Original Article

# Scanning electron microscopy evaluation of smear layer removal using ethylenediaminetetraacetic acid, etidronic acid, and chitosan nanoparticle solution as root canal irrigants

**Sunheri Bajpe, Chitharanjan Shetty, Aditya Shetty, Gurmeen Kaur[1], Shalin Ann Saji[2], Chandra Prabha**

Department of Conservative Dentistry and Endodontics, AB Shetty Memorial Institute of Dental Sciences, Mangalore, Karnataka, [1]Department of Conservative Dentistry and Endodontics, D.Y. Patil Vidyapeeth, Pune, Maharashtra, [2]Sarah Dental Clinic, Kollam, Kerala, India

## ABSTRACT

**Aim:** The purpose of this study is to evaluate and compare the efficacy of 17% EDTA, 18% etidronic acid, and 0.2% chitosan nanoparticle solution in smear layer removal using SEM image analysis.

**Methods:** Thirty freshly extracted mandibular premolars were used. After biomechanical preparation, the samples were divided into Group I (17% EDTA), Group II (18% etidronic acid), and Group III (0.2% chitosan nanoparticle solution) containing 10 samples each. Longitudinal sectioning of the samples was done. The samples were observed under SEM at apical, middle, and coronal levels. The images were scored according to the criteria by Hullsman. Statistical analysis was done, with the significance level set at $P < 0.05$, and performed with SPSS 16.0 statistical package for Windows.

**Results:** Smear layer removal at coronal, middle, and apical thirds was more effective when final irrigation was performed using 0.2% chitosan solution, followed by 17% EDTA. At the apical third, all the irrigants showed poor smear layer removing property, but chitosan showed comparatively better results.

**Conclusion:** 0.2% chitosan nanoparticle solution was more effective in removing the smear layer when compared to 17% EDTA and 18% etidronic acid irrigants.

**Keywords:** 0.2% chitosan, ethylenediaminetetraacetic acid, etidronic acid, irrigants, smear layer

## INTRODUCTION

The success of endodontic treatment is determined by the eradication of microbes and prevention of reinfection of the root canal system. This can be achieved by cleaning and shaping the root canal under constant irrigation, thereby eliminating the inflamed/necrotic tissues, microbial biofilms, and debris within the root canal space.[1]

When dentin is cut during the process of instrumentation, the mineralized tissues are ruptured, generating large amounts of debris.[2] The material appears amorphous, grainy, and uneven under the scanning electron microscope (SEM).[3] This smear layer includes organic components such pulp tissue, odontoblast processes, necrotic debris, microorganisms, and their metabolic byproducts in addition to inorganic dentin debris.[4]

**Address for correspondence:** Dr. Sunheri Bajpe,
Department of Conservative Dentistry and Endodontics,
AB Shetty Memorial Institute of Dental Sciences, Mangalore,
Karnataka, India.
E-mail: shunri@gmail.com

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Whether to remove or maintain the smear layer has been found to be debatable, but a systematic review by Shahravan *et al*. concluded that the removal of the smear layer can improve the fluid-tight seal of the root canal system.[5] The smear layer has also been shown to hinder intracanal medicament and sealer penetration into the dentinal tubules.[4] Various methods to remove this layer have been advocated, such as the usage of ultrasonic instruments, lasers, and chelating agents for mechanical and chemical root canal debridement.[6]

Ethylenediaminetetraacetic acid (EDTA), a chelating agent which is commonly used as a final irrigating solution, affects only the inorganic part of dentine and hydroxyapatite of the smear layer. Complete removal of the layer can only be achieved when NaOCl has been used before the final rinse with EDTA.[7] When it reacts with calcium ions in dentine, it results in calcium chelation, promoting decalcification of dentine within 5 min at approximate depths of 20–30 ∝m. Aiming at minimizing the harmful effects, it has, on the periapical tissues, the search for more biocompatible solutions continues.[8]

Etidronic acid is also known as 1-hydroxyethylidene-1,1-bisphosphonate, and is a biocompatible chelator. Bisphosphonates have adequate calcium-chelating capacity, and hence are systemically administered to patients suffering from osteoporosis or neoplastic diseases involving osteolytic bone destruction.[9]

Chitosan is a natural, cationic amino polysaccharide copolymer of glucosamine and N-acetyl glucosamine. These compounds are obtained by partial deacetylation of chitin, a substance obtained from the shells of crustaceans and shrimps.[10] It is endowed with many beneficial properties such as biocompatibility, biodegradability, bioadhesion, and antimicrobial activity.[11] Its use is ecologically interesting as it has been found to be the most abundant substance in nature, after cellulose.[12]

The present study evaluates and compares the efficiency of 17% EDTA, 18% etidronic acid, and 0.2% chitosan nanoparticle solution in their ability to remove smear layer following root canal instrumentation on human extracted teeth using a SEM.

## MATERIALS AND METHODS

### Sample selection and preparation

Thirty freshly extracted single-rooted human mandibular premolars with single root canal and closed apex were selected. The selection of teeth was based on their relative dimensions and morphology. Buccal and lingual radiographs of the teeth were taken to ensure that they had only a single canal. The teeth were cleaned of debris and soft tissue remnants, and were then stored in a sterile saline solution. Protocols for infection control as per OSHA and CDC guideline regulations in collection, storing, sterilization, and handling were followed.

In order to standardize canal instrumentation, each tooth was decoronated and the length of the root was standardized to 16 mm using a low-speed diamond disk under water as a coolant. The working length of each root canal was established 1 mm short of the apical foramen with #15 K-file after gauging with #10 K-file.

### Root canal preparation

The canals were instrumented in a standardized crown-down manner with sequentially sized K-files (Dentsply-Maillefer, Ballaigues, Switzerland) up to size 40, followed by instrumentation using rotary ProTaper instruments at 250 rpm up to F3 file. Root canal irrigation was performed with 2 mL of 2.5% NaOCl solution throughout instrumentation and between each file. Finally, the root canals were rinsed with 5 mL of saline and randomly divided into five groups ($n = 10$) according to the final irrigating solution used for smear layer removal.

The samples were divided into Groups I, II, and III containing 10 samples each. Group I: 5 ml 17% EDTA solution was used for 3 min; Group II: 5 mL of 18% etidronic acid was used for 3 min; Group III: 5 mL 0.2% chitosan nanoparticle solution for 3 min. The 0.2% chitosan nanoparticle solution was prepared by dissolving 0.2 g of chitosan nanopowder (Sisco Research Laboratories Pvt. Ltd, India) in 100 mL of 1% acetic acid. The mixture was agitated using a magnetic agitator for 2 h to obtain a homogenous clear solution. The irrigating solutions were delivered using a sterile nickel–titanium needle of 30 gauge. A stopper was placed on the needle such that it was restricted to penetrate only up to 2 mm of the working length. The root canals were then flushed with 5 mL of distilled water, and dried with sterile absorbent paper points.

### Scanning electron microscope evaluation

Diamond discs were used at a low speed to cut deep grooves on the buccal and lingual surfaces of the roots, without perforating the root canals. The roots were then split into two equal halves with a chisel and mallet. One-half of each tooth was selected and prepared for SEM examination.

The specimens were secured on metal stubs, desiccated, sputter coated with gold, and examined under SEM at ×4000 magnification. The dentinal surfaces were observed at cervical, middle, and apical thirds with a magnification of ×4,000 for the presence/absence of smear layer and visualization of the

entrance to dentinal tubules. Photomicrographs (×4000) of these areas were taken.

The root canal was qualitatively assessed at the coronal, middle, and apical regions of each root half of each specimen using a graded scale from 1 to 5 to assess the quality of smear layer removal according to Hulsmann criteria *et al.*[13]

| Score | Description |
|---|---|
| Score 1 | No smear layer, orifices of dentinal tubules open |
| Score 2 | Small amount of smear layer, some dentinal tubules open |
| Score 3 | Homogenous smear layer covering the root canal walls, only a few dentinal tubules open |
| Score 4 | Complete root canal wall covered by a homogenous smear layer, no open dentinal tubules |
| Score 5 | Heavy, homogenous smear layer covering the entire root canal walls |

The degree of evaluation was scored in a blind manner based on a five-grade scale by an examiner who was not privy to the true nature and purpose of this study.

### Statistical analysis

Nonparametric data of smear layer scores were presented as a percentage distribution and their mean ranks were calculated for each group at each root section. Kruskal–Wallis test was used to compare between final irrigation solutions at each section and Mann–Whitney *U*-test was used for pair-wise comparisons between the groups whenever indicated. Friedman test was used to compare between root canal thirds at each group followed by Wilcoxon signed-rank test for pair-wise comparisons between root canal thirds if necessary. The significance level was set at $P = 0.05$. Statistical analysis was performed with IBM SPSS 16.0 software for Windows.

## RESULTS

A comparison of smear layer covering the dentinal surfaces at coronal, middle, and apical root canal levels between the groups was performed [Figures 1-3].

Wilcoxon test demonstrated statistically significant differences among all tested sections for the EDTA group. Friedman test denoted that there was a statistically significant difference among all the root sections for the three groups [Table 1].

Intragroup comparison showed a highly significant difference in the middle versus coronal region in the EDTA group and a significant difference in the apical versus coronal and apical versus middle region in the chitosan nanoparticle solution group. In the etidronic acid group also, a significant change in apical-middle and coronal-apical regions was found.

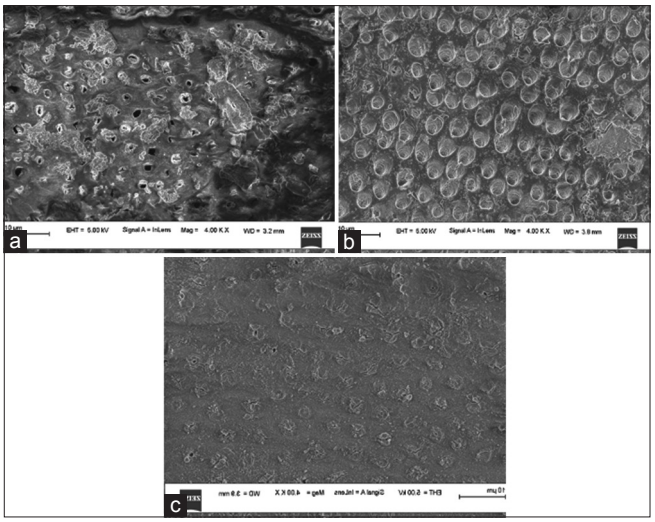


**Figure 1: Coronal third (a) EDTA, (b) Chitosan nanoparticle solution, (c) Etidronic acid, EDTA: Ethylenediaminetetraacetic acid**

Mann–Whitney *U*-test recorded no statistically significant differences among the EDTA and etidronic acid in the middle third ($P > 0.05$). Smear layer removal at the coronal, middle, and apical thirds was more effective when final irrigation was performed using 0.2% chitosan solution, recording the significantly lowest mean ranks of scores compared to the other groups. This was followed by EDTA with the second lowest mean score in relation to all thirds of the root canal [Table 2].

At the apical third, all the irrigants showed poor smear layer removing property, but chitosan showed comparatively better results.

## DISCUSSION

Although most of the contents in the root canal are removed by the instruments, irrigation plays an indispensable role in all areas of the root canal system, particularly in the areas inaccessible for instrumentation.

In this study, we compared the efficacy of 17% EDTA, 18% etidronic acid, and 0.2% chitosan nanoparticle solution as a final irrigant in the removal of the smear layer from the coronal, middle, and apical thirds of the human root canal system.

The effect of smear layer removal of 0.2% chitosan solution used in this study was better than all tested chelating agents at coronal, middle, and apical thirds.

There are three main factors responsible for the elimination of dentin calcium ions adsorption, ionic exchange, and chelation. The chitosan polymer is hydrophilic, and this favors its

**Table 1: Comparison of smear score between tooth sections in each study group**

| Group | *n* | Mean (SD) | Range | Median (Q1-Q3) | Friedman test | | Wilcoxon sign rank test (*P*) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $\chi^2$ | *P* | Middle - Coronal | Apical - Coronal | Apical - Middle |
| EDTA | | | | | | | | | |
| Coronal | 10 | 2 (0.67) | 1-3 | 2 (1.75-2.25) | 15.50 | <0.001* | 0.03* | 0.005* | 0.004* |
| Middle | 10 | 2.8 (0.42) | 2-3 | 3 (2.75-3) | | | | | |
| Apical | 10 | 3.8 (0.42) | 3-4 | 4 (3.75-4) | | | | | |
| Chitosan | | | | | | | | | |
| Coronal | 10 | 1.2 (0.42) | 1-2 | 1 (1-1.25) | 10.75 | 0.005* | 0.56 (NS) | 0.01* | 0.008* |
| Middle | 10 | 1.1 (0.32) | 1-2 | 1 (1-1) | | | | | |
| Apical | 10 | 1.8 (0.42) | 1-2 | 2 (1.75-2) | | | | | |
| Etidronic acid | | | | | | | | | |
| Coronal | 10 | 3.7 (0.48) | 3-4 | 4 (3-4) | 15.24 | <0.001* | 0.06 (NS) | 0.02* | 0.004* |
| Middle | 10 | 3.2 (0.42) | 3-4 | 3 (3-3.25) | | | | | |
| Apical | 10 | 4.7 (0.48) | 4-5 | 5 (4-5) | | | | | |

*$P$<0.05 statistically significant, $P$>0.05 NS. NS: Nonsignificant; EDTA: Ethylenediaminetetraacetic acid; SD: Standard deviation

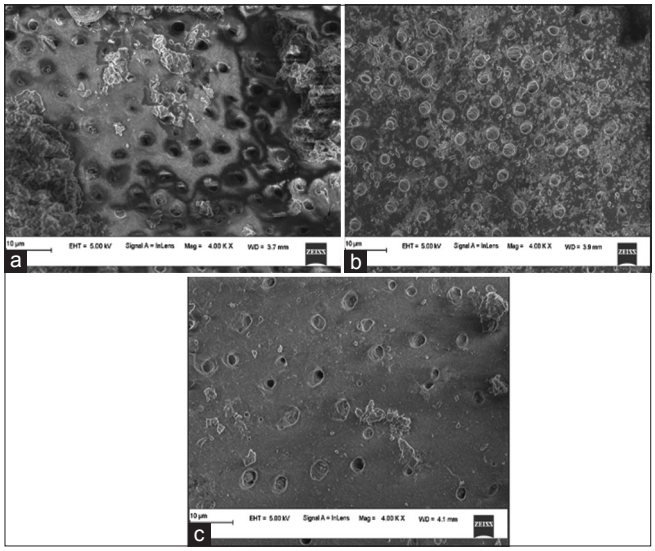


**Figure 2: Middle third. (a) EDTA, (b) Chitosan nanoparticle solution, (c) Etidronic acid, EDTA: Ethylenediaminetetraacetic acid**

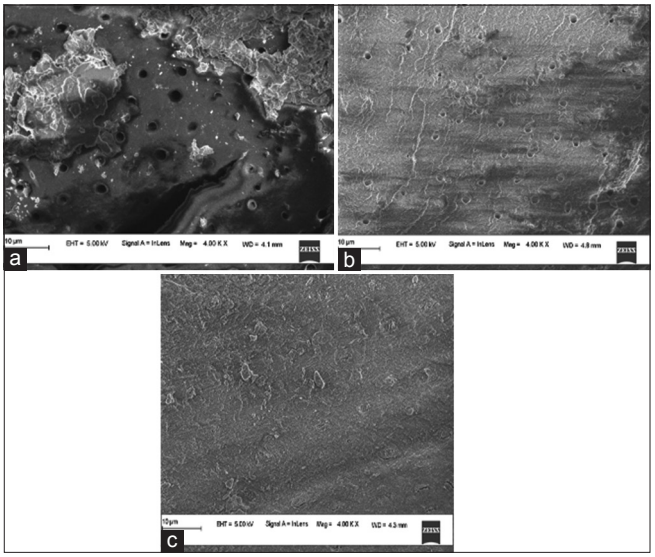


**Figure 3: Apical third. (a) EDTA, (b) Chitosan nanoparticle solution, (c) Etidronic acid, EDTA: Ethylenediaminetetraacetic acid**

intimate contact with the root canal dentin, thereby leading to its adsorption to the root canal wall. The ionic interaction between the dentin calcium ions and the chelating agent is due to the presence of a large number of free hydroxyl and amino groups in the polymer, making it cationic in nature.[10]

Previous studies have assessed the chelating capacity of chitosan on root canal dentin, and these showed that the irrigation of the root canals with a chitosan nanoparticle solution for 3 min effectively removed the smear layer from the root canals. These results were in accordance with those obtained in the present study, where the final irrigation with chitosan nanoparticle solution for 3 min effectively removed the inorganic contents from the dentin.[8,14,15]

In the present research, chitosan was dissolved in 1% acetic acid to form the solution because it is insoluble in water. Thus, it could be speculated that the chelating effect observed in this study would be due to the acid and not of chitosan. However, previous studies have shown that the capacity of 5% acetic acid for reducing dentin microhardness, removing the smear layer, and chelating calcium ions in the root canal is insignificant in relation to 15% EDTA. In this way, it is highly evident that the effect caused by chitosan on dentin microhardness is due exclusively to the substance and not to the acid.[16]

Analysis of the dentinal walls of all the specimens demonstrated that cleaning was more effective in the coronal and middle thirds of the root canal than in the apical third. This can possibly be attributed to the increased depth and reduced diameter in that area of the root canal. The flowability and backflow of the fluid were thus found to be poor in the apical third.[10,17]

**Table 2: Comparison of smear score between study groups in each tooth section**

| Group | *n* | Mean (SD) | Range | Median (Q1-Q3) | Kruskal-Wallis test | | Mann-Whitney *U*-test (*P*) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $\chi^2$ | *P* | 1 versus 2 | 1 versus 3 | 2 versus 3 |
| Coronal | | | | | | | | | |
| EDTA | 10 | 2 (0.67) | 1-3 | 2 (1.75-2.25) | 22.75 | <0.001* | 0.07* | <0.001* | <0.001* |
| Chitosan | 10 | 1.2 (0.42) | 1-2 | 1 (1-1.25) | | | | | |
| Etidronic acid | 10 | 3.7 (0.48) | 3-4 | 4 (3-4) | | | | | |
| Middle | | | | | | | | | |
| EDTA | 10 | 2.8 (0.42) | 2-3 | 3 (2.75-3) | 24.18 | <0.001* | <0.001* | 0.05 (NS) | <0.001* |
| Chitosan | 10 | 1.1 (0.32) | 1-2 | 1 (1-1) | | | | | |
| Etidronic acid | 10 | 3.2 (0.42) | 3-4 | 3 (3-3.25) | | | | | |
| Apical | | | | | | | | | |
| EDTA | 10 | 3.8 (0.42) | 3-4 | 4 (3.75-4) | 25.10 | <0.001* | <0.001* | 0.001* | <0.001* |
| Chitosan | 10 | 1.8 (0.42) | 1-2 | 2 (1.75-2) | | | | | |
| Etidronic acid | 10 | 4.7 (0.48) | 4-5 | 5 (4-5) | | | | | |

*$P<0.05$ statistically significant, $P>0.05$ NS. NS: Nonsignificant; EDTA: Ethylenediaminetetraacetic acid; SD: Standard deviation

The presence of more abundant and larger dentinal tubules coronally exposes the dentin to a larger volume of irrigants, thus allowing better flow of the solution.[18] Hence, further improvement in the efficiency of smear layer removal can be observed. This was confirmed by several other researchers who concluded that greater amounts of smear layer were found remaining at the apical third of the canal.[10,19-21] This study concentrated on the efficacy of the irrigants alone in order to highlight the actions of each without activation as an adjunct.

Studies done on the efficacy of removal of smear layer using 18% etidronic acid have shown that it is an effective irrigant.[9,22] In a study by De-Deus *et al*., this chelator solution was utilized in concentrations of 9% and 18% to remove smear layer. 18% concentration was found to provide better results,[23] and hence, the higher concentration was used in our study. Etidronic acid was found to have a lower smear layer removal efficacy in all areas of the root canal when compared to EDTA and chitosan nanoparticle solution.

As per the observations made in the samples of this study, chitosan worked better at the apical third than 17% EDTA solution. As described in a study by Kamble *et al*., a neutral EDTA solution has the ability to reduce the noncollagenous protein (NCP) component and mineral of dentin.[11] Due to the lower content of NCPs in the apical third, the degree of chelation of EDTA is low in this part.[24] Paqué *et al*., in their study, reported that dentin in the apical third of the root canal is sclerosed; hence, EDTA may not have such a pronounced action on sclerosed dentin in the apical third.[25] This is in agreement with various other studies that have reported EDTA to be effective in smear layer removal only in coronal and middle thirds, but not in the apical third.[5,9]

## CONCLUSION

A moderate concentration of 0.2% chitosan nanoparticle solution removes the smear layer with greater efficiency than 17% EDTA and 18% etidronic acid at the coronal, middle, and apical thirds of the root canals.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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