Efficacy of MTAD on the root canal surfaces … Sridevi N et al

**Abstract:**
Effective removal of smear layer aids in penetration of intra-canal medicaments into the dentinal tubules and also enhances the adaptation of root canal sealers with the radicular dentin. Various irrigating solutions are used in the effective removal of the smear layer. In this study, distal roots of 60 freshly extracted human mandibular molars were used. Protaper rotary systems were used for cleaning and shaping with Glyde File Prep and NaOCl as a working solution. The samples were divided into three groups and subjected to final irrigation protocol with different irrigants, Group 1: 5% NaOCl for 5 min; Group 2: 17% EDTA at pH 9 for 1 min followed by 5% NaOCl for 1 min; Group 3: MTAD for 5 min. The teeth were split longitudinally into two halves and then these sections were observed under SEM for evaluation of smear layer and erosive effects on underlying dentin structure.

**Results:** Results were tabulated and statistically analyzed using Kruskal–Wallis test and multiple comparison between each group was carried out using Mann–Whitney U-test. Significantly effective removal of smear layer was present in specimens irrigated with 17% EDTA followed by 5% NaOCl and MTAD as compared to the specimens where 5% NaOCl is used as final irrigant. High degree of dentinal erosion was observed in Group 2 where 17% EDTA was used as irrigant.

**Conclusion:** In this study, it was observed that MTAD was effective in removing the smear layer without detrimentally affecting the dentin. The combined use of EDTA followed by NaOCl effectively removed the smear layer but had a detrimental effect on the dentinal wall. Use of NaOCl alone was in effective in removing the smear layer.

**Key Words:** Dentinal tubules, erosion, root canal irrigants, root canal surface, smear layer

**Introduction**
Irrigating solutions enhance bacterial elimination and facilitate removal of organic and inorganic debris from the canal which aid in the success of root canal treatment. Eick et al. had first used scanning electron microscope (SEM) to identify the smear layer on the instrumented root canal surfaces.1 Since then it had been an inevitable tool in evaluating the efficiency of irrigants on root canal surfaces. The commonly used root canal irrigants during cleaning and shaping were saline, sodium hypochlorite, and ethylenediaminetetraacetic acid (EDTA). Moorer and Wesselingk stated that sodium hypochlorite used in concentration varying from 0.5% to 5% has been widely accepted as an adjunct to improve cleaning due to antisepic property and organic tissue dissolving capability,7 never the less it has been observed that NaOCl alone is in effective in the removal of the smear layer.3–5 EDTA forms soluble calcium chelates with inorganic components of the smear layer formed on the root canals thus aid in the removal of them.6 The effective removal of the smear layer demands for combining the efficacy of multiple irrigants as presently the dissolution of organic and inorganic debris cannot be established with one irrigation solution. Consequently, Goldman and associates in 1982 recommended the use of sodium hypochlorite during instrumentation and use of high volume final flush of 17% EDTA followed by sodium hypochlorite to be effective in achieving complete debridement.3,7

Torabinejad et al. claimed that a mixture of citric acid, doxycycline (MTAD and Tween 80) is the most biocompatible material most effective in the disinfection of root canals and removal of smear layer.8 Penetration and efficiency of irrigating solutions have been enhanced by using manual irrigating needles, cannulas or the machine - assisted agitation systems with sonic and ultra-sonic energy sources.9

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Hence, in this study, a comparative evaluation was done on the efficacy of 5% NaOCl, 17% EDTA followed by 5% NaOCl solution and with MTAD as a final rinse on smear layer removal in relation to coronal, middle and apical third of the radicular dentin and also to evaluate the effect of the irrigant on the root dentinal surface using scanning electron microscopic analysis.

Materials and Methodology
Sixty freshly extracted human mandibular molars with fully formed apices were cleaned and then they were stored in normal saline. Distal roots were sectioned with a diamond disc at cemento-enamel junction. Lengths of distal roots were standardized to 12 mm from the root apex. The presence of a single canal in the distal roots was confirmed by a radiograph. After checking the patency with size 10 k file the working length was measured. The apical foramen was closed with modeling wax in order to confine the irrigants within the root canals. Protaper NiTi (Dentsply, Switzerland) rotary systems were used for cleaning and shaping of all the samples with Glyde™ File Prep (Dentsply, USA) and NaOCl as a working solution and enlarged up to size F3 (30) to allow flushing of irrigating solution.

The specimens were randomly divided into three groups based on various irrigants, which were used as a final rinse.

Group 1 (n=20): 5% NaOCl (Prime dental products India) was used for 4 min (at a flow rate of 5 ml/min, 15 ml in total). Then, the canal was irrigated with 10 ml of distilled water.

Group 2 (n=20): 17% EDTA (Roydent Dental products, USA) was used for 1 min (at a flow rate of 1 ml/min, 15 ml in total). The final rinsing was done with 5 ml of 5% NaOCl for 1 min, followed by irrigation with 10 ml of distilled water.

Group 3 (n=20): MTAD (Biopure MTAD, USA) was prepared according to manufacturers instructions. 1 ml of MTAD solution was used for irrigating the canal no. 15 size file is placed into the canal and moved up and down 4-5 times along the course of the canal and left in place for 4 min and then withdrawn. The canal is irrigated with the remaining 4 ml of MTAD gradually for 1 min (at a flow rate of 5 ml/min).

All the roots were dried with paper points, and then longitudinal grooves were made on the buccal and lingual surfaces of the root with a diamond disc. All the roots were split longitudinally into two halves using chisel and mallet. 20 halves were randomly selected and then divided into coronal, middle, and apical. The samples were gold sputtered and observed under SEM (Stereoscan, UK) at ×5000 magnification and photomicrographs were taken.

Scoring of the specimens
The samples were scored for the presence or absence of smear layer on the surface of root canals or in the dentinal tubules on the coronal, middle, and apical portion of each canal according to the following criteria:

0 = No smear layer. No smear layer on the surface of the root canals and all tubules were clean and open.

1 = Moderate smear layer. No smear layer on the surface of the root canals, but tubules contained debris.

2 = Heavy smear layer. Smear layer covered the root canal surface and tubules.

The samples were scored for the presence or absence of a degree of erosion of the dentinal tubules.

0 = No erosion. All tubules looked normal in size and appearance.

1 = Moderate erosion. The peri tubular dentin was eroded.

2 = Severe erosion. The intertubular dentin was destroyed, and tubules were connected with each other.

Scoring was done from the photomicrographs obtained. Results were tabulated and statistically analyzed. Mean values were compared between three-thirds within every group and between groups by Kruskal–Wallis (non-parametric) One-way ANOVA test. Mann–Whitney U-test was used to identify the significant groups. In this study, \( p<0.05 \) was considered as the level of significance.

Results
The SEM pictures (Figures 1-3) and a statistical value recorded clearly showed that:

The smear layer was present in dentinal tubules in the coronal, middle, and apical third in Group 1 where 5% NaOCl, (Figure 1) is used as final irrigant. Significantly effective removal of smear layer was present in Group 2 (17% EDTA followed by 5% NaOCl) (Figure 2), and Group 3 (MTAD) (Figure 3) as compared to Group 1.

High degree of dentinal erosion was observed in SEM pictures of Group 2 (Figure 2) than compared to Group 3.

Within the groups (Table 1) in Group 1 all the three areas showed equal mean values for smear layer and no statistical significance was observed between the three areas. In Group 2 apical third showed a higher mean value for smear layer than other two-thirds and there was no statistical significance was observed between the coronal, middle and apical thirds. In Group 3, all the three areas showed an equal mean value for smear layer and there was no statistical significance was observed between the coronal, middle and apical thirds. In Group 2, (Table 2) middle third showed the higher mean value of erosion.
Between the groups (Table 3) Group 1 showed a highest mean value for smear layer in all the three areas compared to Group 2 and Group 3. Statistical significance was observed for smear layer in Group 1 compared to Group 2 and Group 3. There was no statistical difference for smear layer between Group 2 and Group 3. In all the three areas of Group 2 (Table 4) showed higher mean value for erosion. There was statistical significance observed between Group 2 and Group 3 in all the three areas.

Discussion

Current methods of smear layer removal include chemical, ultrasonic and laser techniques. None of these are totally effective in the complete elimination of smear layer without damaging the underlying dentin structures along the entire course of the pulp space. Yet the most commonly used method for cleaning the root canal is the use of NaOCl alone or in combination with EDTA. Torabinejad et al. developed an irrigating solution MTAD that showed complete removal of smear layer without damaging the underlying dentin when it is used as a final rinse. Hence, in this study, the effect of MTAD on the root canal surfaces was compared with the conventional irrigating solutions.

In Group 1, 5% NaOCl was used as a final irrigant as it is widely accepted as an adjunct to improve cleaning due to its antiseptic property and has organic tissue-dissolving capability. SEM pictures of the dentinal tubules were completely covered by smear layer which clearly demonstrates the inability of 5% NaOCl to remove the smear layer in all the three sections which were also conformed in a number of previous studies. This is explained that its solvent action on organic pulp tissue does not efficiently remove the inorganic portion of the smear layer produced during instrumentation of the root canal. As the dentinal tubules were completely covered with smear layer the underlying alteration in dentin structures could not be identified in this group.

In Group 2 17% EDTA was used for 1 min to remove the smear layer. According to the study by Calt and Serper, the time limit is specific because the rapid action of EDTA in removing the smear layer occurs during initial 1 min, beyond which it affects the dentin structures. This was followed by 5% NaOCl for 1 min which removed the superficial debris. The combination of EDTA and NaOCl has been shown to remove most of the inorganic and organic components of the smear layer, respectively.
Thus, the dentinal erosion in the Group 2 could be explained by the action of EDTA results in the softening of peritubular dentin. EDTA demonstrated an aggressive action with root canal dentin in this study.17

In Group 3, the final rinse was 5 ml MTDA which was used for 5 min according to manufacturer’s instructions to remove the smear layer and to impart an antimicrobial effect.18

Group 3 specimens irrigated with MTAD also demonstrated smear layer removal which was close to that of Group 2 (17% EDTA followed by 5% NaOCL). This could be substantiated by the fact that tetracycline which is a chelating agent has a high pH, thereby resulting in demineralization.8 Further citric acid, another chelating agent also reacts which calcium to form a non-ionic soluble chelate.18 Previous studies have reported that the efficacy of citric acid in terms of dissolution of Ca2+ ions from the dentin were greater than those of EDTA.19 Haznedaroglu reported that lower concentration of citric acid and with lower pH removed the smear layer more efficiently, and this fact suggests that the lower concentration of citric acid present in BioPure MTAD would aid in the removal of smear layer efficiently.20

Hence as far as MTAD is concerned, the chelating action of tetracycline and citric acid were responsible for the removal of smear layer which recorded values close to that EDTA.

Group 3 displayed the least erosive effects on radicular dentin compared to Group 2. Torabinejad et al. observed that when MTAD is followed by a final flush with of NaOCl a chemical reaction occurred between them which resulted in the formation of a brown solution in the canal due to dentinal absorption and release of doxycycline. Further, he demonstrated that SEM examination of the root canal surface treated with these combined regimens showed a higher degree of erosion than when compared to the usage of MTAD alone.10 Hence, in thus study empty MTAD was used alone without NaOCl as a final flush.

Citric acid, which was one of the active ingredients of MTAD, has the ability to enlarge the dentinal tubules by a mechanism referred to as “preferential demineralization” of peritubular dentin. Owing to this action the diameter of tubules on the

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Crumpton et al. have demonstrated that the volume of 17% EDTA does not influence the quality of smear layer removal since this is a function of time;13 hence in this study 5ml of 17% EDTA with a contact time of 1min was used.

SEM pictures in Group 2 and Group 3 revealed the absence of smear layer leaving the dentinal tubules clean and open all the three portions of the canal. In Group 2 17% EDTA acts on the loose dental debris thus transforming into soluble calcium chelate which is washed off using other irrigants.13 At the same time, organic matter is removed by its interaction with HOCl which active principle behind NaOCl.14 Thus the maximum effect of smear layer removal was obtained using EDTA followed by NaOCl as the final rinse.3,7

SEM pictures in Group 2 resulted in high degree of dentinal erosion compared to Group 3. Dentinal tubular orifices where irregular, enlarged and rough in appearance this is due to the removal calcium ions from the dentin which softens the peritubular dentine, increasing the diameter of exposed dentinal tubule.15 Trowbridge and Kim showed that peritubular dentin is highly mineralized and it is harder than intertubular dentin16 but the low collagen content of peritubular dentin makes it more quickly dissolvable than intertubular dentin.16

Table 2: Comparison of mean, SD and test of significance of smear layer values between the groups.

<table>
<thead>
<tr>
<th>Third</th>
<th>NaOCl</th>
<th>EDTA followed by NaOCl</th>
<th>MTAD</th>
<th>P value</th>
<th>Significance between the groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Coronal</td>
<td>2.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.22</td>
<td>0.00</td>
</tr>
<tr>
<td>Middle</td>
<td>2.00</td>
<td>0.00</td>
<td>0.05</td>
<td>0.22</td>
<td>0.00</td>
</tr>
<tr>
<td>Apical</td>
<td>2.00</td>
<td>0.00</td>
<td>0.15</td>
<td>0.37</td>
<td>0.00</td>
</tr>
</tbody>
</table>

ETDA: Ethylenediaminetetraacetic acid, MTAD: Mixture of citric acid, doxycycline, SD: Standard deviation

Table 3: Comparison of mean, SD and test of significance of erosion values at the coronal, middle and apical thirds within and between Groups II and III.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Third</th>
<th>Mean</th>
<th>SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group II</td>
<td>Coronal</td>
<td>1.10</td>
<td>0.31</td>
<td>0.857</td>
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<tr>
<td>Middle</td>
<td>1.15</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical</td>
<td>1.10</td>
<td>0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>Coronal</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apical</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: Standard deviation

Table 4: Comparison of mean, SD and test of significance of erosion values between the groups.

<table>
<thead>
<tr>
<th>Third</th>
<th>EDTA followed by NaOCl</th>
<th>MTAD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>Coronal</td>
<td>1.10</td>
<td>0.31</td>
<td>0.00</td>
</tr>
<tr>
<td>Middle</td>
<td>1.15</td>
<td>0.37</td>
<td>0.00</td>
</tr>
<tr>
<td>Apical</td>
<td>1.10</td>
<td>0.31</td>
<td>0.00</td>
</tr>
</tbody>
</table>

EDTA: Ethylenediaminetetraacetic acid, MTAD: Mixture of citric acid, doxycycline, SD: Standard deviation
surface treated with citric acid was greater but the percentage of total dentin surface area occupied by the openings of the dentinal tubules were fewer which indicates the minimal action of a citric acid in MTAD on the intertubular dentin. Tetracycline, the other ingredient of MTAD, resulted in fewer tubule openings and a layer of organic debris was often found as a partial covering in tetracycline treated surfaces. The diminished potential of action of tetracycline is due to its high pH when compared to the other irrigating solutions like EDTA. This supports that tetracycline seems to be more desirable for use as a root canal surface conditioner rather than a demineralizing agent.

Tween 80, yet another ingredient of MTAD, also known as polysorbate 80, is a non-ionic surfactant and emulsifier derived from sorbitol, which is obtained from various types of fruits. Moreover, they are non-irritating, readily biodegradable and do not possess any action on radicular dentin.

Based on all these facts one can justify that MTAD does not result in erosion of the underlying dentin that was further confirmed by Torabinejad et al.

Conclusion
Within the limitation of this study, it was observed that MTAD was effective in removing the smear layer from dentin surface and does not detrimentally affect the dentin. The combined use of EDTA followed by NaOCl also removed the smear layer effectively but had detrimental effect on the dentinal wall. Use of NaOCl alone was in effect in removing the smear layer.

References