An in vitro comparative evaluation of smear layer removal using NaOCl, EDTA and MTAD solution as a final rinse- a scanning electron microscopic study

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Abstract

Background: During instrumentation of the root canals a smear layer is produced that covers the root canal walls & the opening to the dentinal tubules. Presence of smear layer prevents penetration of intracanal & complete adaptation of obturation materials to the prepared root canal surfaces. Various organic acids, EDTA, ultrasonic instruments have been used to remove the smear layer. Presently a new irrigating solution (MTAD) containing a mixture of a tetracycline isomer, an acid and a detergent is used in instrumented root canals for removal of smear layer. Aims: To evaluate and compare effects of NaOCl, EDTA and MTAD on removal of smear layer and their effects on dentinal tubules. Methods: Sixty extracted single rooted teeth were divided into four groups and root canals were prepared with hybrid technique, using different solutions; sterile distilled water, 5.25% NaOCl, 17% EDTA and MTAD. The teeth were then split and the canal surfaces were viewed under SEM (Scanning Electron Microscopy) for removal of smear layer. Results: The results showed that NaOCl failed to remove the smear layer. Smear removing ability of MTAD was significantly better than EDTA in the apical third. Conclusions: MTAD is an effective solution for the removal of smear layer when used as a final rinse without changing the structure of the dentinal tubules.

Key words: Smear layer; EDTA; MTAD.
**Introduction**

Thorough debridement of the root canal system is essential for successful endodontic treatment (1). However, removal of dentin always gives rise to the formation of a thin layer covering the entire root canal wall. This layer known as the ‘smear layer’ was first observed by McComb and Smith in 1975. This thick organomineral layer contains organic material derived from necrotic and/or vital pulp tissue, odontoblastic processes, bacteria and blood cells (2). Although there is some controversy regarding the desirability of retaining the smear layer in adhesive dentistry, in endodontics, its removal is considered to be advantageous and highly desirable (3).

Historically, countless compounds in aqueous solutions have been suggested as root canal irrigants including inert substances such as saline or acids like citric acid, lactic acid, tannic, polyacrylic acid to chelator solutions like Bis-dequalinium acetate, EDTA, broad spectrum antibiotics like tetracyclines and chlorine compounds like sodium hypochlorite.

However several invitro and in vivo studies have shown that NaOCl does not effectively remove the smear layer. The most widely used chelating agent for the removal of smear layer is ethylene diamine tetraacetic acid (EDTA) and it was initially used in root canal therapy by Nygaard-Ostby in 1957. EDTA is mainly used as a final flush at a concentration varying from 15% to 17% and as a disodium salt solution. It removes minerals from the dentinal wall by chelation (4).

MTAD has been reported to be effective in removing endodontic smear layers, eliminating microbes that are resistant to conventional endodontic irrigants and dressings, and providing sustained antimicrobial activity through the affinity of doxycycline to bind to dental hard tissues (6). Thus, an invitro study was carried out to evaluate the effect of various irrigants on removal of smear layer & dentinal erosion in instrumented root canals.

**Material and methods**

Sixty single rooted permanent maxillary anterior teeth with complete root formation having no coronal restorations or root canal treatment done were selected. Conventional access cavities were prepared and canal preparation was done using the step down-step back technique or the hybrid technique. The apical preparation of each tooth was carried out to a size 30 file. 5.25% NaOCl was used as an intracanal irrigant during instrumentation except in control group (Group I) where sterile distilled water was used. The teeth were then divided into four main groups of 15 teeth each. Then a final rinse was carried out with following solutions.

I. Sterile distilled water (positive control)

II. 5.25% NaOCl

III. 17% EDTA

IV. MTAD, a new solution containing a mixture of a tetracycline isomer (doxycycline), an acid (citric acid), and a detergent (Tween – 80)

After instrumentation each canal was initially irrigated with 1ml of one of the solutions. To ensure a uniform & direct contact of each irrigant with the root canal walls, a no. 15K file was wrapped with cotton and soaked with one of the solutions and placed to the working length for 4min. Then each canal was irrigated with 4 ml of one of the experimental or control solutions as a final rinse. The total exposure time to the final solution was approximately 5min.
Irrigants on smear layer removal

After instrumentation and flushing, samples were split into 2 halves. Each sample was placed in a 2% glutaraldehyde solution for 24hours. Then the specimens were dried with ascending concentrations of ethanol solution at 30, 50, 75, 90, 95, 99.9% concentration. Specimens were placed in a dessicator for at least 24hrs. Samples were then mounted on aluminum stubs and coated with 25µm thick layer of gold palladium and viewed under a scanning electron microscope at x5000 magnification. Photomicrographs were obtained from coronal, middle & apical levels of each root canal and were qualitatively evaluated according to the following criteria (7):

1 = No smear layer. No smear layer on the surface of the root canals; all tubules were clean and open.
2 = Moderate smear layer. No smear layer on the surface of root canal, but tubules contained debris.
3 = Heavy smear layer. Smear layer covered the root canal surface and the tubules.

Statistical analysis was performed using Mann-Whitney test to assess the significant difference between the groups for mean scores of smear removal.

Results

Photomicrographs obtained from coronal, middle and apical levels of each root canal were qualitatively evaluated which depicted the following observations
Figure 1: Specimen surface at the coronal third irrigated with Distilled water (Group I) Smear layer evident
Figure 2: Specimen surface at the coronal third irrigated with 5.25% NaOCl (Group II) Smear layer evident. Hardly any tubules are visible.
Figure 3: Specimen surface at the coronal third irrigated with EDTA (Group III) Complete removal of smear layer achieved. Erosion of peritubular dentin seen.
Figure 4: Specimen surface at the middle third irrigated with EDTA (Group III).

Complete removal of smear layer achieved, erosion of peritubular dentin seen.
Figure 5: Specimen surface at the apical third irrigated with EDTA (Group III), root surface is clean but smear layer is present in tubules
Figure 6: Specimen surface at the coronal third irrigated with MTAD (Group IV). Complete removal of smear layer achieved.
Figure 7: Specimen surface at the middle third irrigated with MTAD (Group IV) Surface appears clean. Opening of the tubules are free of debris.
Figure 8: Specimen surface at the apical third irrigated with MTAD (Group IV), complete removal of smear layer achieved.

Based on the comparison of mean scores for smear layer removal between distilled water (I) and sodium hypochlorite (II), a non-significant difference (p>0.05) was observed between groups I and II for coronal, middle and apical thirds of root canals as depicted in table. It is evident from the table that a highly significant difference
Irrigants on smear layer removal

(P<0.01) was observed between group I and group III along with group I and group IV for coronal, middle and apical thirds of the root canal.

**Table 1**: Comparison of mean scores for smear layer removal between the groups

<table>
<thead>
<tr>
<th>Categories</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Distilled water (n = 15)</td>
<td>5.25% NaOCl (n = 15)</td>
<td>17% EDTA (n = 15)</td>
</tr>
<tr>
<td>Coronal</td>
<td>3.0±0.0</td>
<td>3.0±0.0‡∞</td>
<td>1.1±0.3*</td>
</tr>
<tr>
<td>Middle</td>
<td>3.0±0.0</td>
<td>3.0±0.0‡∞</td>
<td>1.1±0.4*</td>
</tr>
<tr>
<td>Apical</td>
<td>3.0±0.0</td>
<td>3.0±0.0‡∞</td>
<td>2.0±0.0*#</td>
</tr>
</tbody>
</table>

* Significantly different (p<0.01) from group I
‡ Significantly different (p<0.01) from group I
∞ Significantly different (p<0.01) from group III
# Significantly different (p<0.01) from group IV

Highly significant differences were observed (p<0.01) when comparing the mean scores for smear layer removal between groups II and III in addition to group II and group IV for coronal, middle and apical thirds. Highly significant difference was noted (p<0.01) at apical third for smear removal between the groups III and IV.

**Discussion**

Studies have shown that the removal of smear layer promotes dentin permeability allowing and producing greater penetration of filling material into lateral canals & dentinal tubules. Unfortunately, no irrigating solution is capable of acting simultaneously on the organic and inorganic elements of the smear layer without affecting the root canal wall (8).

Recently, Torabinejad and associates introduced an irrigant (MTAD) which is a mixture of tetracycline isomer, an acid, and a detergent for the removal of smear layer (9). The present study was conducted to evaluate and compare the efficacy of various irrigants including MTAD to remove the smear layer and debris at 3 different levels of root canal.

In group I, sterile distilled water was totally ineffective in removal of smear layer and was thus used as a positive control. Specimens irrigated with 5.25% NaOCl showed presence of an irregular, granular, smear layer under SEM. These findings are consistent with the findings of the previous studies (7, 10-12). Under SEM, the specimens irrigated with 5.25% NaOCl followed by 17% EDTA solution revealed that the surfaces of the root canals and the dentinal tubules in the coronal and middle thirds were seen free of smear layer and debris which is in agreement with a previous study (12). Baumgartner and Mader (11) observed that alternating the use of EDTA and NaOCl is effective method for smear layer removal.

When the specimen surface at the apical third irrigated with EDTA was viewed, root surface was found to be clean with smear layer present in the tubules. Decline in the smear layer removal efficacy of EDTA along the apical third of the root canals might be attributable to inadequate volume and or penetration of the solution in narrow portion of the canal during instrumentation. These findings are in agreement with the findings of O’Connel and Morgan et al (13) and Torabinejad et al (7). Severe erosion was noted in coronal and middle thirds of the specimens of this group.

MTAD has the ability to remove organic and inorganic substances from the surfaces of roots. Its ability to bind to dentin and be released gradually over time, is facilitated by the presence of citric acid and the presence of a detergent Tween-80 that aids its propensity to diffuse into the root canal and the dentinal tubules. Reduction of the surface tension by detergents has been shown to improve the penetrating ability of the irrigating solutions and increasing the capability of removing smear layer from the apical 3rd of canals (10, 14).

Under SEM the specimens irrigated with 5.25% NaOCl followed by MTAD solution revealed complete removal of smear layer and patent dentinal tubules in the
Irrigants on smear layer removal

coronal, middle and even in the apical 3rd of root canals. In addition, no significant dentinal erosion was observed in the coronal and middle thirds of these specimens. These results are consistent with the findings of past studies (7, 9, 15).

Conclusions

Based on the results of this study, it seems that MTAD is an effective solution for the removal of smear layer when used as a final rinse. It does not significantly change the structure of the dentinal tubules when used in conjunction with NaOCl as a root canal irrigant compared with EDTA when used as a final irrigant. However, further studies and clinical trials are necessary to substantiate the results of this study.

References