# Assessment of Root Canal Smear Layer Removal Efficacy of Ethylenediaminetetraacetic acid, Etidronic Acid, and Chlorhexidine using Scanning Electron Microscope

<sup>1</sup>F Rejula, <sup>2</sup>Dennis Mohan, <sup>3</sup>M Sunith, <sup>4</sup>Sujay Gopal, <sup>5</sup>Dinesh F Swamy, <sup>6</sup>Rashmi P Yadahalli, <sup>7</sup>Joseph Joy

#### ABSTRACT

**Background:** The smear layer is an amorphous film, i.e., always formed by the action of endodontic instruments inside the root canal during chemomechanical preparation. Different solutions have been used and tested for its removal. For the removal of smear layer, demineralization, and softening of root dentin, chelating agents have been preferred.

**Aim:** The current study aims to evaluate the root canal smear layer removal efficiency of different irrigating solutions with the use of a scanning electron microscope (SEM).

**Materials and methods:** A total of 75 freshly extracted singlerooted mandibular first premolars were taken for the study. A random distribution of samples was done among group 1 with 17% ethylenediaminetetraacetic acid (EDTA) irrigation, group II with 18% etidronic acid irrigation, and group III with 2% chlorhexidine irrigation. After instrumentation and irrigation, the samples were observed under SEM at coronal, middle, and apical levels.

**Results:** Using the methods in the present study, it was analyzed that the majority of the smear layer removal was observed with 17% EDTA at coronal ( $2.38 \pm 0.40$ ), middle ( $2.48 \pm 0.44$ ), and apical ( $2.54 \pm 0.41$ ) followed by 18% etidronic acid. The least smear layer removal was observed with 2% chlorhexidine at all the three levels. A statistically significant difference between 17% EDTA and 18% etidronic acid, 17% EDTA, and 2% chlorhexidine at coronal, middle, and apical levels was found.

<sup>1</sup>Associate Professor, <sup>2,3</sup>Reader, <sup>4</sup>Consultant, <sup>5</sup>Lecturer <sup>6,7</sup>Senior Lecturer

<sup>1</sup>Department of Conservative Dentistry and Endodontics Government Dental College, Alappuzha, Kerala, India

<sup>2,3</sup>Department of Conservative and Endodontics, Pariyaram Dental College, Kannur, Kerala, India

<sup>4</sup>Department of Oral Implantology, Sujay's Dental Care, Bengaluru Karnataka, India

<sup>5</sup>Department of Pedodontics and Preventive Dentistry, Goa Dental College & Hospital, Bambolim, Goa, India

<sup>6</sup>Department of Conservative and Endodontics, SJM Dental College and Hospital, Chitradurga, Karnataka, India

<sup>7</sup>Department of Conservative Dentistry and Endodontics, Mar Baselios Dental College, Kothamangalam, Kerala, India

**Corresponding Author:** F Rejula, Associate Professor Department of Conservative Dentistry and Endodontics Government Dental College, Alappuzha, Kerala, India, e-mail: rejula@hotmail.com **Conclusion:** The present study shows that 17% EDTA efficiently removes the smear layer from root canal walls.

**Keywords:** Apical, Coronal, Middle, Scanning electron microscope, Smear layer.

**How to cite this article:** Rejula F, Mohan D, Sunith M, Gopal S, Swamy DF, Yadahalli RP, Joy J. Assessment of Root Canal Smear Layer Removal Efficacy of Ethylenediaminetetraacetic acid, Etidronic Acid, and Chlorhexidine using Scanning Electron Microscope. Int J Prev Clin Dent Res 2017;4(4):275-278.

#### Source of support: Nil

Conflict of interest: None

#### INTRODUCTION

The critical factors for a successful endodontic therapy are root canal cleaning and disinfection. Hence, chemical auxiliary agents are required for the inaccessible areas of the root canal system for periapical tissue repair.<sup>1</sup>

The smear layer is an amorphous film, i.e., always formed by the action of endodontic instruments inside the root canal during chemomechanical preparation. Different methods and solutions have been used and tested for the removal as the residual layer, which can intervene with the root canal filling quality. For removal of the smear layer, demineralization and softening with root dentin chelating agents have been preferred. However, demineralization might have a negative influence on the chemical and structural composition of dentin.<sup>2</sup>

During root canal treatment, for removal of the smear layer, many materials have been used (ultrasonic instruments, lasers, and chelating agents) for chemical and mechanical debridement. Among chelating agents, EDTA is the most commonly used chelator in endodontics. Several studies have shown that the use of a combination of sodium hypochlorite (2.5-5%) and EDTA with the concentration between 10 and 17% is very effective in the removal of organic and inorganic debris. The EDTA is a Ca<sup>2+</sup> chelating agent, and, therefore, capable of removing smear layer. It has been found that a final flush of EDTA can open up the dentinal tubules, and thus, it increases the number of lateral canals to be filled.<sup>3</sup> Etidronic acid (also known as 1-hydroxyethylidene-1,1-bisphosphonate or HEBP) can be used with the combination of sodium hypochlorite as it is a good biocompatible chelator.<sup>4</sup>

Recently, 2% chlorhexidine solution has been considered as an effective root canal irrigant. It also has the antimicrobial activity similar to sodium hypochlorite as it is of a broad spectrum, along with a substantive antimicrobial activity. Chlorhexidine has been studied for its various properties with the objective of being an alternative to sodium hypochlorite.<sup>5</sup>

Hence, the present study was undertaken to assess the root canal smear layer removal efficacy of irrigating solutions by using SEM.

## MATERIALS AND METHODS

Single-rooted 75 freshly extracted mandibular first premolars were included in the study. The samples were collected from the Department of Oral and Maxillofacial Surgery. The samples were distributed in each group as follows.

Group I: 25 premolars with 17% EDTA irrigation

*Group II*: 25 premolars with 18% etidronic acid irrigation

*Group III*: 25 premolars with 2% chlorhexidine irrigation

Premolars with calcifications or accentuated curvatures, carried or fractured teeth, and teeth with open apices, with resorption, or craze line were not selected for the study.

Endo-Access Bur was used to access coronally. K files were used with a stepback technique for chemomechanical preparation of root canals. The International Organization for Standardization size number 40 was introduced into the canals to enlarge apically. A Gates Glidden drill number 2–4 was used to enlarge the coronal third of the root canal. About 2 mL of 2.5% sodium hypochlorite solution irrigation was done between each instrument change. After instrumentation, 25 premolars were allotted to each group and different irrigation protocols were used in each group.

## Group I: 17% EDTA Irrigation

The canals were irrigated with 3 mL of 17% EDTA irrigation for 1 minute followed by 5 mL of distilled water. The EDTA was allowed to remain in the canal for 1 minute only.

# Group II: 18% Etidronic Acid Irrigation

The canals were irrigated with 3 mL of 18% etidronic acid irrigation for 1 minute followed by 5 mL of distilled water. An 18% etidronic acid was allowed to remain in the canal for 1 minute only.

## Group III: 2% Chlorhexidine Irrigation

The canals were irrigated with 3 mL of 2% chlorhexidine for 1 minute followed by 5 mL of distilled water. A 2%

chlorhexidine was allowed to remain in the canal for 1 minute only.

The canals were dried with absorbent paper points and the entrance to each of the canals was protected with a cotton pellet. The teeth were stored in a plastic bag placed in a humidor. Longitudinal grooves were prepared on buccal and lingual surfaces of each root using a diamond disk 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 a SEM at coronal, middle, and apical levels.

## Scoring Criteria

*Score 1*: No smear layer (no smear layer on the surface of the root canals with all tubules clean and open)<sup>6</sup> *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 Methods**

The statistical analysis was done by using Statistical Package for the Social Sciences software 20. Comparing the smear layer removal between the three different groups was done by Kruskal–Wallis analysis of variance followed by Mann–Whitney *U* test for individual comparisons. A p-value less than 0.05 was considered statistically significant.

# RESULTS

Table 1 reveals the mean smear layer removal by different irrigants at coronal, middle, and apical levels. Majority of the smear layer removal was observed with 17% EDTA at coronal ( $2.38 \pm 0.40$ ), middle ( $2.48 \pm 0.44$ ), and apical ( $2.54 \pm 0.41$ ) levels, followed by 18% etidronic acid. The least smear layer removal was observed with 2% chlorhexidine at all the three levels. A highly statistically significant difference was present between the root canal irrigants at all the three levels.

Table 2 reveals the intergroup comparisons of different root canal irrigants at the coronal third. There was

 
 Table 1: Different irrigating solutions used at coronal, middle, and apical levels

Irrigating solutions		Coronal	Middle	Apical
17% EDTA	Mean	2.38	2.48	2.54
	SD	0.40	0.44	0.41
18% etidronic	Mean	1.26	1.30	1.30
acid	SD	0.52	0.52	0.53
2% chlorhexidine	Mean	1.10	1.13	1.13
	SD	0.34	0.42	0.42
p-value		p<0.001**	p<0.001**	p<0.001**
p<0.05; **Highly significant; SD: Standard deviation				



Table	2:	Intergroup	comparison	at	coronal	third
IUNIO		micigioup	oompunoon	u	ooronai	u in u

	• • •		
Comparison		Mann–Whitney	
between	Mean rank	U test	p-value
17% EDTA vs 18% etidronic acid	18.60–9.30	33.40	0.001**
17% EDTA vs 2% chlorhexidine	19.80–10.20	16.00	0.0001**
18% etidronic acid vs 2% chlorhexidine	15.62–12.42	87.90	0.346 NS

p<0.05; \*\*Highly significant; NS: Nonsignificant

Table 4: Intergroup comparison at apical third level	
--	--

Comparison between	Mean rank	Mann–Whitney U test	p-value
17% EDTA vs 18% etidronic acid	22.10-10.13	24.10	0.0001**
17% EDTA vs 2% chlorhexidine	23.44-8.00	18.00	0.0001**
18% etidronic acid <i>vs</i> 2% chlorhexidine	15.54–15.64	101.42	0.452 NS

p<0.05; \*\*Significant; NS: Nonsignificant

statistically significant difference between 17% EDTA and 18% etidronic acid, 17% EDTA and 2% chlorhexidine, but there was no statistical difference between 18% etidronic acid and 2% chlorhexidine.

A highly statistically significant difference was found for the comparison of different root canal irrigants at middle third and apical third (between 17% EDTA and 18% etidronic acid, 17% EDTA and 2% chlorhexidine), but a statistical significant difference was not found between 18% etidronic acid and 2% chlorhexidine (Tables 3 and 4).

#### DISCUSSION

Smear layer formation is a microscopic layer appearing from the scoured dentin due to the use of files (rotary instruments and endodontic) during root canal treatment. Under SEM, the smear layer is viewed as a uniform, dense layer of an amorphous structure that completely obliterates the entrance to the dentin tubules and drastically reduces the permeability of the dentin.<sup>7</sup>

The presence of smear layer on permanent root canal walls was first reported by McComb and Smith. The smear layer is an amorphous structure composed of an organic portion, which is coagulated proteins, necrotic and normal pulpal tissue, saliva, microorganisms, etc., and an inorganic portion consisting minerals from the dentinal structure.<sup>8</sup>

The present study shows that 17% EDTA is more effective than 18% etidronic acid and 2% chlorhexidine. The studies have shown that 17% EDTA efficiently removes the smear layer from root canal walls.<sup>9,10</sup> Similar results were noted from the present study. The EDTA reacts with the calcium ions in dentin and forms soluble calcium

Smear Layer	Removal	and	Different	Irrigating	Solutions
-------------	---------	-----	-----------	------------	-----------

Table 3: Intergroup comparison at middle third level					
		Mann–Whitney			
Comparison between	Mean rank	U test	p-value		
17% EDTA <i>vs</i> 18% etidronic acid	20.27–10.22	28.50	0.0001**		
17% EDTA vs 2% chlorhexidine	22.43–10.45	21.10	0.0001**		
18% etidronic acid <i>vs</i> 2% chlorhexidine	16.27–16.42	90.00	0.481 NS		
n < 0 0E, **I light signi	finanti NC: No.	anianificant			

p<0.05; \*\*Highly significant; NS: Nonsignificant

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. 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>11</sup>

In the current study, etidronic acid is less effective compared with 17% EDTA. However, it is better than the 2% chlorhexidine. Etidronic acid was found to have smear layer removal efficacy in coronal and middle third. But, it showed less smear layer removal in the apical third when compared with EDTA. This might be because of the lesser chelating action of etidronic acid than EDTA. The lower efficacy of etidronic acid on sclerosed dentin can also be an attributing factor.<sup>12</sup>

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 and 0.2% are recommended for this purpose, whereas 2% is the concentration for root canal irrigating solution usually found in endodontic literature.<sup>13</sup> Only a few in vivo studies have investigated the antimicrobial efficacy of chlorhexidine as an irrigant.<sup>14</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>15</sup>

#### CONCLUSION

The present study shows that 17% EDTA efficiently removes the smear layer from root canal walls. 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.

# REFERENCES

- Siqueira JF Jr, Rôças IN, Santos SR, Lima KC, Magalhães FA, Uzeda M. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. J Endod 2002 Mar;28(3):181-184.
- Baldasso FER, Roleto L, Silva VDD, Morgental RD, Kopper PMP. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. Braz Oral Res 2017 May;15:31:e40.
- 3. Kuruvilla A, Jaganath BM, Krishnegowda SC, Ramachandra PM, Johns DA, Abraham A. A comparative evaluation of smear layer removal by using EDTA, etidronic acid, and maleic acid as root canal irrigants: an *in vitro* scanning electron microscopic study. J Conserv Dent 2015 May-Jun;18(3): 247-251.
- 4. Girard S, Paque F, Badertscher M, Sener B, Zehnder M. Assessment of a gel type chelating preparation containing 1-hydroxyethylidene 1, bisphosphonate. Int Endod J 2005 Nov;38(11):810-816.
- Yamashita JC, Tanomaru Filho M, Leonardo MR, Rossi MA, Silva LA. Scanning electron microscopic study of the cleaning ability of chlorhexidine as a root canal irrigant. Int Endod J 2003 Jun;36(6):391-394.
- Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, Kim J, Shabahang S. A new solution for the removal of the smear layer. J Endod 2003 Mar;29(3): 170-175.
- Pashley DH, Michelich V, Kehl T. Dentin permeability: effects of smear layer removal. J Prosthet Dent 1981 Nov;46(5): 531-537.

- McComb D, Smith DC. A preliminary scanning electron microscopic study of root canals after endodontic procedures. J Endod 1975 Jul;1(7):238-242.
- 9. Vlad R, Kovacs M, Sita D, Pop M. Comparison between different endodontic irrigating protocols in smear layer removal from radicular dentin. Eur Sci J 2016 May;12(15):38-43.
- 10. Chhabra N, Gyanani H, Kamatagi L. Smear layer removal efficacy of combination of herbal extracts in two different ratios either alone or supplemented with sonic agitation: an *in vitro* scanning electron microscope study. J Conserv Dent 2015 Sep-Oct;18(5):374-378.
- 11. Silva PV, Guedes DF, Pécora JD, da Cruz-Filho AM. Timedependent effects of chitosan on dentin structures. Braz Dent J 2012;23(4):357-361.
- 12. De-Deus G, Zehnder M, Reis C, Fidel S, Fidel RA, Galan J Jr, Paciornik S. Longitudinal co-site optical microscopy study on the chelating ability of etidronate and EDTA using a comparative single-tooth model. J Endod 2008 Jan;34(1):71-75.
- 13. Eugeniu MC, Radu C, Vlad P. Chlorhexidine as endodontic cleaning solution compared to sodium hypochlorite: an *in vivo* study. Int J Dent Med Spec 2015 Jan-Mar;2(1):2-6.
- 14. Makkar S, Aggarwal A, Pasricha S, Kapur I. Comparative evaluation of octenidine hydrochloride and chlorhexidine as antibacterial root canal irrigant. Indian J Oral Sci 2015;6(1): 10-13.
- 15. 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 Community Dent 2016 Aug;6(Suppl 2):S160-S165.