parts that are not accessible for instrumentation, even though the instruments remove majority of the contents in the root canal.[8]

The study evaluated the effectiveness of 0.2% chitosan nanoparticle solution, 17% EDTA, and 18% etidronic acid as a final irrigant for removing smear layer from the coronal, middle, and apical thirds of root canal system. The 0.2% chitosan solution had a greater removal impact than all other chelating agents. The formation of complexes between chitosan and metal particles (Ca+ ions) likely involves ionic exchange, adsorption, and chelation.[3] Because the chitosan polymer is hydrophilic, it makes intimate contact with the dentin of the root canal wall due to adsorption. [8] Because the contact angle of 0.2% chitosan solution is lower, it may better penetrate the dentin to enhance its benefits.[6] The two hypotheses used to explain chitosan chelating mechanism are as follows:

i. The bridge model states that Chitosan has two or more amino groups that interact with the same metal ion.
ii. According to the pendant model, just one amino group is involved in the binding process, and the metal ion is suspended from the amino group in the same way as a pendant.[4]

Etidronic acid was the next effective irrigant in the study, which was in accordance with Yadav HK et al, (2015), as it can be used as a single irrigant before and after instrumentation without temporarily losing its intended qualities.[9] Girard et al (2005) found that HEBP inhibits smear layer formation during instrumentation, is compatible with hypochlorite, and has superior calcium binding and smear-preventing capacity compared to other available products.[10] The NaOCl/HEBP combination shows increased activity compared to NaOCl alone due to HEBP's ability to remove the smear layer and facilitate NaOCl's penetration into dentin structure. NaOCl maintains its biological properties, killing microorganisms and dissolving organic tissue, while HEBP reduces and removes inorganic matter. This combination produces less smear layer and dentinal debris, lowering NaOCl's reactivity with residue, reducing free chlorine usage, and increasing the stability of the combined solution.[11] Despite its benefits, HEBP is a weak chelator which might be the reason for lower efficacy than 0.2% chitosan.

EDTA, introduced in 1957, is a widely used chelator in endodontics, creating soluble calcium chelates when combined with dentin ions, effectively removing smear layers and making most commonly used chelating solutions.[12] EDTA, when combined with NaOCl, maintains its ability to complex calcium but reduces chlorine content, affecting tissue dissolving ability by up to 4%.[6] EDTA can effectively separate biofilms from root canal walls, making an alternating irrigation schedule with NaOCl more effective in reducing bacterial load in root canal systems.[13] Short-term use of NaOCl treatment after EDTA treatment severely erodes root canal wall dentin, decalcifying it to 20-30µm depth within 5 minutes.[14]

This study found that all irrigants significantly removed the smear layer in the coronal and middle aspect of the root canal, but none completely removed it in the apical part, indicating that the effectiveness of smear layer removal is compromised by a small canal diameter because it exposes the dentin to a lower volume of irrigants.[15] Paque et al. suggest that EDTA may not be as effective on sclerosed dentin in the apical third of the root canal.[12]

As this study was an invitro research it needs to be validated in in-vivo conditions and requires further research. Various factors can influence the behaviour of these agents like blood, tissue remnants etc., The present study was conducted on single, wide and straight root canal systems and the chelating agent's efficacy might be altered in curved and narrow canals of the posterior teeth.

Conclusion

Within the limitations of the current study, it can be concluded that all three tested irrigants removed the smear layer from the coronal, middle, and apical third. However, among these irrigants, 0.2% chitosan was most efficient followed by 18% etidronic acid. Therefore, 0.2% Chitosan solution and 18% Etidronic acid can be thought of as alternatives to EDTA when used as a chelating agent.

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