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Original Article

Comparative Evaluation Of Smear Layer Removal Using Three Chelating Agents And Their Effect On The Penetrability Of Epoxy Resin-Based Sealer Into Dentinal Tubules Using SEM And CLSM -In Vitro Study

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ABSTRACT:
Introduction: The aim of this study was to evaluate and compare the effect of 17% EDTA, 7% Maleic acid and 1% Phytic acid on removal of smear layer using SEM and their effect on the penetrability of AH Plus sealer into dentinal tubules using Confocal Laser Scanning Microscope (CLSM). **Methods:** 30 extracted teeth were selected for this study. The FRP for the experimental groups were 5 mL of each irrigant for 2 min followed by 2mL of saline. According to the sealer and final irrigation used as mentioned above, all samples were divided into 3 groups. 15 samples were split longitudinally to obtain 2mm slices and were examined on SEM. Another 15 samples were filled using AH Plus sealer and was sectioned horizontally at 4 mm from the apex using a diamond disc to obtain a 2-mm-thick slice. The samples to be submitted to confocal laser microscopy for analysis. **Statistical analysis used:** ONE WAY ANOVA TEST & Tukey –Kramer Multiple comparison tests were used. **Result:** The findings of the present study suggest that there was a significant difference between 17% EDTA and 7% maleic acid, 7% Maleic acid was not found to be as effective as 17% EDTA. However when 17% EDTA and 1% Phytic Acid were compared, the difference in the smear layer removal at all the three levels was statistically not significant. In this study 1%Phytic Acid and 7% Maleic acid effectively removed smear layer from coronal, middle and apical levels. **Conclusion:** Sealer penetration was seen higher in 17% EDTA group as compared to 1% phytic acid and 7% maleic acid.
Keywords: Endodontic, Confocal laser scanning microscopy, Scanning electron microscopy, Smear layer, Root canal irrigant, Final irrigation solution.

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INTRODUCTION

Most important requisite of endodontic treatment is proper cleaning, shaping and a three-dimensional obturation with adequate seal of root canal.[1, 2] Throughout the chemo-mechanical preparation, abundant irrigation is done to sterilize the root canal and dentinal tubules and to eliminate loose, infectious, and desecrated materials.[3] A layer of sludge material is always formed in addition to superficial debris over the surface of the instrumented root canals. In year 1975 it was termed as "smear layer" by McComb and Smith.[4] For effective removal of smear layer, acid solutions are used nowadays and commonly used irrigants for this purpose are EDTA (17%) and citric acid (1–50%).[5] EDTA is a very effective root canal irrigant for amputation of smear layer from root canal walls.[6] But studies have shown that EDTA is not readily biocompatible, in consideration of these fact, a substitute agent for smear layer removal is warranted, and the research for more biodegradable material to replace EDTA is still going on.[7] Ballal *et al*, in his study stated that maleic acid (7%) cause highest surface irregularity on dentine when compared to EDTA (17%), which assumes a critical part in

micromechanical holding of sealers.[8] Phytic acid (1%) is a relatively new solution used for irrigation. Nassar *et al*, found that, 1% phytic acid has ability to remove the smear layer from NaOCl-treated flat coronal dentin surfaces and stated that it is a less cytotoxic and biocompatible irrigant when compared with EDTA.[9] It is imperative for the sealer to infiltrate into the dentinal tubules to totally fill the root canal inclusive of lateral & accessory canal, isthmus and apical delta. From an imperceptible point, infiltration of sealer into dentinal tubules is exceptionally fundamental on the grounds that it may maintain the microorganism far from nutrient sources and it might entomb residual debris and root canal pathogens.[8] The infiltration of sealer into the dentinal tubules builds the association between the material and the dentine, which, may improve the mechanical bonding of the material by mechanically looking it into place; and that's how microleakage reduces.[10]

Hence the present study compare and evaluates removal of smear layer using EDTA (17%) , Maleic acid (7%) and Phytic acid (1%) using SEM analysis and their impact on the penetrability of AH-Plus Sealer in dentinal tubules utilizing CLSM.

MATERIALS AND METHODS

30 Single rooted extracted human teeth were selected for the study (Figure 1).

Figure 1: DECORONATION OF TOOTH

Radiographs were taken to ensure that the teeth have a single canal. All teeth were autoclaved for disinfection and were then be stored in normal saline (Baxter). The teeth were decoronated using a diamond disk (Bosch) to obtain uniform root length of 18 mm for all samples. The canals were accessed and the working length (WL) was established 1 mm short of the total canal length via the insertion of a #15 K-file (DENTSPLY). Before root canal preparation, all roots were randomly divided into three groups (n=10) according to the solution used in the final rinse protocol (FRP) as follows: 1) Group I: 17% EDTA (PRIMEDENT), 2) Group II: Phytic Acid, 3) Group III: Maleic Acid. The root canals were prepared at WL using the ProTaper rotary system (DENTSPLY) up to F2 file. After each instrument change, 2 mL of saline was delivered into the root canals. The FRP for the experimental groups were 5 mL of the test solution for 2 min followed by 2mL of saline. Finally, the canals were dried with paper points.

TOOTH PREPARATION FOR SEM ANALYSIS

Five teeth of each group were prepared for scanning with an electron microscopy (SEM) operated at 10 kV. 15 Specimens were split longitudinally through the root canal in a bucco-lingual direction with a diamond disk at a low speed, thus making 30 dentine sections of 2mm thickness (Figure 2).

FIGURE 2: BUCCOLINGUAL SECTIONING OF THE TOOTH

Each sample was dehydrated in graded series of ethanol solutions, mounted on stubs, gold-sputtered, and was examined on SEM. Photomicrographs of these areas on each of the coronal, middle and apical thirds were taken (Figure 3).

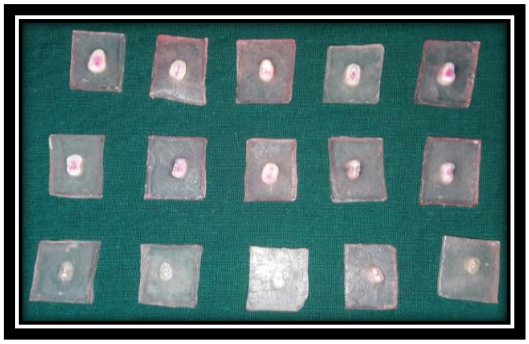
FIGURE 3: SAMPLES MOUNTED FOR ANALYSIS

TOOTH PREPARATION FOR CLSM ANALYSIS

The remaining 15 teeth were filled using equal parts of paste A and paste B of an epoxy resin-based sealer (AH Plus, DENTSPLY) which is mixed with 0.1 % Rhodamine B. The endodontic sealer was placed in the canal 1 mm shorter than the WL using a 400-rpm lentulospiral (Mani) for 5 seconds. The specimens were stored at 37 °C and 100% humidity for 7 days to allow the sealer to set. Each specimen was horizontally sectioned under water cooling at 4 mm

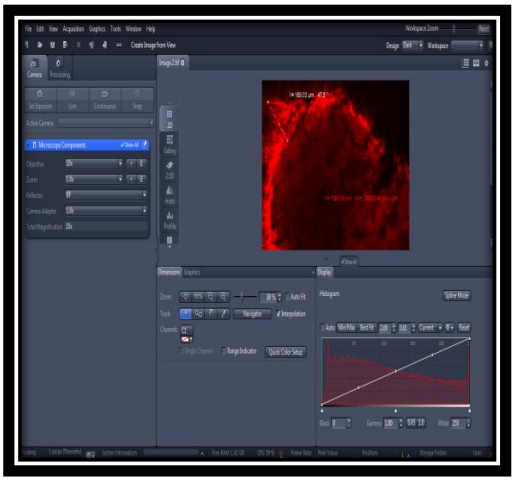
from the apex using a diamond disc to obtain a 2-mm-thick slice (Figure 4).

FIGURE 4: HORIZONTAL SECTIONING OF THE TOOTH



The samples to be submitted to confocal laser microscopy were having 2 mm thickness. The dentin segments were examined on a confocal microscope (Zeiss LSM 710 confocal). The respective absorption and emission wave lengths for the Rhodamine B were 540 nm and 590 nm. Dentin samples were analyzed using the 10× lens. To calculate the percentage of sealer penetration around the root canal, first each image was imported into the ZEN 710 software and the circumference of root canal measured. Next, areas along the canal walls in which the sealer penetrated into dentinal tubules were outlined and measured using the same method. Subsequently, the percentage of root canal sealer penetration in that section was established. Using the ruler tool of the ZEN 710 software, depth of sealer penetration was measured and recorded at four standardized points of each 10×picture. The canal wall was serving as the starting point and sealer penetration into dentinal tubules were measured to a maximum depth of 1,000 µm. These data points were averaged to obtain a single measure for each section. The images taken in a CLSM are shown in (Figure 5).

FIGURE 5: ZEN 710 SOFTWARE



RESULTS

Distribution of mean and SD of SEM values in 17% EDTA , 1% Phytic Acid and 7% Maleic Acid groups at Coronal, Middle and Apical canal levels is given in table 1.

Table 1:

Canal levels	17% EDTA	1% Phytic Acid	7% Maleic Acid
	Mean ± SD	Mean ± SD	Mean ± SD
Coronal	1.20±0.44	1.60±0.54	2.0±0.70
Middle	2.0±0.70	2.40±0.54	2.60±0.54
Apical	2.40±0.54	2.80±0.44	2.80±0.44

17% EDTA effectively removed smear layer compared to 7% Maleic acid and 1% Phytic acid. Similar results were seen with CLSM analysis given in table 2.

Table 2:

Canal levels	17% EDTA (µm)	1% Phytic Acid (µm)	7% Maleic Acid (µm)
	Mean ± SD	Mean ± SD	Mean ± SD
Coronal	619.06±82.27	421.59±59.21	337.44±62.04
Middle	412.36±64.88	203.40±99.85	189.12±80.3
Apical	260.93±64.79	139.98±93.02	102.72±12.74

By applying Tukey–Kramer Multiple comparison test there is a significant difference between mean values of SEM and CLSM values in 17% EDTA , 1% Phytic Acid and 7% Maleic Acid groups compared together at Coronal, Middle and Apical canal levels ($p<0.001$) given in table 3.

Table 3:

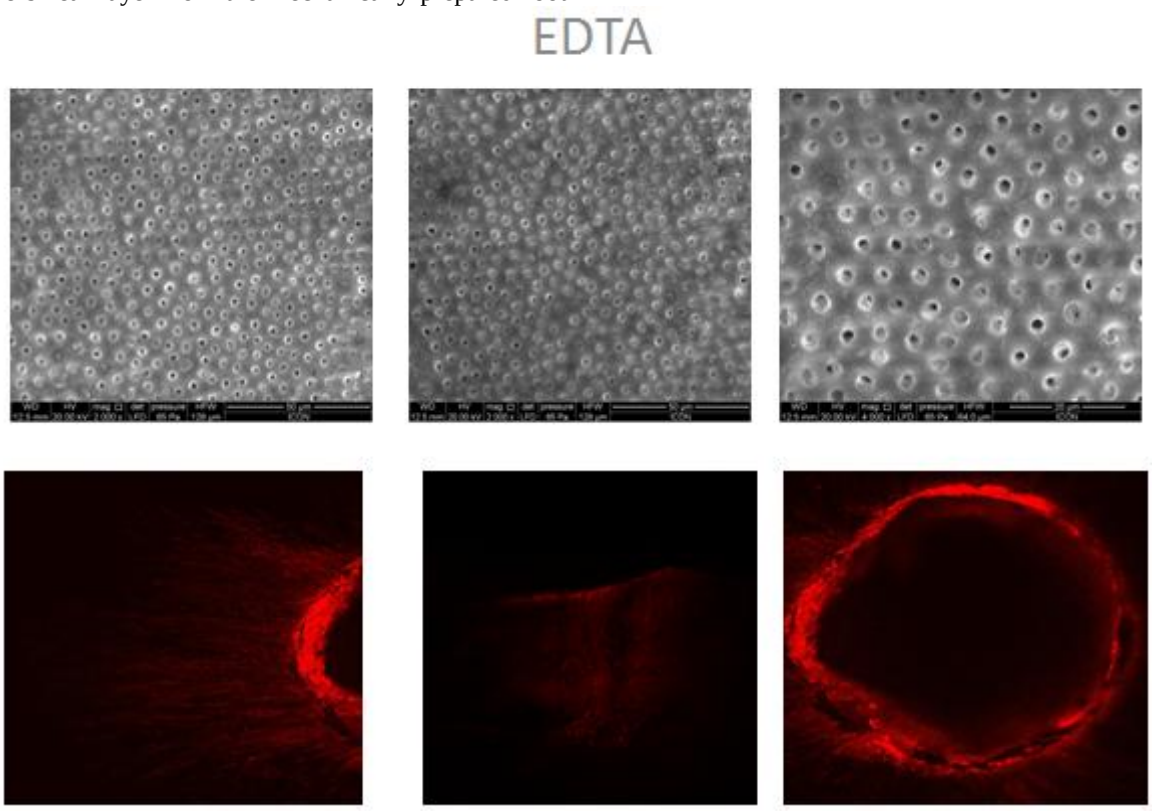
Source of variation	Degrees of freedom (d.f.)	Sum of squares	Mean squares
Treatments (between columns)	17	3067197	180423
Residuals (within columns)	12	194467	27009
Total	**89**	**3261664**	

DISCUSSION

Decease tissue residue in the root canals serve as a nutritional source for any remaining microorganisms. For successful endodontic treatment, there is always a requirement for chemo-mechanical debridement though it is impossible to create a sterile environment in infected teeth after chemo-mechanical preparation.[2] Morphology of root canal system is extraordinarily complex due to this there are chances of leaving behind the residual pulpal tissue and inorganic debris in such areas which are difficult to be instrumented.[8] In relation to this, irrigants have a major fundamental role to play. However, not a single irrigant meet all these requirements individually. Therefore, alternatives have been proposed.[7]

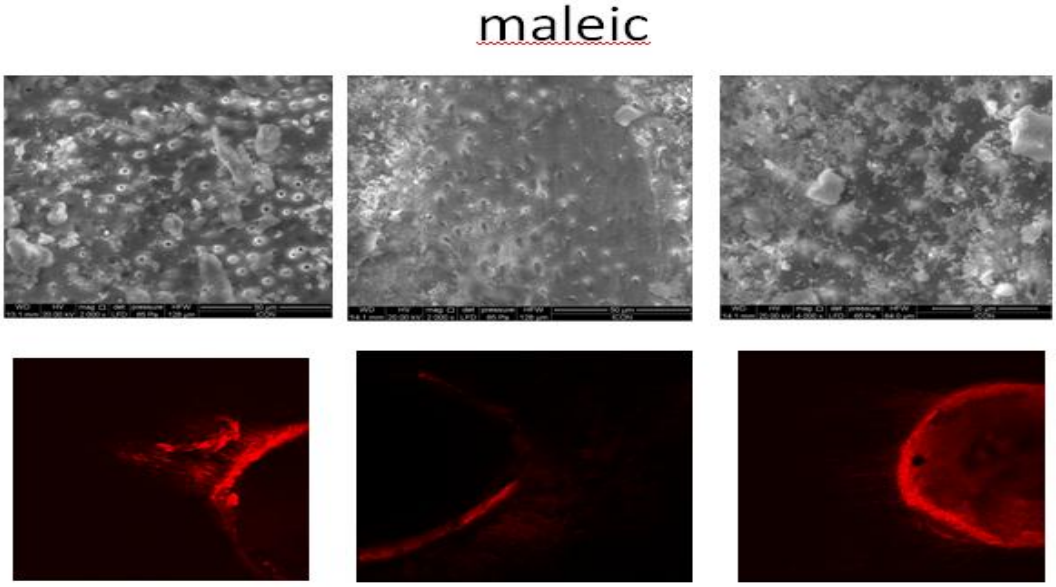
The smear layer associated with cavity preparation and that present within root canal cannot compare directly. *McComb & Smith* were the first to portray about the smear layer present on the surface of mechanically prepared root canal in year 1975.[4,5] It is a very debatable topic whether to keep or get rid of the smear layer from the mechanically prepared root canal. Some authors have focused on its removal whilst others have concluded its effect on microleakage, bacterial infiltration of the dentinal tubules and the adaptation of intracanal medicaments and root canal sealers.[5]

In a concentration of 17%, EDTA has been the most commonly used irrigant since 1957 and an application time of 1–5 minutes.[9] EDTA is a very effective root canal irrigant for complete eradication of smear layer from the instrumented walls.[7] Similar results were originated from this research. EDTA forms soluble calcium chelates when it reacts with calcium ions in dentine.[6] But studies have shown that EDTA is not readily biocompatible, there have been some concerns about the leakage of this irrigant into the periapical tissue and because of that extrusion of EDTA beyond the root canal system should be avoided.[7] In consideration of these facts, a substitute agent for smear layer removal is warranted, and the research for more biodegradable material to replace EDTA is still going on.[9]

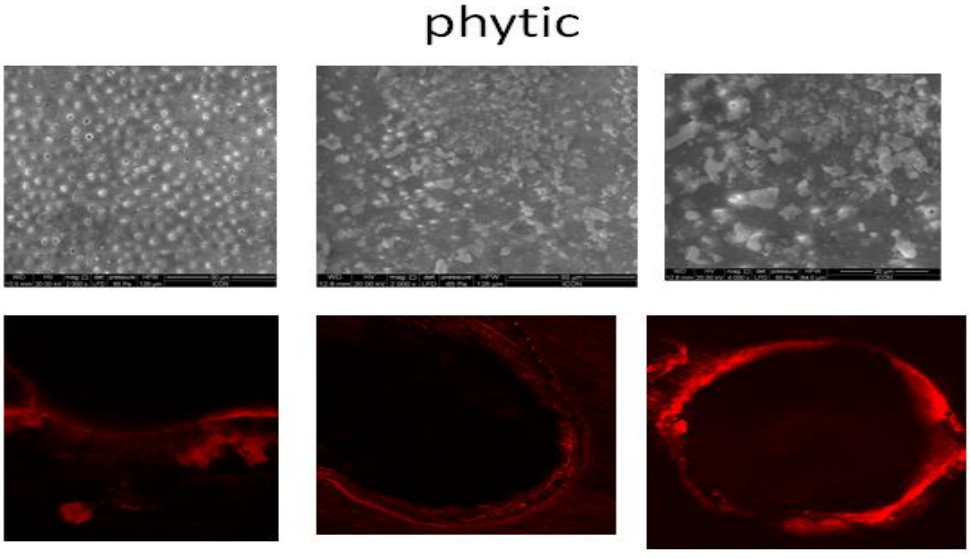


The result of this study indicates that EDTA is efficient eradicating smear layer mainly from the coronal third and middle third as compared to 1% Phytic acid and 7% Maleic acid, its action in apical third is however, very much afflicted. This is in favour with Ciucchi *et al*[22] study who concluded that there was a definite diminish into

the effectiveness of irrigating solution along the apex of the canals. Reason behind this must be the number of dentinal tubules present in apical third of root canal is less, also dentin in apical third is much more sclerosed.[17] Maleic acid in adhesive dentistry used as an acid conditioner as it is a very mild organic acid. At 5% & 7% concentration maleic acid effectively eradicates smear layer from the root canal, on the other hand at 10% or more it is capable of removing minerals and destruction to root canal surfaces.[11] Ballal *et al*. in his study stated that final irrigation using 7% maleic acid for 1min of application time was better when compared to 17% EDTA in elimination of smear layer from the apex of root canal. However, in the present study, maleic acid was not found to be as effective as 17% EDTA.[5, 6] The reason behind this could be, the ability of maleic acid to remove smear layer is less in vital teeth, due to the presence of the dentinal fluid which can apply pressure and so affect its complete removal.[17] However there was no considerable discrepancy seen in smear layer removing ability between 1% Phytic acid and 7% maleic acid, in coronal, middle and apical third of root canal.



Phytic acid moreover known as inositol hexakisphosphate or IP6 is inundated cyclic acid.[15] Phytic acid has great affection to essential natural resources for example, calcium, iron, and zinc, although the binding of phytic acid with calcium iron is pH-dependent.[13] Nassar *et al,* found that, phytic acid has ability to remove the smear layer from NaOCl-treated flat coronal dentin surfaces and stated that it is a less cytotoxic and biocompatible irrigant, when compared with EDTA. In this study also, 1% phytic acid was proved to be successful in eradicating smear layer from coronal as well as middle third of root canals. Root canal surfaces treated with 1% phytic acid were cleaner with more open dentinal tubules when compared with 7% maleic acid. On root canal surfaces, the effect of both 1% phytic acid and 17% EDTA in cleaning the apical third was less than that in the middle third, and this is attributed to the anatomy of the former region.[9]



To determine the effectiveness of different root canal chelating agents to remove the endodontic smear layer scanning electron microscopy (SEM) has been used extensively. [5] Scanning electron microscopy allows an examination of morphologic details of the surfaces of prepared root canal. Other than SEM , the smear layer can also be scored by using digital image analysis. It can overcome the potential of evaluator bias, requires less time, and other parameters of interest like density and average diameter of dentinal tubules can be measured, but SEM was opted in this study because it is a commonly available tool for evaluating the smear layer. [11]

It is very important for the sealer to infiltrate into the dentinal tubules, it has a clinical importance. Mamootil and Messer in 2007, concluded that it is very advantageous to the filling when sealer cements penetrate into dentinal tubules properly. The infiltration of sealer into the dentinal tubules builds the association between the material and the dentine, which, may improve the mechanical bonding of the material by mechanically looking it into place; and that's how microleakage reduces.[2] In the present study AH Plus sealer is used as resin sealer and it has shown deeper penetration into the dentinal tubules other than conventional root canal sealers. Their physical properties such as viscosity, flow, chemical composition, surface tension, solubility, setting and working time make it effective sealer.[21]

CLSM was used in the present study to examine the deepness of penetration of sealers in tubules of radicular dentin. Patel *et al* and Gharib *et al* stated that Confocal microscopy offers a number of benefits other than conventional wide-field optical microscopy and scanning electron microscopy. The advantages of CLSM includes complete removal of background information away from the focal plane, which leads to image disintegration, control depth of field, the ability to collect serial optical sections even from thick specimens.[24,25]

Based on the consequences of the present investigation, sealer penetration was influenced by the irrigant used at the final rinse protocol. Sealer penetration was seen into the dentinal tubules, which indicated elimination of smear layer from the canal; the sealer would not penetrate into the canal, if the smear layer has blocked the tubule openings.[23] In this investigation, sealer penetration was seen higher in 17% EDTA group as compared to 1% phytic acid and 7% maleic acid.

There is a need for further in-vivo research with regards to the smear layer removal with the irrigants used in the study. Also different methods should be tried in-vivo apart from SEM for assessing the effective smear layer removal. Of late, different new sealers have been incorporated in endodontic for three dimensional obturation. Their penetration into the dentinal tubules should be assessed both in vivo and in vitro using different techniques.

CONCLUSION

Within the limitation of the study conclusions drawn from this study is as followed:

1) The findings of the present study suggest that there was a significant difference in the smear layer removal between 17% EDTA and 7% maleic acid, 7% Maleic acid was not found to be as effective as 17% EDTA.

2) When 17% EDTA and 1% Phytic Acid were compared, the difference in the smear layer removal at all the three levels was statistically not significant.

3) 1%Phytic Acid and 7% Maleic acid effectively removed smear layer from coronal, middle and apical levels. And also when their smear layer removing abilities were compared the difference was not statistically significant.

4) AH Plus® sealer after using 17% EDTA has shown the best results among all groups.

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