The Effect of EDTA and Citric Acid on Smear Layer Removal of Mesial Canals of First Mandibular Molars, A Scanning Electron Microscopic Study.

A. Khademi DDS. MS*, M. Feizianfard DDS. MS*

Abstract

Background: The purpose of this in vitro study was to determine the effect of EDTA and citric acid on smear layer removal in different regions of root canals.

Methods: In this study, mesial roots of 48 freshly extracted human mature mandibular first molar teeth with curved mesial roots of about 15-45 degrees and lengths of 20-23 mm were used. Instrumentation was done using the crown down technique by hand and rotary filing. Irrigant used during instrumentation was NaOCl. The teeth were divided in three groups. The mesial canals of teeth were irrigated by 17% EDTA in group I, 7% citric acid in group II and 5.25% NaOCl in group III as the control group. Then, the mesial roots were split into two parts and studied under scanning electron microscopy.

Results: The degree of cleanliness by 17% EDTA and 7% citric acid were 96.55% and 95% respectively. Although both solutions seem to be appropriate, their difference was statistically significant (P<0.05) and EDTA proved better than citric acid especially in middle and apical thirds of canals. The smear layer removal in apical area was less than that in other areas and was maximum in the middle third. However, the removal of smear layer in apical area was acceptable in both groups.

Conclusion: It seems that use of both 17% EDTA and 7% citric acid offer desired results and they can remove smear layer from narrow and curved canals especially from apical region.

Keywords: EDTA, citric acid, smear layer, irrigation

Thorough debridment of the root canal system is essential for successful endodontic treatment. Canal preparation should not only remove the pulp tissue, necrotic debris, microorganisms, and affected dentin from the treated tooth, but also prepare it to receive the filling material that will seal the apical foramen. However, removal of dentin will always give rise to formation of a thin smear layer covering the entire root canal wall. McComb and Smith first demonstrated this layer in instrumented root canals.

The smear layer consists of organic and inorganic substances, including fragments of odontoblastic processes, microorganisms, and necrotic materials.

Presence of this smear layer prevents penetration of intracanal medication into the irregularities of the root canal system and the dentinal tubule and also prevents complete adaptation of obturating materials to the prepared root canal surface. In an in vitro study, Orstavik and Haapasalo showed the importance of the smear layer removal and the presence of patent dental tubules for decreasing the time necessary to achieve the disinfecting effect of intracanal medications. Bystrom and Sundqvist also showed that the presence of a smear layer can inhibit or significantly delay the penetration of antimicrobial agents such as intracanal irrigants and medications into the dentinal tubules.

Although the effect of smear layer removal on a successful root canal treatment is still controversial, it seems that its removal is better than keeping it.

Smear layer removal requires a combination of NaOCl (an organic solvent) and acids such as, citric acid, tannic, polyacrylic, or phosphoric acid, or chelating agents such as EDTA or REDTA for the removal of the inorganic part.

*Associate Professor, Department of Endodontics, Isfahan University of Medical Sciences, Isfahan, Iran
Correspondence to: Dr. Abbassali Khademi, Faculty of Dentistry, Isfahan University of Medical Sciences and Health Services, Isfahan, Iran.
Goldman et al\textsuperscript{11} and Yamada et al\textsuperscript{12} found that the use of a copious final flush with 17\% EDTA, followed by sodium hypochlorite (NaOCL) effectively removes the smear layer. In addition to acids, tetracycline Hcl has been recommended to remove the smear layer from the surfaces of instrumented canals and root end cavity preparations\textsuperscript{13, 14}.

Different studies have shown that EDTA and citric acid can remove the smear layer, as a result dentinal tubules are opened and medicaments and filling material can penetrate better. However, only a few studies have compared these two different materials. The objective of this study was to compare the ability of these two irrigants in smear layer removal from mesial canals of first mandibular molar teeth, which are the most difficult canals for penetration of irrigants, especially in apical regions.

In this study, the crowns were not cut in order to carry out this research under clinical conditions.

**Materials and Methods**

Forty-eight freshly extracted human mature mandibular first molar teeth with intact crown were used. Their lengths were between 20-23 mm and their mesial roots had the curvature of 15-45 degrees (according to Schneider classification\textsuperscript{15}).

After preparing a conventional access preparation for each canal, a k-type file (size 08 or 10) was used to determine the working length by penetrating the apical foramen and pulling back into the clinically visible apical foramen. The working length of mesial canals were 20 – 23 mm. The instrumentation of mesial canals were performed primarily by hand filing with nickel titanium k-type files (Dentsply Maillefer, Ballaigues, Switzerland\textsuperscript{16} up to number 20, followed by flaring the cervical region by gates glidden drill (Mani Japan) numbers 2, 3, 4 via crown down technique and finally filing and flaring by rotary system of profile ( Dentsply Maillefer, Ballaigues, Switzerland )\textsuperscript{16} in the following order:

\begin{itemize}
  \item 20 (04), 20 (06), 25 (04), 25 (06), 30 (06)
\end{itemize}

Between each instrument, patency file and 1 ml 5.25\% NaOCl as irrigant were used. The apical foramen of each canal was enlarged to the size of 30 (06) rotary profile. After instrumentation, the teeth were divided into 3 groups and final irrigation was done by a 27 – gauge needle syringe, these include:

- **Group 1**: 22 teeth: at first irrigation with 5 ml of 17\% EDTA which was buffered with PH=7.8 for 5 minutes, then by 5 ml of 5.25\% NaOCl and finally by 5 ml distilled water.
- **Group 2**: 22 teeth: at first irrigation with 5 ml of 7\% citric acid for 5 minutes, then by 5 ml of 5.25\% NaOCl and finally by 5 ml distilled water.
- **Group 3** ( control ): 4 teeth, In this group the canals were irrigated only with 5ml of 5.25\% NaOCl and then by 5 ml distilled water.

Final irrigation with distilled water was done to terminate any irrigating activity and also for prevention of any sedimentation such as citrate crystals.

After irrigation, first a small piece of cotton was inserted in mesial orifices to avoid entrance of dentinal chips into the canals during root splitting. The mesial roots were split longitudinally in buccolingual direction and half of each root was placed in a 2 \% glutaraldehyde solution for 24 hours and the other half of each root was discarded.

The fixed specimens were rinsed three times with a sodium cacodylate buffered solution (0.1 M, PH 7.2) incubated in osmium tetroxide for 1 hour, dehydrated with ascending concentration of ethyl alcohol (30\% – 100\%), and placed in a desiccator for at least 24 hours.

Each specimen was mounted on an aluminum stub and coated with 25 \textmu m of gold palladium and was examined under a scanning electron microscope.

The magnification of all the photomicrographs were \times 2500 and the surface unit of all photomicrographs were the same.

The specimens were then coded based on the final irrigation solution. In a blind manner, two investigators scored the presence or absence of smear layer on the surface of the root canal or in the dentinal tubules and cleanliness percentage at the coronal, middle, and apical portion of each canal according to the following criteria:

1- The number of opened tubules = The number of all tubules – The number of closed tubules.
2- Cleanliness percentage = (The number of opened tubule / the number of all tubules) \times 100.

The data were analyzed statistically with Student t, ANOVA and Duncan tests.
Results
The results obtained from this study are summarized in Table 1 and Figures 1-9 show the scanning electron photomicrographs of control and irrigated samples by 17% EDTA and 7% citric acid and NaOCl 5.25%.

According to this study, compared to 7% citric acid, 17% EDTA removed the smear layer more effectively. The superiority of EDTA over citric acid was especially observed in middle and apical regions, although, in cervical region, these two solutions showed no significant differences. It was also noted that the middle third had the highest degree of cleanliness compared to other areas, while the apical third had the lowest.

Discussion
In this study, the degree of cleanliness with 17% EDTA was higher than 7% citric acid. Although the difference between these two materials was statistically significant, from the clinical standpoint, both of them seem to be acceptable. The results of this research were somehow similar to results obtained in previous studies12, 17, 18. Yamada et al12 reported that 17% EDTA is more effective than 25% citric acid; In their study which was carried out on single rooted teeth, neither statistical comparison nor quantity evaluation was used and the samples were only evaluated qualitatively. On the other hand, the hand instrumentation and Gates Glidden drilling were used in canal preparation.

Takeda et al18 observed no differences between 17% EDTA and 6% citric acid. The preparation method on canals in their study was also hand instrumentation and a four grade scale (0-3) was used to evaluate photomicrographs.

SceIza et al17 reported no differences between EDTA-T (a combination of 17% EDTA and tergentol) and 10% citric acid. The study was done on straight single rooted teeth and the canals were prepared only by hand instrumentation. Lenarda et al19 made a comparison between 1 mol/L citric acid and 15% EDTA. They prepared some of the samples by hand instrumentation and others by rotary profile system. They believed that citric acid was more effective than EDTA, especially in samples that were prepared via profile rotary system and EDTA was more effective in samples that were prepared by hand instrumentation. Their study showed that the instrumentation method could change the ability of smear layer removal with different solutions.

The amount of smear layer removal in different regions of canals in our study showed no significant differences between 17% EDTA and 7% citric acid in cervical region. 17% EDTA should be preferred to 7% citric acid in the middle and apical regions.

It was observed that the 17% EDTA cleaned the middle region better than in other canal regions, whereas 7% citric acid had cleaned the cervical and middle regions better than the apical region.

In our study the apical region had the least amount of cleanliness. These results are completely different from the result obtained by Yamada et al12 who had found the apical region as the cleanest region; Besides, our finding does not accord with SceIza’s study17 who found that the middle and apical regions are the same. Takada et al18 also reached the same conclusion.

Taking into account that apical region is the most important and most difficult region in smear layer removal, the degree of cleanliness obtained with both solutions (95.9% EDTA and 92.5% citric acid) was highly satisfactory. This shows that the method of canal preparation and final irrigation in this study has been effective in smear layer removal. If we could increase the penetration of irrigation into the apical region, we could obtain more cleanliness in this area. For example the use of a more slender needle, a miniator brush or detergents for decreasing the surface tension may give a much better result.

Based on the results of this study, using both EDTA or citric acid in smear layer removal can provide satisfactory results; We suggest that more clinical studies in this field should be planned in future.

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Table 1. The amount of smear layer removal (on percentage) in two groups: 17% EDTA and 7% citric acid. Data are mean ± SD.

<table>
<thead>
<tr>
<th>Region</th>
<th>Scanned Regions</th>
<th>17% EDTA</th>
<th>7% Citric acid</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical</td>
<td>88</td>
<td>95.87 ± 7.00</td>
<td>95.44 ± 3.57</td>
<td>0.657</td>
</tr>
<tr>
<td>Middle</td>
<td>88</td>
<td>97.9 ± 2.36</td>
<td>96.03 ± 4.69</td>
<td>0.031</td>
</tr>
<tr>
<td>Apical</td>
<td>88</td>
<td>95.87 ± 4.52</td>
<td>92.52 ± 6.11</td>
<td>0.008</td>
</tr>
<tr>
<td>Total</td>
<td>264</td>
<td>96.55 ± 5.05</td>
<td>95 ± 5.17</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Figure 1. Photomicrograph of cervical region of canal irrigated with 5.25% NaOCl (control sample) showing the presence of the smear layer.

Figure 2. Photomicrograph of middle region of canal irrigated with 5.25% NaOCl (control sample) showing the presence of the smear layer.
Figure 3. Photomicrograph of apical region of canal irrigated with 5.25% NaOCl (control sample) showing the presence of the smear layer.

Figure 4. Photomicrograph of cervical region of canal irrigated with 17% EDTA + 5.25% NaOCl showing the removal of the smear layer and open dentinal tubule.
Figure 5. Photomicrograph of middle region of canal irrigated with 17% EDTA + 5.25% NaOCl showing the removal of the smear layer and open dentinal tubule.

Figure 6. Photomicrograph of apical region of canal irrigated with 17% EDTA + 5.25% NaOCl showing the removal of the smear layer and open dentinal tubule.
Figure 7. Photomicrograph of cervical region of canal irrigated with 7% citric acid + 5.25% NaOCl showing the removal of the smear layer and open dentinal tubule.

Figure 8. Photomicrograph of middle region of canal irrigated with 7% citric acid + 5.25% NaOCl showing the removal of the smear layer and open dentinal tubule.
Figure 9. Photomicrograph of apical region of canal irrigated with 7% citric acid + 5.25% NaOCl showing the removal of the smear layer and open dentinal tubule.

References