

# Comparative analysis of endodontic smear layer removal efficacy of 17% ethylenediaminetetraacetic acid, 7% maleic acid, and 2% chlorhexidine using scanning electron microscope: An *in vitro* study

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Received: 02-07-2016

Accepted: 25-07-16

Published: 02-09-16

## Abstract

**Aim:** The aim of the present study was to evaluate the efficiency of different endodontic irrigants in the removal of smear layer through scanning electron microscopic image analysis. **Materials and Methods:** The present *in vitro* study was carried out on 45 single-rooted extracted human mandibular premolar teeth with single canal and complete root formation. Teeth were randomly assigned to three groups with 15 teeth in each group. Group I samples were irrigated with 17% ethylenediaminetetraacetic (EDTA) irrigation, Group II with 7% maleic acid irrigation, and Group III with 2% chlorhexidine irrigation. Scanning electron microscope evaluation was done for the assessment of smear layer removal in the coronal, middle, and apical thirds. Comparison of the smear layer removal between the three different groups was done by Kruskal–Wallis test, followed by Mann–Whitney *U* test for comparing individual groups. A *P* value less than 0.05 was considered to be statistically significant. **Results:** Statistically significant difference was seen between the two test groups (17% EDTA vs. 7% maleic acid and 17% EDTA vs. 2% chlorhexidine) in smear layer removal at coronal, middle, and apical thirds of the root canal. The most efficient smear layer removal was seen in Group I with 17% EDTA irrigation compared with other groups (*P* < 0.05) and the least by 2% chlorhexidine. **Conclusion:** The present study shows that 17% EDTA efficiently removes the smear layer from root canal walls.

**Key words:** Chlorhexidine, EDTA, maleic acid, root canal irrigants, scanning electron microscope, smear layer

## INTRODUCTION

The purpose of endodontic treatment is the elimination of microorganisms from the root canal system and the prevention of reinfection.<sup>[1]</sup> One of the most important procedures during root canal treatment is the

chemomechanical preparation. The current root canal instrumentation methods produce a granular amorphous layer covering the dentin, referred to as smear layer.<sup>[2]</sup>

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**How to cite this article:** Attur K, Joy MT, Karim R, Anil Kumar VJ, Deepika C, Ahmed H. Comparative analysis of endodontic smear layer removal efficacy of 17% ethylenediaminetetraacetic acid, 7% maleic acid, and 2% chlorhexidine using scanning electron microscope: An *in vitro* study. *J Int Soc Prevent Communit Dent* 2016;6:S160-5.

Access this article online	
Quick Response Code:	Website: www.jispcd.org
	DOI: 10.4103/2231-0762.189755

The smear layer consists of both organic and inorganic substances such as fragments of odontoblastic processes, microorganisms, and necrotic material covering the root canal walls and openings of the dentinal tubules. The smear layer itself may be infected and may protect the bacteria within the dentinal tubules. It can hinder the penetration of intracanal medicaments and sealers into the dentinal tubules.<sup>[3]</sup> The smear layer can also act as a barrier between obturating materials and the canal wall, and thus, interfere with the formation of an appropriate seal.<sup>[4]</sup>

Hence, thorough debridement of the root canal system for removing smear layer is crucial for long-term success of root canal treatment.

Ethylenediamine tetraacetic acid (EDTA) is the most widely used irrigant for smear layer removal. In addition to the cleansing function, it acts on inorganic material by reacting with calcium ions in dentine, resulting in calcium chelation, promoting decalcification of dentine at depths of 20–30  $\mu\text{m}$  within 5 minutes. Because of its harmful effect on periapical tissues, the search for more biocompatible solutions other than EDTA continues. Weak acids, such as citric acid, maleic acid, and apple cider vinegar have been evaluated at different concentrations for the removal of smear layer.<sup>[5]</sup>

Maleic acid is a mild organic acid which has been found to possess smear layer removing quality when used as an acid etchant in restorative dentistry.<sup>[3]</sup> At different concentrations, it has also been found to remove the endodontic smear layer, indicating that it can be used as an alternative to routine use of 17% EDTA at concentrations of 5 and 7%.<sup>[6]</sup>

2% chlorhexidine solution has been gaining popularity as an efficient root canal irrigant. Chlorhexidine has broad spectrum antimicrobial activity similar to sodium hypochlorite along with a substantive antimicrobial activity.<sup>[7]</sup> Chlorhexidine has been studied for its various properties with an objective of being an alternative to sodium hypochlorite. However, its capacity to clean root canal walls requires further investigation.<sup>[8]</sup>

Hence, the present study was undertaken to evaluate the efficiency of 17% EDTA, 7% maleic acid, and 2% chlorhexidine endodontic irrigants in smear layer removal through scanning electron microscopic image analysis.

### Objectives of the study

To compare and evaluate the efficacy of 17% EDTA, 7% maleic acid and 2% chlorhexidine in the removal of

smear layer after chemomechanical preparation using scanning electron microscope.

## MATERIALS AND METHODS

The present *in vitro* study was carried out on 45 single-rooted extracted mandibular premolar teeth, with single canal and complete root formation, collected from the Department of Oral and Maxillofacial surgery. Carried or fractured teeth, teeth with open apices, with resorption, craze line, or calcified canals were excluded. Superficial soft tissues were removed with a brush and all the teeth were stored in distilled water. Sample size was calculated using the formula

$$n = \frac{z_{1-\alpha/2}^2 2\sigma^2}{d^2}$$

where  $n$  is required sample size,  $z_{1-\alpha/2}$  is a constant, its value for a two-sided test is 1.96 for 95%,  $d$  is absolute precision 20% = 0.2, and  $\sigma$  is pooled variance.

Teeth were randomly assigned to three groups with 15 teeth in each group.

Group I: 15 samples with 17% EDTA irrigation.

Group II: 15 samples with 7% maleic acid irrigation.

Group III: 15 samples with 2% chlorhexidine irrigation.

The teeth were decoronated to standardize root length of 17 mm for all the samples. Working length was observed under magnifying loupes. It was estimated using a number 10 K file until it was visible at the apical foramen of each root canal and subsequently by subtracting 1 mm from this point. Chemomechanical preparation of root canals was performed in a step back technique using K files. The canals were enlarged apically up to ISO size number 40. A Gates Glidden drill number 2–4 was used to enlarge the coronal third of the root canal. Irrigation was done using 1 ml of 3% sodium hypochlorite solution between each instrument change, and subsequently the specimens were divided into three groups.

Group 1: (17% EDTA)–Final irrigation of the canal was done using 5 ml of 17% EDTA for a minute followed by 3 ml of distilled water.

Group 2: (7% Maleic acid)–Final irrigation of the canal was done using 5 ml of 7% maleic acid for a minute followed by 3 ml of distilled water.

Group 3: (2% Chlorhexidine)–Final irrigation of the canal was done using 5 ml of 2% chlorhexidine for 1 minute followed by 3 ml of distilled water.

All the irrigating solutions were introduced into the canal using stainless steel 26 gauge beveled needle. The needle was placed within 1–2 mm of working length within each canal and irrigation was performed.

After irrigation, all the root canals were dried with absorbent paper points, and a sterile cotton pellet was placed in the access cavity. The teeth were stored in a plastic bag placed in a humidior. Longitudinal grooves were prepared on buccal and lingual surfaces of each root using a diamond disc at a slow speed without penetrating the canal. The roots were then split into two halves using a chisel, and then the samples were observed under scanning electron microscope at apical, middle, and coronal levels.

The images were scored according to the criteria given by Torabinejad *et al.*,<sup>[9]</sup> which measure the presence, quantity, and distribution of the smear layer.

Score 1 = no smear layer (no smear layer on the surface of the root canals with all tubules clean and open)

Score 2 = moderate smear layer (no smear layer on the surface of root canals but tubules contain debris)

Score 3 = heavy smear layer (smear layer covers the root canal surface and the tubules)

### Statistical analysis

Comparing the smear layer removal between the three different groups was done by Kruskal–Wallis analysis of variance (ANOVA) followed by Mann–Whitney *U* test for individual comparisons. *P* value less than 0.05 was considered to be statistically significant.

## RESULTS

Table 1 and Graph 1 show the mean smear layer removal by different irrigants at different root levels. 17% EDTA showed the maximum smear layer removal at coronal ( $2.40 \pm 0.50$ ), middle ( $2.46 \pm 0.51$ ), and apical ( $2.60 \pm 0.50$ ) followed by 7% maleic acid; 2% chlorhexidine showed the least smear layer removal at all the three levels. Statistical analysis by using Kruskal–Wallis ANOVA revealed statistically highly significant difference among the root canal irrigants at all the three levels.

Table 2 revealed an intergroup comparison among the root canal irrigants at coronal third by using Mann–Whitney *U* test. There was statistically highly significant difference between 17% EDTA and 7% maleic acid and 17% EDTA and 2% chlorhexidine, however, there was no statistical difference between 7% maleic acid and 2% chlorhexidine.

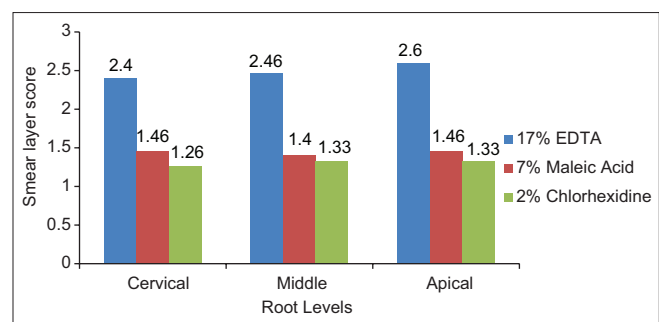
Table 3 shows intergroup comparison among the root canal irrigants at middle third by using Mann–Whitney *U* test. There was statistically highly significant difference between 17% EDTA and 7% maleic acid and 17% EDTA and 2% chlorhexidine, however, there was no statistical difference between 7% maleic acid and 2% chlorhexidine.

Table 4 revealed an intergroup comparison among the root canal irrigants at apical third by using Mann–Whitney *U* test. There was statistically highly significant difference between 17% EDTA and 7% maleic acid and 17% EDTA and 2% chlorhexidine, however, there was no statistical difference between 7% maleic acid and 2% chlorhexidine.

Table 5 shows comparison of smear layer removal by each irrigants at different root levels by using Kruskal–Wallis ANOVA. There was no statistical difference among any of the irrigants at different root levels ( $P > 0.05$ ).

## DISCUSSION

Necrotic tissue remnants in the root canals serve as a nutrient source for any remaining microorganisms.<sup>[10]</sup> The successful outcome of an endodontic treatment depends on the complete eradication of the microorganisms from the root canal system prior to obturation. Irrigation is a crucial step during and after instrumentation for effective removal of smear layer as well as for lubrication of root canal system.<sup>[11]</sup>



**Graph 1:** Mean smear layer removal by different irrigants at coronal, middle, and apical levels

**Table 1: Mean smear layer removal by different irrigating solutions at coronal, middle, and apical levels**

Irrigating solutions	Coronal (Mean±SD)	Middle (Mean±SD)	Apical (Mean±SD)
17% EDTA	2.40±0.50	2.46±0.51	2.60±0.50
7% Maleic Acid	1.46±0.63	1.40±0.63	1.46±0.63
2% Chlorhexidine	1.26±0.45	1.33±0.48	1.33±0.48
Kruskal-wallis ANOVA and <i>P</i> value	21.00	21.445	26.909
	<i>P</i> <0.001**	<i>P</i> <0.001**	<i>P</i> <0.001**

*P*<0.05, \*\*=Highly Significant

**Table 2: Mann-Whitney *U* test for inter group comparison at coronal third**

Comparison between	Mean Rank	Mann-Whitney <i>U</i>	<i>P</i> value
17% EDTA vs 7% Maleic Acid	20.70-10.30	34.50	0.001*
17% EDTA vs 2% Chlorhexidine	21.80-9.20	18.00	0.000**
7% Maleic Acid vs 2% Chlorhexidine	16.63-14.37	95.50	0.486 NS

*P*<0.05, \*\*=Highly significant, NS=Non significant

**Table 3: Mann-Whitney *U* test for inter group comparison at middle third**

Comparison between	Mean Rank	Mann-Whitney <i>U</i>	<i>P</i> value
17% EDTA vs 7% Maleic Acid	21.17-9.83	27.50	0.000**
17% EDTA vs 2% Chlorhexidine	21.67-9.33	20.00	0.000**
7% Maleic Acid vs 2% Chlorhexidine	15.67-15.33	110.00	0.481NS

*P*<0.05, \*\*=Highly Significant, NS=Non significant

**Table 4: Mann-Whitney *U* test for inter group comparison at apical third**

Comparison between	Mean Rank	Mann-Whitney <i>U</i>	<i>P</i> value
17% EDTA vs 7% Maleic Acid	21.30-9.70	25.50	0.000**
17% EDTA vs 2% Chlorhexidine	22.00-9.00	15.00	0.000**
7% Maleic Acid vs 2% Chlorhexidine	16.17-14.83	102.50	0.683 NS

*P*<0.05, \*\*=Significant, NS=Non significant

**Table 5: Comparison of smear layer removal by 17% EDTA solution at different tooth levels**

Irrigating solutions	Coronal	Middle	Apical	K ANOVA and <i>P</i> value
17% EDTA	2.40±0.50	2.46±0.51	2.60±0.50	1.217, <i>P</i> =0.544
7% Maleic Acid	1.46±0.63	1.40±0.63	1.46±0.63	0.156, <i>P</i> =0.925
2% Chlorhexidine	1.26±0.45	1.33±0.48	1.33±0.48	0.203, <i>P</i> =0.904

*P*>0.05, Non significant

Scanning electron microscope is one of the most commonly used technique for evaluating smear layer removal,<sup>[3]</sup> and hence, was used in the present study. Before using root canal irrigants on human beings, laboratory studies have to be conducted to determine the benefits and consequences.<sup>[12]</sup> Hence, the efficiency of 7% maleic acid and 2% chlorhexidine gluconate was evaluated.

The results of this study show that 7% maleic acid and 2% chlorhexidine gluconate did not promote an adequate cleaning of the root canal as considerable quantity of smear layer adhered to the dentin walls when compared to 17% EDTA. Studies have shown that 17% EDTA efficiently removes the smear layer from root canal walls.<sup>[13-15]</sup> Similar results were noted from the present

study. EDTA reacts with the calcium ions in dentine and forms soluble calcium chelates. It is known that the efficiency of a chelating agent depends on several factors including application time, pH, concentration, and amount of the solution.<sup>[16]</sup> In addition, the relationship between the concentration of the chelating agent and the application time seems to be important since it was found that highly concentrated solutions applied for a long period cause roughness of dentin surface.<sup>[17]</sup>

Maleic acid has been reported to be a mild organic acid used as an acid conditioner in adhesive dentistry. Ballal *et al.*<sup>[3]</sup> reported that final irrigation with 7% maleic acid for 1 min was more effective than 17% EDTA in smear layer removal from the apical third of the root canal. However, in the present study, maleic

acid was not found to be as effective as 17% EDTA but was efficient than 2% chlorhexidine gluconate solution as an irrigant in removing smear layer. Application time may be a factor in smear layer removal.

Chlorhexidine has been used in various concentrations (0.002–2%) with different periods of contact time between the disinfectant and various microorganisms. According to these results, 2% chlorhexidine solution was far more efficient in the shortest period of time than were all other concentrations tested. Chlorhexidine is a potent antiseptic, which is widely used for chemical plaque control in the oral cavity. Aqueous solutions of 0.1–0.2% are recommended for this purpose, whereas 2% is the concentration for root canal irrigating solution usually found in endodontic literature.<sup>[18]</sup> Only a few *in vivo* studies have investigated the antimicrobial efficacy of chlorhexidine as an irrigant.<sup>[19]</sup> Moreover, 2% chlorhexidine, used for subgingival irrigation is nontoxic to periodontal tissue at this concentration, a fact that also justifies its use as an irrigating solution in the root canal system in terms of biocompatibility.<sup>[20]</sup>

Chlorhexidine is active against a wide range of yeast, fungi, facultative anaerobes, aerobes, gram negative organisms and gram positive such as *Enterococcus faecalis*.<sup>[21]</sup>

Studies from previous literature have shown that the chlorhexidine gluconate solution can be an effective endodontic irrigant. Chlorhexidine could maintain the canal free of microorganisms, even after biomechanical preparation because of its adsorption capacity and slow liberation of active cations by the dental tissues.<sup>[22]</sup> However, in the present study the results are contrast as chlorhexidine showed least smear layer removal.

Ferraz *et al.*<sup>[23]</sup> (2001) observed that chlorhexidine was not capable of dissolving pulp tissue, which is an essential property for instrumentation and preparation of teeth with pulp necrosis.

Because chlorhexidine is active against a wide range of microorganisms, it can help in preventing reinfection of the root canal; hence, further long-term *in vivo* studies may be needed to conclude the efficiency of chlorhexidine as root canal irrigant.

According to this study, 2.0% chlorhexidine gluconate solution combined with 17% EDTA promoted an effective cleaning of the dentin walls, and hence, due to its excellent antimicrobial activity, can be used as an

alternative irrigating solution. There is no consensus on the optimum contact time which an irrigant solution to be kept in root canals for smear layer removal. However, some of the studies suggested a duration for 1 min with EDTA is sufficient.<sup>[24,25]</sup>

The choice and use of the appropriate and most efficient irrigating agent, however, requires better understanding of their action. Moreover, smear layer removal is controversial and, certainly, not the only factor affecting root canal because *in vitro* conditions may not reflect *in vivo* conditions accurately.<sup>[26,27]</sup>

## CONCLUSION

The present study shows that 17% EDTA efficiently removes the smear layer from root canal walls. The results obtained from the present *in vitro* study do not necessarily allow any definite actions of the tested substances *in situ*. Blood, tissue remnants, and various other variables may affect the actions of irrigating agents in the root canal system. Curved canals are more challenging and make effective cleaning of the root canal system more difficult. As deeper penetration of the needle takes place in the single-rooted premolar tooth because of wider canals, the results may vary in posterior teeth with narrow canals. Further studies are necessary to confirm the results, preferably of longer duration, to validate their effectiveness and contribution in the quality of treatment.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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