Effect of Final Irrigating Solutions on Smear Layer Removal and Penetrability of an Epoxy Resin based Sealer into Dentinal Tubules

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ABSTRACT

Introduction: During instrumentation smear layer formation occurs on dentinal wall and for a successful root canal its removal is necessary. Aim of the study was to compare effect of 17% EDTA, MTAD & 18%Etidronic acid (HEBP) + 5% NaOCl, irrigating solutions on smear layer removal and penetrability of AH Plus sealer into dentinal tubules using scanning electron microscopy (SEM).

Material and methods: Sixty single rooted mandibular premolars, were divided into three groups (n=20).Group -I 17% EDTA, Group -II MTAD, Group -III 18%Etidronic acid+ 5% NaOCl. The final rinse was done by 5ml of solution for 2 minutes. Ten samples from each group were evaluated at middle and apical thirds for smear layer removal using SEM. Remaining ten samples of each group were obturated with gutta-percha & AH PLUS sealer and evaluated at 5mm above from the root apex for sealer penetration using SEM.

Results: At middle 3rd no difference in smear layer removal was seen between Group –I and Group -II For apical 3rd Group- II showed better smear layer removal than Group –I and Group- III. Thus both in middle and apical thirds MTAD showed better smear layer removal. For mean sealer penetration Group -II showed a highest depth of sealer penetration i.e, 231.37±18.12µm followed by Group -I 154.95±22.53µm and Group –III 125.99±17.39µm

Conclusion: MTAD is effective in smear layer removal from both middle and apical thirds. Maximum depth of sealer penetration was seen in MTAD group followed by EDTA group and least in Etidronic acid group.

Keywords: AH Plus; EDTA; Etidronic Acid; Sealer penetration; SEM; Smear layer; MTAD

INTRODUCTION

A bacteria tight seal is an important part of the root canal therapy, improper seal between core obturating material and root canal dentine can lead to micro leakage.[1] This may be attributed to the presence of microcrystalline layer of debris known as smear layer.[2] The depth of sealer penetration depends on the extent of smear layer removed which in turn, depends on effectiveness of irrigating solutions used.[3] Thus smear layer removal has a positive impact on the prognosis of the endodontic treatment.

Chemical irrigation is frequently considered as the method of choice to remove smear layer.[4] Sodium hypochlorite (NaOCl) is an irrigant of choice. It has excellent antimicrobial action and capacity to dissolve organic material but it alone is not effective in removing smear layer. NaOCl should be used along with chelating agents like Chitosan, Maleic acid, EDTA (Ethylene Diamine Tetra-acetic Acid), citric acid, MTAD (mixture of tetracycline acid and detergent) and Etidronic acid or HEBP (1- Hydroxethylidene-1,1-Bisphosphonate) that have ability to remove the inorganic phase of smear layer.

EDTA is chelating agent which removes smear layer leading to opening of the dentinal tubules thus increasing the number of lateral canals to be filled. MTAD consists of an antibiotic, a chelating agents and a detergent. It is effective in removing the smear layer along the entire length of the prepared root.

HEBP also known as Etidronic acid, is a biocompatible chelator that has calcium chelating capacity and can be used for effective smear layer removal in combination with sodium hypochlorite without having any short-term reactivity with it.[5] AH Plus is an epoxy –resin based sealer which has significant dentinal wall penetration positive handling characteristics and superior physical properties.

The aim of this study was to evaluate the effectiveness of three final irrigating solutions (17%EDTA, BioPure MTAD, and 18% Etidronic acid+ 5% sodium Hypochlorite) in removing smear layer from the middle and apical third of instrumented canals, and to evaluate penetration depth of AH PLUS sealer within the dentinal tubules

MATERIAL AND METHODS

This study was approved by an institutional ethics committee. Sixty freshly extracted single-rooted caries free mandibular premolar teeth that had a single straight canal and mature apex were collected. Teeth having multiple canals,

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radicular resorption, endodontic filling were excluded. Selected teeth were cleaned and stored in saline until use. Teeth were decoronated and root length was standardized to 16 mm. Instrumentation was performed with a working length of 15 mm using crown-down technique with Mtwo rotary (VDW Gmbh MUNICH GERMANY) Ni-Ti instruments. The canals were prepared to apical size (35, 0.04). Between each change of instrument, the canals were irrigated with 2ml 5.25% sodium hypochlorite (AMD Labs Bangalore India Pvt., Ltd.) with a 30-gauge needle (Canal clean Biodent Co India ltd). After biomechanical preparation canals were irrigated with 5ml of 0.9% saline to minimize interaction of NaOCl with irrigants that will be employed as a final rinse.

60% aqueous solution of Etidronic acid (HEBP) (Code H6773) obtained from a commercial source (Sigma Aldrich) was customized for this study. It was mixed with 30 ml of deionised water to get a resultant weight/volume of 18% of Etidronic acid (pH:10.5) and stored in a dark glass- bottle in at room temperature before use. A fresh 1:1 mixture of 5% NaOCl and 18% Etidronic acid was prepared immediately before the experiments, resulting in a solution that contained both 2.5% NaOCl and 9%Etidronic acid.[5]

Smear layer removal assessment

To simulate in- vivo conditions apical foramen and apical 2mm of teeth were sealed with wax. 60 samples were divided into 3 groups(n=20). The samples in Group-I(n=20) were irrigated with 17% EDTA, Group -II(n=20) irrigated with MTAD and Group –III (n=20) irrigated with 18% Etriodonic acid + 5% NaOCl. The final rinse protocol was 5ml of irrigant for 2 minutes. After final irrigation and drying, ten samples from each Group-I, Group-II, and Group-III (n=30) were split into two halves longitudinally in coronal to apical direction. The sectioning was done with water cooled Mini tome diamond Saw. One split tooth half of the each sectioned samples was dried in the critical point dryer, mounted on a aluminium stub, sputter coated with 20µm of gold and analysed under SEM

Photomicrographs were taken at apical and middle thirds at magnification of 2000X.

Images were scored using a three-score system described by Torabinejad et al.[6]

Score 1 No smear layer, all tubules were clean and open.

Score 2 Moderate smear layer, tubules contained debris.

Score 3 Heavy smear layer that covered the root canal surface and the tubules.

Sealer penetration assessment

In the remaining 10 samples of each group (n=30) Sealer was applied using lentulo spiral and obturation was done using lateral condensation technique. The coronal access of all samples was sealed with temporary restorative material Cavit. The samples were stored for 1 week at 37°C in 100% humidity to ensure setting of the sealer. Each specimen was horizontally sectioned with Mini tome Saw under water cooling at 3mm and 5mm from apex to obtain a 2mm thick slice. Same procedure was followed for sample preparation to be examined under SEM at 500x magnification at the 5mm level of the dentine slice. Maximum depth of sealer penetration in the dentinal tubules was measured in microns, using a calibrated measuring tool, which was incorporated into the SEM control system.

STATISTICAL ANALYSIS

Statistical analysis was performed using [SPSS] 22.0 Armonk, NY: IBM Corp.,

The non-parametric Kruskal Wallis Test followed by Mann Whitney Post hoc Analysis was used to compare mean smear layer removal scores between 3 groups in Middle and Apical 3rd region and Sealer Penetration depth (in micrometres) between all the 3 groups. Wilcoxon Signed Rank Test was used to compare the mean Smear Layer Removal Scores between Middle & Apical 3rd region in each study group. The level of significance was set at P<0.05.

RESULTS

The means and standard deviations of the smear layer scores of the groups are listed in

Table 1. 17% EDTA and MTAD removed smear layer

Region	Groups	Mean	SD	P-Value[a]	Sig. Diff	P-Value[b]
Middle Third	Group -I	1.30	0.48	0.01*	G-I Vs G-II	0.62
	Group -II	1.20	0.42		G-I Vs G-III	0.02*
	Group -III	2.00	0.67		G-IIVs G-III	0.007*
Apical Third	Group –I	1.60	0.52	<0.001*	G-I Vs G-II	0.02*
	Group –II	1.10	0.32		G-I Vs G-III	0.002*
	Group -III	2.60	0.52		G-II Vs G-III	<0.001*

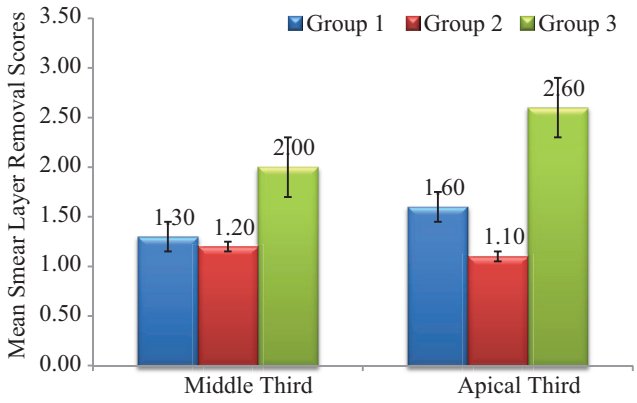
* - Statistically Significant; a. P-Value obtained by Kruskal Wallis test, b. P-Value obtained by Mann Whitney Post hoc Analysis

Table-1: Comparison of mean smear layer removal scores between 03 groups in Middle and Apical 3rd region

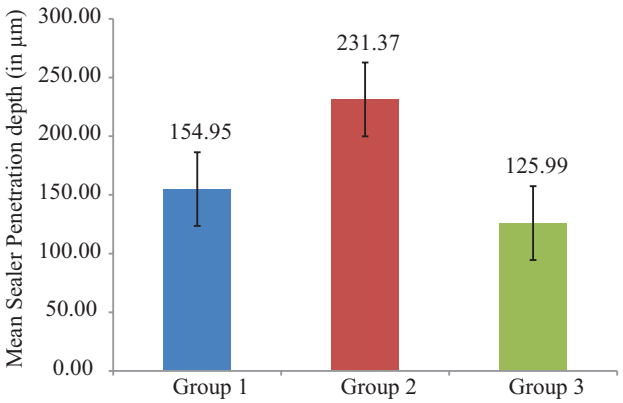
Groups	N	Mean	SD	Max	Min	P-Value[a]	Sig. Diff	P-Value[b]
Group –I	10	154.95	22.53	181.0	122.2	<0.001*	G-I Vs G-II	<0.001*
Group –II	10	231.37	18.12	255.1	206.6		G-I Vs G-III	0.008*
Group -III	10	125.99	17.39	154.0	102.1		G-II Vs G-III	<0.001*

a. P-Value obtained by Kruskal Wallis test, b. P-Value obtained by Mann Whitney Post hoc Analysis

Table-2: Comparison of mean Sealer Penetration depth (in µm) at 5mm above apex level between 03 groups



Graph-1: Comparison of mean smear layer removal scores between 03 groups in Middle and Apical 3rd region



Graph-2: Comparison of mean sealer Penetration depth (in mm) at 5mm above apex level between 03 group

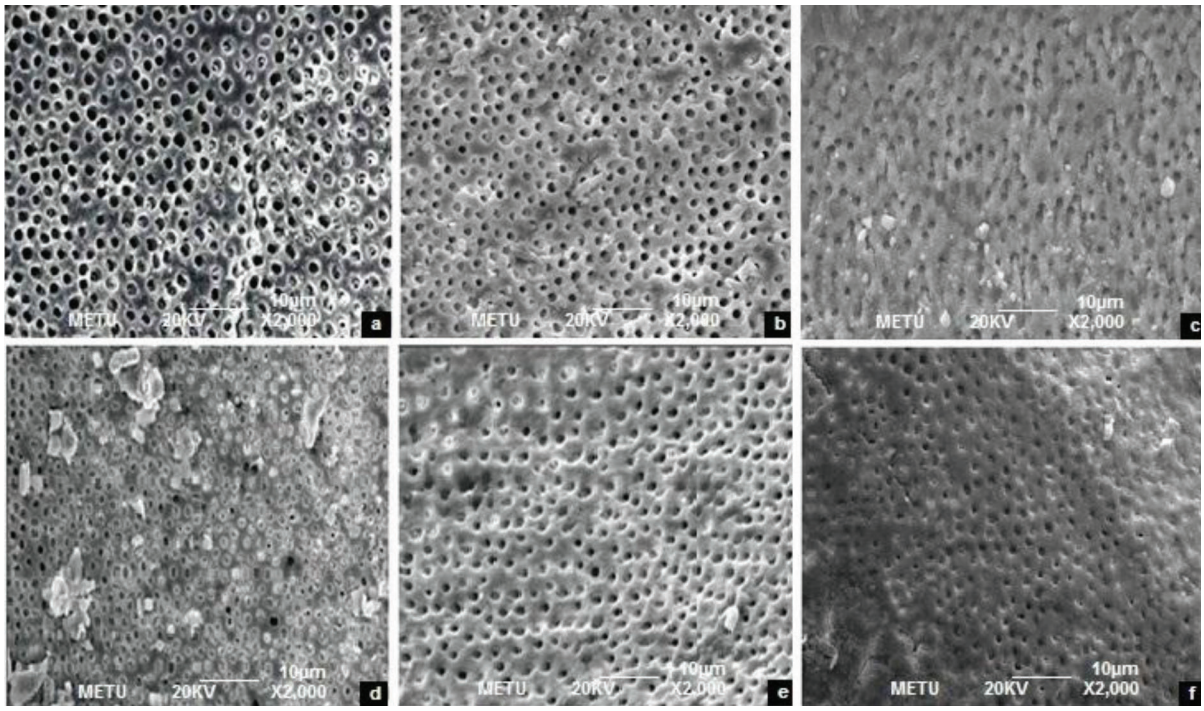


Figure-1 Scanning electron microscope images (x2000) showing smear layer removal from root canal walls after irrigation with (a) 17% EDTA (middle third), (b) MTAD (middle third), (c) 18% Etidronic acid+ 5% NaOCl (middle third), (d) 17% EDTA (apical third), (e) MTAD (apical third) (f) 18% Etidronic acid+ 5% NaOCl (apical third)

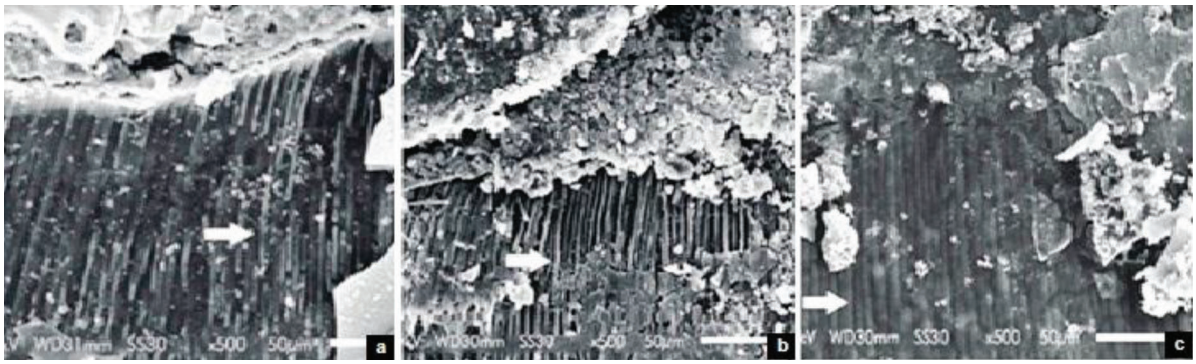


Figure-2: Scanning electron microscope images (x500) showing sealer penetration after smear layer removal using (a) 17% EDTA, (b) MTAD, (C) 18% Etidronic acid + 5% NaOCl

efficiently from middle thirds of the root canal and no statistical significant difference was found between Group-I and Group-II, A statistically significant difference was noted between Group-I and Group –III P=0.02 as well as between Group –II and Group –III P=0.007 (Figure 1a,b,c). For apical thirds only MTAD succeeded in eliminating the smear layer. A statistically significant difference was found between Group –I & Group –II P=0.02, Group –I & Group-

III P=0.002 and Group –II & Group -III P<0.001 (Figure 1d,e,f). (Graph 1)

Table 2 shows depth of sealer penetration in the dentinal tubules at 5mm above the apex level. A significant difference between the groups in depth of sealer penetration after use of different final irrigating solutions (P< 0.001). Group -I irrigated with 17% EDTA showed a maximum depth of sealer penetration at 181µm (Fig2 a). Group –III irrigated with combination of 18% HEBP +5% NaOCl showed Least depth of sealer penetration ie, 154 µm (Fig 2c) Group –II with MTAD a final irrigant showed highest and maximum sealer penetration at 255.1µm (Fig 2b), as compared to Group –I and Group-II (Graph 2)

DISCUSSION

To achieve desirable outcomes of endodontic treatment total removal of smear layer and a deep penetration of root canal sealers into the dentinal tubules is required.[7,8] According to Mamootil and Messer[9] penetration of sealer into dentinal tubules increases the interface between the filling material and dentine thus improving the sealing ability and retention of material by mechanical locking.

Mohsen et al.,[10] suggested 17% EDTA was more effective in the removal of smear layer from coronal and middle third as compared to apical thirds, Gharib et al.,[11] in a similar study found that there was significantly less depth of penetration of sealer in the apical sections than in coronal and middle third. Our study is also in consensus with findings that smear layer removal and sealer penetration is more in middle thirds as compared to apical thirds.

Torabinejad et al.,[12] Mozayeni et al.,[13] and Mancini et al.,[14] showed that MTAD is an effective solution for the removal of the smear layer. The dentinal tubules in the apical third of canals treated with MTAD were significantly cleaner than those treated with EDTA.

In a study done by Kuruvilla et al,.[15], and Tartari et al.,[16] in which the surface of root canals irrigated with Etidronic acid was found to have shown less smear layer removal in the apical third when compared with EDTA. Similar observations were noted in this study.

In Group -I, where 17% EDTA was used as final irrigant, middle thirds showed complete smear layer removal in 6 out of 10 samples (60%). In apical thirds none of the samples showed a complete smear layer removal and moderate smear layer was seen in 7 out of 10 samples. Whereas 3 samples showed presence of heavy smear layer. Smear layer removal action of EDTA can be attributed to its chelating action on the root canal. The moderate smear removal observed in the apical third may be due to incomplete penetration of EDTA in the apical area. Paque et al.,[17] 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 apical third.[18]

In Group -II, where Bio pure MTAD was used, most surfaces in middle, and apical thirds had no smear layer. The middle third showed a complete smear layer removal in 8 of 10 samples ie (80%) and apical third showed complete smear layer removal in 7 out of 10 samples ie (70%). MTAD can dissolve the organic portion of smear. This may be attributed to the synergistic action of citric acid, doxycycline, and detergent present in MTAD. 4.25% citric acid acts a chelator. The detergent (0.5% polysorbate 80) decreases the surface tension and increases the penetrating ability of MTAD.

In Group -III where a combination of 5% NaOCl + 18% Etidronic acid was used 3 samples out of 10 ie 30% showed complete smear layer removal in the middle thirds of root canal. In the apical third moderate smear layer was seen in 4 out of 10 samples and the rest of the samples showed presence of heavy smear layer. Freshly mixed 18% Etidronic acid +5% NaOCl was found to be less efficient in removing the smear layer among all the tested groups. Etidronic acid is a weak chelating agent, does not contain surfactant and its limited efficacy on sclerosed dentine can be a contributing factor.

De-Deus et al.,[19] stated this combination required approximately five minutes to completely remove the smear layer. In our study there was reduced time of contact of combination irrigation solution i.e. for two minutes compared to previous study of five minutes to achieve the desired effect.

On comparison of smear layer removal in the middle third. Group –I (EDTA), Group- II (MTAD) had no statistically significant difference. A significant difference was found in smear layer removal between Group-II (MTAD) and Group III (Etidronic acid + NaOCl) as well as between Group I (EDTA) & Group –III (Etidronic acid + NaOCl).

Significant difference was noted between the three groups in apical thirds. Groups-II (MTAD) showed best smear layer removal and Group –III (Etidronic acid+NaOCl) showed least smear layer removal. These results are in agreement with those of Akhlaghi et al.[20] This could be attributed to the anatomy and lack of penetration of irrigants in the apical portion of the canals.[21] As found by this study MTAD performs better than EDTA and Etidronic acid+NaOCl in apical thirds.

Among the entire root canal sealers AH Plus is known for its adhesive property to root canal wall. With the added advantages like low solubility, low shrinkage and tissue compatibility.[22] Factors that influence sealer penetration are the presence or absence of smear layer, number and diameter of dentinal tubules, presence of water, and properties of the sealer.[23]

According to the results of the study, there was significant difference in the maximum depth of sealer penetration amongst all the three groups. Maximum depth of the sealer penetration were observed in the Group-II irrigated by MTAD (255.1µm) followed by

Group -I EDTA(181.0µm) and Group -III Etidronic acid+ NaOCl groups(154.0µm)

(Table 2).

Better penetration of sealer in MTAD group can be because of the fact that it dissolves both organic and inorganic remnants in the root canal. As the surface area of dentin exposed to the sealer increases, the adhering and penetrating capacity of

sealer also improves and a better seal is expected

The penetration of AH plus into dentinal tubules for EDTA group in our study was found to be 181µm. A similar depth of penetration was observed in study done by A Rouhani et al[24] ie 172.22µm

The penetration depths of AH plus sealers for Group II (MTAD) is 231.37µm which is greater than reported by shokouhinejed et al.,[25] ie 22.07µm. Reason for this discrepancy could be attributed to the different mode of tooth section ie longitudinal sectioning, which introduces possibility of missing areas of deeper penetration because the dentin surrounding the canal cannot be fully observed. Horizontal sectioning followed in the present study eliminates the aforementioned possibility of missing areas to understand the depth of sealer penetration.

In study done by Shenoy et al.,[26] the depth of sealer penetration in EDTA group was 384.8µm and 425 µm in MTAD group, which is different from our study of 154.95µm in EDTA group and 231.37µm in MTAD group. The difference in the values can be because of the type of sealer used ie Acroseal (Septodont) a calcium hydroxide based sealer and method of assessing sealer penetration using confocal laser scanning microscope as compared SEM and use of resin based AH plus sealer in the present study.

In this study we used lateral condensation for obturation whereas in a study done by Saraf et al.,[27] they checked AH plus penetration after obturation using thermoplasticised gutta percha and the mean sealer penetration values being 1011.9 ± 315.752µm.The difference could be because of different obturation techniques. The heated gutta percha pushed with greater condensation pressure could have spread the sealer deep inside the dentinal tubules.

Sealer penetration in Group -III was the least 125.99µm. It can be because of poor smear removal ability of this irrigant resulting in orifices of dentinal tubules blocked by debris explaining the poor sealer penetration in this group.

Further studies are required to evaluate the combined used of 18% Etidronic acid +5% NaOCl on various aspects of smear layer removal and its effect on sealer penetration.

SEM under high magnification shows highly detailed structure of dentinal tubules with the smear layer removed and extent of sealer penetration. However, this method has some limitations such as sectioning of the samples before SEM examination, and possibility of artifacts during preparation.

Further studies are recommended to throw light on use of various sealers and irrigants using different and latest methods of assessing.

CONCLUSION

Within the parameters of this in-vitro study it can be concluded that use of a final irrigating solution has a definite effect on smear layer removal and improves sealer penetration into dentinal tubules. 17% EDTA and MTAD are both effective in smear layer removal from the middle thirds but for apical thirds only MTAD was found to be effective in smear layer removal. Maximum depth of sealer penetration (255.1) µm was seen in samples irrigated with MTAD, followed by EDTA (154.95)µm and Etidronic acid + sodium hypochlorite (125.99)µm

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