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Comparative Evaluation of Smear Layer Removal Using 17%EDTA, MTADand &7%Maleic Acid- An Invitro Study

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Abstract:
Background:*The study was aimed to evaluate the efficacy of 17% EDTA, MTAD, and 7% maleic acid in smear layer removal using scanning electron microscopic image analysis.*
Materials and Methods: *Thirty freshly extracted mandibular premolars were collected and prepared after decoronation to obtain working length of 17mm and was prepared till F 3 rotary protaper file with 3% sodium hypochlorite after each instrument change. The samples were divided into Groups I (17% ethylenediaminetetraacetic acid (EDTA)), II (MTAD), and III (7% maleic acid) containing 10 samples each. Longitudinal sectioning of the samples was done. Then the samples were observed under scanning electron microscope (SEM) at apical, middle, and coronal levels. The images were scored according to Torabijenad et al criteria:*
Statistical Analysis: *Data was analysed statistically using Kruskal-Wallis analysis of variance (ANOVA) followed by Mann-Whitney U test for individual comparisons. The level for significance was set at 0.05.*
Results: *At the coronal level the amount of smear layer removal was similar in all the groups. There was no much difference in smear layer removal in the middle third and apical third of root canals with irrigation with 7% maleic acid and MTAD. Due to the non-availability and high cost of MTAD, the use of 7% Maleic acid seems promising as a smear layer removalirrigant.*

Keywords:*Scanning electron microscope; Smear layer, EDTA; MTAD; Maleic acid*

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I. Introduction

The formation of smear layer is an inevitable part of root canal instrumentation. Smear layer removal is an asset and could help to achieve a successful outcome of the root canal treatment.[(1)]Smear layer contains both organic and inorganic components. The smear layer has been recommended to be removed as it may be having mixture of bacteria and their by-products. [(2)]The presence of this layer not only hinders the penetration of intracanal medicaments, sealers, root canal irrigants and obturating material into the dentinal tubules but also risk micro leakage[(3)]Ethylenediaminetetraacetic acid (EDTA)is the most frequently used chelator in endodontics.[(4)] EDTA is a calcium chelating agent and therefore capable of removing smear layer. It has been found that a final flushof EDTA can openup the dentinal tubules and thus increasesthe number of lateral canals to be filled.[(5).]

MTAD is a mixture of doxycycline, citric acid and a detergent(Tween 80).[(6)]Maleic acid is a mild organic acid used as an acid conditioner in adhesive dentistry. [(7)] It has been found to possess the smear layer removing quality when used as an acid etchant in restorative dentistry [(8).]Ballal et al have suggested 7% maleic acid as a mild organic acid found to have the smear layer removing ability from root canal dentine and to be more effective than EDTA at the apical third of root canal.[(9)]

The apical portion of the root canals is very complex and difficult to clean and shape because of the ever-increasing anatomic complexity. The presence of lateral canals in the apical third is 73 %.[10] They serve as avenues for the passage of irritants primarily from the pulp to the periodontium. This highlights the advantages of cleaning in the apical third of the canal. Moreover the intricacies and fine aspects of root canal anatomy compromise the physical removal of bacterial load through instrumentation alone, shedding responsibilities in disinfection of the root canal space mainly on irrigants.

The presentstudy evaluates and compares the efficiency of 17% EDTA, MTAD and 7% maleic acid in their ability to remove smear layer following root canal instrumentationon human extracted tooth using scanning electron microscope (SEM).

II. Materials And Methods

STUDY DESIGN- in vitro study

STUDY SETTING: Study was conducted in the Department of Conservative Dentistry and Endodontics, Government Dental College, Kozhikode. And Department ofNanotechnology, Amrita Institute, Ernakulam after obtaining ethical clearance from the institution

SAMPLE SIZE CALCULATION

The sample size will be calculated by the formula

$$n=\frac{(Z\alpha + Z\beta)^2 \times SD^2 \times 2}{d^2}$$

where

n= sample size

Zα= 1.96 for an α error 5%

Zβ=0.84 for a power of 80 %

SD= Standard Deviation

FOR THE PRESENT STUDY

SD= 19.5

d=10

$$n=\frac{(1.96+0.84)^2 \times 19.5^2 \times 2}{10^2} = 29.8$$

In order to detect a clinically relevant difference of 10% at 5% level of significance with 80 % power, the required sample size was arrived at 10 samples in each group rounded to 30.

INCLUSION CRITERIA

1. Teeth with straight roots
2. Teeth with fully formed apices
3.Non carious mandibular premolar extracted for orthodontic reasons

Exclusion Criteria

1. Teeth that was previously endodontically treated
2. Fractured teeth
3. Teeth with curved roots
4. Teeth with previous coronal restorations
5. Teeth with calcified canals

METHODOLOGY [11]

Selection of samples

Thirty freshly extracted single rooted human mandibular premolars which were extracted for orthodontic purpose was selected for the study. All the teeth selected wereasper the inclusion criteria. Ultrasonic scaling wasdone to clean the outside surface of root.Teeth with cracks or fracture lines were eliminated after examining using loupes.Collected teeth werebe placed in 5.25% NaOCl for 1 h in order to disinfect the root surfaces and the samples was stored in 0.9% physiological saline.

Teeth preparation for the study

An open end model was used in the study. The teeth weredecoronated to obtain uniform working length of 17 mm for all the samples using a diamond disc and water coolant. The root canals were accessed and standardised

crown down preparation was done till rotary Protaper F3, with 3% NaOCl irrigation between each file, followed by irrigation with 5 ml of saline.

The samples are divided into Groups I, Group II, and Group III containing 10 samples each

Groups I - 17% EDTA irrigation

Group II – MTADirrigation(Biopure, Dentsply)

Group III- 7 % maleic acid irrigation

A total of 10 ml of the irrigantswas used in each root canal. The irrigantswere delivered using 28 guage, side vented prorinse needles that will penetrate to within 1-2mm from the working length in each canal. The instrumentation time for each canal was 15-20 min.

To determine the effects of experimental and control solutions as a final rinse on the surface of root canal, 5 ml of experimental solutions was used after instrumentation. Initially 1ml of the solution was introduced into the canal and agitated with a No15K file 100 times per minute with the help of a stop watch, followed by4ml of the irrigant which was also agitated for 2 minutes. Thespecimens were finally irrigated with 10 ml of distilled water to remove any precipitate that may have formed from the test irrigants.

This procedure was conducted identically for all the groups. After instrumentation the canals was be dried with sterile paper points.

Teeth Preparation for SEM analysis

After the root canals were dried with paper points, the entrance to each canal was protected with a cotton pellet. Using diamond disc longitudinal deep grooveswas made on the buccal and lingual surfaces, with precaution not to perforate the root canals. Following this, the roots weresplit into two halves with chisel and mallet. One half of each tooth, containing greater part of the apex was selected as the representative sampleandwas coded and scheduled for SEM Examination

SEM Analysis

The scanning electron microscope analysis was done from Department of Nanotechnology Amrita Institute Ernakulum.Thespecimens were dehydrated by ethyl alcohol: 30 %for 10 min, 50 % for 20 min, 70 % for 20 min, 90 % for 30min, 100% for 30 min. After that the specimens was mounted on coded stubs, air dried, placed in a vacuum chamber, and sputter coated with 300A gold layer. .The specimens was then analysed using a SEM. 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 will be taken on each coronal, middle and apical thirds.

SEM images was then be analysed for the amount of smear layer present using a three score system by a teaching faculty who will be blinded to the irrigation regimens employed for each group. The scores will be attributed according to the rating system developed by Torabinejad et al [12]

Score 1 - No smear layer all tubules clean and open

Score2.Moderate smear layer (no smear layer on surface of root canals but tubules contain debris)

Score 3. Heavy smear layer (smear layer covers the root surface and the tubules)

Statistical Analysis

Statistical Package for social science (SPSS) version 16 was used for analysis. Owing to the nonparametric nature of the data, nonparametric tests were used for statistical analysis.Kruskal – Wallis analysis of variance (ANOVA) was used for intragroup comparisons. Mann- Whitney U test testwas used for intergroup comparisons

III. Results

Results of **Table 1** and **Graph 1** shows that in the coronal third ,the smear layer removal of 17% EDTA, MTAD and 7% Maleic acid were not statically significant as the p value was 0.339. In the middle third there was statistically significant difference in the smear layer removal efficacy with 7% Maleic acid (mean value 1.2) showing more efficacy when compared to MTAD and 17% EDTA. In the apical third also there was statistically significant difference in the smear layer removal efficacy as the p value wasless than <0.001. From the above data, 7% Maleic acid and MTAD showed equally good smear layer removal efficacy in the middle third and apical third.

Table 1.

	Irrigating solutions	N	Mean	Std. Deviation	Minimum	Maximum	p-value	Inference
Coronal third	17% EDTA	10	1.2	0.42164	1	2	**0.339**	There is no significant difference
	MTAD	10	1.2	0.42164	1	2		
	7%Maleic Acid	10	1	0	1	1		
Middle third	17% EDTA	10	1.8	0.42164	1	2	**0.012**	There exists significant difference
	MTAD	10	1.3	0.48305	1	2		
	7%Maleic Acid	10	1.2	0.42164	1	2		
Apical third	17% EDTA	10	2.7	0.48305	2	3	**<0.001**	There exists significant difference
	MTAD	10	1.3	0.48305	1	2		
	7%Maleic Acid	10	1.2	0.42164	1	2		

Graph 1.

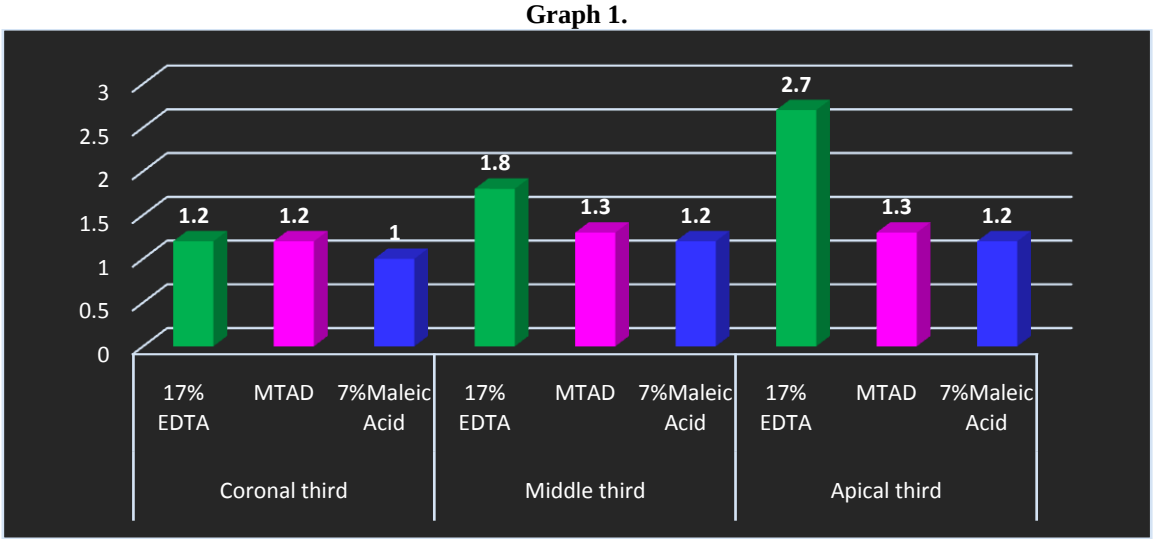


Table: 2

Coronal third			
Comparison between	**Mean rank**	**Mann-Whitney U**	**p-value**
17% EDTA vs MTAD	10.5-10.5	50	**1.00**
17%EDTA vs 7% Maleic acid	11.5-9.5	40	**0.146**
MTAD vs 7% Maleic acid	11.5-9.5	40	**0.146**

From **Table 2** it was observed that when Mann Whitney test was used for comparison of smear layer removal efficacy between the three test irrigants in the coronal third , the results were not statistically significant as p value is greater than 0.05

Table: 3

Middle third			
Comparison between	**Mean rank**	**Mann-Whitney U**	**p-value**
17% EDTA vs MTAD	13-8	25	**0.03**
17%EDTA vs 7% Maleic acid	13.5-7.5	20	**0.009**
MTAD vs 7% Maleic acid	11-10	45	**0.615**

From **Table no 3** it can be observed that the smear layer removal efficacy in the middle third showed statistically significant difference when MTAD and 7% Maleic acid was compared with 17% EDTA and there was no statististical difference between the smear layer removal efficacy between MTAD and 7%Maleic acid.

Table: 4

Apical third			
Comparison between	**Mean rank**	**Mann-Whitney U**	**p-value**
17% EDTA vs MTAD	15.05-5.95	4.5	**<0.001**
17%EDTA vs 7% Maleic acid	15.2-5.8	3	**<0.001**
MTAD vs 7% Maleic acid	11-10	45	**0.615**

From **Table no 4** it was observed that the smear layer removal efficacy in the apical third showed statistically significant difference when MTAD and 7% Maleic acidwas used, when comparedto 17% EDTA.

Table: 5

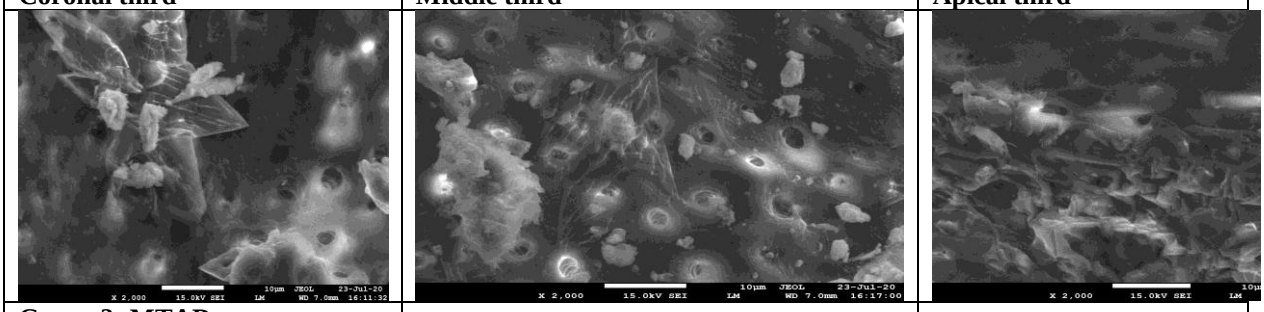
Irrigating solutions		**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**	**Kruskal-Wallis H**	**p-value**
17% EDTA	Coronal third	10	1.2	0.42164	1	2	19.607	**<0.001**
	Middle third	10	1.8	0.42164	1	2		
	Apical third	10	2.7	0.48305	2	3		
MTAD	Coronal third	10	1.2	0.42164	1	2	0.33	**0.848**
	Middle third	10	1.3	0.48305	1	2		
	Apical third	10	1.3	0.48305	1	2		
7 % Maleic Acid	Coronal third	10	1	0	1	1	2.231	**0.328**
	Middle third	10	1.2	0.42164	1	2		
	Apical third	10	1.2	0.42164	1	2		

The observations in Table 5 shows that when Kruskal Wallis was used to compare the smear layer removal efficacy it was seen that all the test irrigants removed smear layer in the coronal third , but the smear layer removal efficacy of 7% Maleic acid and MTAD were more or less similar.

Figure 2 :Scanning Microscopic Images at 2000X

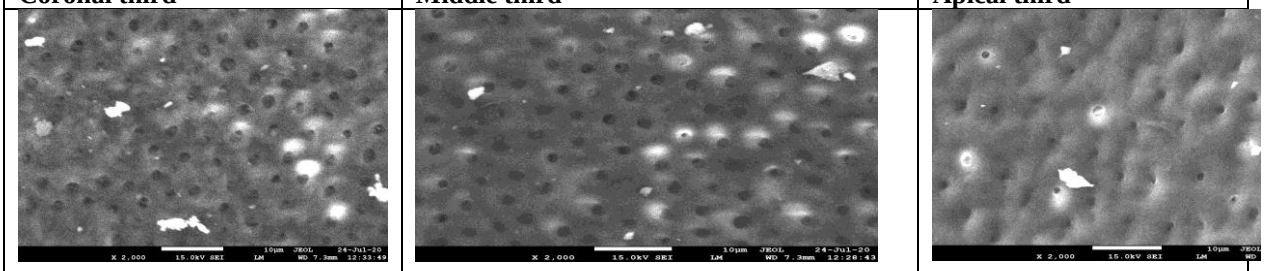
Group 1-17%EDTA

Coronal third **Middle third** **Apical third**

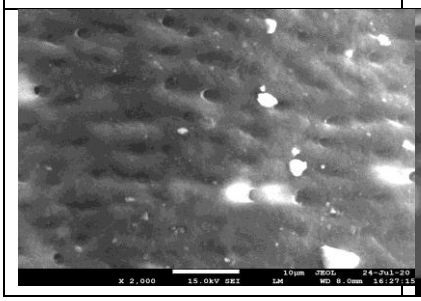
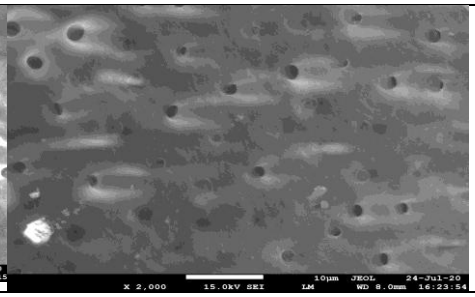
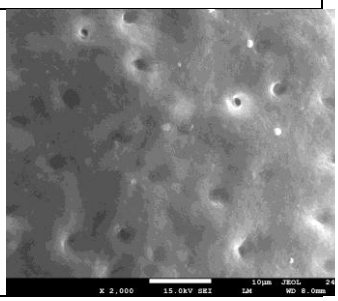


Group 2- MTAD

Coronal third **Middle third** **Apical third**



Group 3-7% Maleic Acid

Coronal Third	Middle Third	Apical Third
		

IV. Discussion

The endodontic smear layer has been described as one that is formed during instrumentation, consisting of not only dentine but also necrotic and viable tissue, including remnants of odontoblastic processes, pulp tissue and bacteria. Pashley et al[(13)] had described the smear layer as a porous structure which was permeable to even large molecules like albumin. Mader et al [(14)] had stated that the smear layer was a non-homogenous and weakly adherent structure and may slowly disintegrate and dissolve around leaking filling margins, thus creating voids between root canal walls and filling material. The smear layer presence plays a significant role in an increase or decrease in apical leakage. Its absence makes the dentine more conducive to a better and closer adaptation of the guttapercha to the canal wall. [(15)]

Scanning electron microscope is one of the most commonly used techniques for evaluating smear layer removal and hence this technique was used to evaluate the smear layer removal efficiency of the three test irrigants.

In the present study, all the three irrigants (17% EDTA, MTAD and 7% Maleic acid) removed smear layer effectively from the coronal third but with no statistically difference between them.

The results of this study shows that 7% Maleic acid removed smear layer in the apical third when compared to 17% EDTA. Similar observation was also found in the study conducted by Ballal et al. [(9)]In the study conducted by Prabhu SG et al [(16)] it was found that the smear layer removal efficacy of 7% Maleic acid was significant in both middle third and apical third of the root canal with little or no debris and the intertubular dentine was not demineralised or damaged.

Another observation in this study was that 17% EDTA showed very little smear layer removal in the apical third of the canal. This is in agreement with Ciucchi et al [(17)] who stated that there was a decline in the efficiency of irrigating solutions along the apical part of the canal. This can be probably be explained to the fact that dentine in the apical third is much more sclerosed and the numberof tubules present there is less

MTAD was first introduced by Torabinejad et al. and they found that it effectively removes smear layer, when it issued as a final rinse, with NaOCL as an initial irrigant. According to them the tetracycline part of MTADremoves the smear layer and other debris and detergentTween- 80, reduces the surface tension of the irrigant,thereby aiding in better penetration of the irrigant. [(18)]

In the present study it was found that the smear layer removal efficacy of MTAD and 7% Maleic acid was comparatively equal in the middle third and apical third of the root canal. But a little better action was seen with 7% Maleic acid. This might be because of the lesser chelating action of MTADthan Maleic acid.

V. Conclusion

According to the findings and within the limitation of the present study, it can be concluded that 7% Maleic acid and MTAD shows efficient smear layer removal in the in the apical and middle third of the root canal. (Table 1and Figure 2).7% Maleic acid seems to be a promising irrigant when compared to 17% EDTA. MTAD has also good smear layer removal action but the high cost and non-availability in India precludes its usage. Nevertheless further long term clinical studies are necessary to confirm these results and evaluate their relevance to treatment outcomes.

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