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Abstract:
Introduction: The purpose of the present study was to determine the effect of 17% Ethylene, di-amine, tetra-acetic acid (EDTA), 6% phosphoric acid and Erbium: Yttrium, Aluminum, Garnet (Er:YAG) Laser in removing the smear layer by scanning electron microscopy.

Methods: In this study, 80 single-rooted human teeth were selected. Instrumentation was done by use of hand files and step-back technique up to file #40 at apical and file #80 at coronal area. During instrumentation, 1ml of 5.25% sodium hypochlorite (NaOCl) was used as irrigation between each file. The teeth were randomly divided into four groups with different methods of smear removal.17% Ethylene, di-amine, tetra-acetic acid was used in group 1, 6% Phosphoric acid in group 2, Erbium: Yttrium, Aluminum, Garnet laser in group 3, and no intervention in group 4 (as control). Roots were then longitudinally sectioned and prepared for scanning electron micrograph in cervical, middle and apical areas. Data were analyzed by Kruskal-Walis, Mann-Whitney, Friedman and Wilcoxson tests (P<0.05).

Results: The results showed a significant difference in smear layer removal between three methods in favor of the EDTA (P<0.001) and Phosphoric groups (P<0.001) with no significant difference between them (P=0.49). Although Er:YAG laser showed some limited ability to remove the smear layer, the effectiveness was not significantly different from the control group (P=0.157).

Conclusion: Based on the findings of this study, EDTA and Phosphoric acid were effective methods to remove smear layer from the root canal walls but Er:YAG laser showed less efficacy compared to the other experimental groups.

Keywords: layer, smear; EDTA; Phosphoric acid; Er:YAG.

Introduction

The success of endodontic treatment depends on cleaning and shaping of root canal system, technique and quality of instrumentation and disinfection, followed by the three-dimensional obturation of this space. Many studies have demonstrated that different methods of canal preparation, either using manual or
Removal of Smear Layer by Endodontic Irrigation Solutions and Er:YAG Laser

rotary instruments will produce a smear layer that covers the dentinal tubules and instrumented walls. McComb and Smith were among the first researchers to describe the smear layer on the surface of instrumented root canals (1). This layer contains organic and inorganic substances, fragments of odontoblastic process, microorganisms and necrotic materials (1-6). Eick et al showed that the smear layer thickness ranges in size from less than 0.5 to 15µm (7). There is still a question whether smear layer should be removed or not (8). The presence of a smear layer can prevent the penetration of disinfectants and medicament into the dentinal tubules (9). Different irrigation solutions have been used to remove the smear layer. Sodium hypochlorite (NaOCl) in different concentration of 1-5.25% is a common irrigant solution widely used because of its bactericidal properties and ability to dissolve organic tissues (10, 11). But, it has never been proved that NaOCl is able to remove the smear layer (12). Decalcifying solutions such as phosphoric acid, citric acid, Ethylene diamine, tetra-acetic acid (EDTA) and Maleic acid have been reported suitable in removing the smear layer (5, 10, 13-17). Pashely has reported that phosphoric acid in different concentration of 30 to 65% for 15sec has the ability to remove smear layer and was able to widen the dentinal tubules. Some studies have shown that 5% phosphoric acid in combination with 2.5% NaOCl have been effective in smear layer removal and have the ability to decalcify dentin (16, 17).

Wayman et al showed that the smear layer in canals, treated by 10%, 25%, 50% citric acid solutions can be removed totally, but best result can be achieved by 10% citric acid and 2.5% NaOCl followed by a final flush with 10% citric acid. Final canal irrigation with EDTA and NaOCl has been recommended for removal of organic and inorganic components of the smear layer by several investigators (13).

Laser has shown promising results in endodontic treatment, and its potential application is being explored by a number of investigators (18-23). Harashima et al have observed that irradiation of Argon laser produces melted dentine surface and vaporizes debris and pulpal tissue remnants on the instrumented root canals (20). Onal et al showed that carbon dioxide (CO$_2$) laser is able to remove organic tissue in the root canals to open dentinal tubules and is able to melt and fuse hydroxyl apatite crystals (21). Dedrich et al, used Neodymium-Doped: Yttrium, Aluminum, Garnet (Nd:YAG) laser on root canal walls and showed melted, recrystallized and glazed surfaces (22). Takahashi et al observed a significant removal of debris and smear layer after irradiation with Erbium: Yttrium, Aluminum, Garnet (Er:YAG) laser (23). Takeda et al have also shown that the Er:YAG laser is effective on removal of debris and smear layer and exposes the orifices of dentinal tubules by photothermal ablation mechanism (18).

The purpose of this in vitro study was to compare the effect of 17% EDTA, 6% Phosphoric acid and Er:YAG laser irradiation on removing the smear layer from the prepared root canal walls.

Methods

Eighty recently extracted human mature permanent single-rooted non-carious teeth, which were extracted for a periodontal disease or orthodontics treatment purposes, were selected for this study. The teeth were radiographed to confirm the absence of complications or existence of an anomaly in the root canal anatomy. A conventional access cavity was prepared for each tooth. The working length of each canal was determined visually 1mm shorter than apical foramen with a size 15 k-type file (Mani, Touchi, Japan). Apex was then covered by soft wax to block the apical foramen during the irrigation. The canal was prepared by step-back technique to the working length up to file #40 at apical and file #80 at coronal area by hand instrumentation. The root canal was irrigated by 1ml of 5.25% NaOCl between each instrument with a 30-gauge needle (Soha, Tehran, Iran) as apically as possible without binding. Crown was removed using diamond disk with non-stop machine (Krupp dental-denta rapid-Germany) and 13 mm of root length remained. Finally, the teeth were randomly divided into 4 experimental groups as followed:

- **Group 1 (n=25):** The root canals were irrigated for 1 minute with 5ml of 17% EDTA (PH: 7.8) (Merck-, Darmstadt, Germany) followed by irrigation of 5ml of NaOCl and 5ml of distilled water as the final rinse.
- **Group 2 (n=25):** The root canals were irrigated for 1 minute with 5ml of 6% phosphoric acid (Merck-, Darmstadt, Germany) followed by the irrigation of 5ml of NaOCl and final rinse of 5ml distilled water to terminate any irrigating activity and preventing any sedimentation.
- **Group 3 (n=25):** The specimens were irradiated with Er:YAG laser ($\lambda=$2,940nm), 300µ fiber tip with a power of 1W, 100 mJ of energy/pulse, frequency of 10Hz and pulse duration of 250µs in SP mode.
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(Fotona, Fidelis plus, Ljubljana, Slovenia) for the total exposure of 15 seconds per canal. The fiber tip was introduced to the canal and was in contact with the canal walls. The laser was activated for 3 seconds at the apex area and four additional exposures of laser were performed, each with 3 seconds intervals with spiral moves from apical to coronal area (Total irradiation was 5 times). A water coolant spray was used during irradiation (18, 19).

Five teeth served as control. In that group, the root canals were irrigated only by 5ml of 5.25% NaOCl followed by final rinse of 5ml distilled water.

After final rinse in each group, the canals were dried by absorbent paper points and then were filled with paper points, orifices were closed by cotton pallet to prevent any contamination with dentinal particles in the following procedures. Two parallel longitudinal grooves were made in the buccal and lingual of the root by a diamond fissure bur (Teezkavan.co, Tehran, Iran) then the specimens were split into two halves by wedging a wax spatula into the groove. One-half of the each root was selected randomly. After preparation of specimens, they were viewed under the Scanning Electron Microscope (Cam Scan MV2300, Oxford instrument, UK). All specimens were evaluated in three areas of coronal, middle and apical thirds of the root canals and photographed at ×2500 magnification. The photographs were evaluated for presence of smear layer in blind manner with two investigators who scored the presence or absence of smear layer using the following chart (Table 1) (24).

The inter examiners’ reliability was verified by using the kappa test after scoring the photomicrographs, the data were recorded and analyzed with Kruskal-Wallis, Mann-Whitney, Fridman and Wilcoxon tests and SPSS 16 (SPSS Inc, Chicago IL, USA). The significance level set at $P=0.05$.

### Results

The results obtained from this study are presented in Table 2, Figure 1. SEM photomicrographs of the control, and irrigated samples by Er:YAG and different irrigation solutions are presented in Figures 2-5. All specimen of control group showed entire covering of smear layer on the canal walls.

Kruskal-Wallis and Mann-Whitney tests showed that 17% EDTA and 6% Phosphoric acid were significantly more effective on removing smear layer compared to Er:YAG laser ($P=0.000$). But no significant difference was found between 17% EDTA and 6% Phosphoric acid groups ($P=0.49$), in all three parts of the canals (coronal, middle, apical).

Fridman and Wilcoxon tests showed a significant difference between smear layer removals in three levels of canal in each group. As follow:

Coronal area in group treated by Er:YAG laser showed the cleanest area compared to middle and apical areas, while the apical was found with the most smear layer and debris (Coronal>Middle>Apical) (Figure 1, Table 2).

17% EDTA showed an equal effect on removing smear layer in coronal and middle areas followed by apical area (Coronal=Middle>Apical) (Figure 1, Table 2).

### Table 1. Smear layer scoring chart

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The surface is free of debris and smear layer.</td>
</tr>
<tr>
<td>2</td>
<td>The surface is free of smear layer, but little debris is presented.</td>
</tr>
<tr>
<td>3</td>
<td>The surface is clean, but both smear layer and dispersed debris are observed.</td>
</tr>
<tr>
<td>4</td>
<td>The surface is clean, but the level of smear layer and debris is also noticeable.</td>
</tr>
<tr>
<td>5</td>
<td>The clean surface is a bit greater than unclean surface.</td>
</tr>
<tr>
<td>6</td>
<td>Almost half of smear layer and debris have been removed.</td>
</tr>
<tr>
<td>7</td>
<td>The greater part of smear layer and debris are left.</td>
</tr>
<tr>
<td>8</td>
<td>The surface is completely covered with smear layer and debris.</td>
</tr>
</tbody>
</table>

### Table 2. Comparison of p values recorded in groups of the apical, middle and coronal regions (Mann-Whitney).

<table>
<thead>
<tr>
<th>Comparing Groups</th>
<th>Overall</th>
<th>Apical</th>
<th>Middle</th>
<th>Coronal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Er:YAG – EDTA</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Er:YAG – Phosphoric acid</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Er:YAG – Control</td>
<td>0.00</td>
<td>0.119</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>EDTA – Phosphoric acid</td>
<td>0.49</td>
<td>0.29</td>
<td>0.47</td>
<td>0.04</td>
</tr>
<tr>
<td>EDTA – Control</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Phosphoric acid – Control</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

### Figure 1. Mean score of smear layer removal at overall, apical, middle and coronal levels in the experiment groups (kruskal-wallis test)
6% Phosphoric acid showed the same behavior in all portions of canal in removing the smear layer (Coronal=Middle=Apical) (Figure 1, Table 2).

Regardless of which technique used for removing smear layer, coronal area was the cleanest, followed by middle, and then apical area (Coronal>Middle>Apical) (Figure 2).

Discussion

The results of this study showed that %17 EDTA and %6 Phosphoric acid methods are significantly more effective in removing smear layer than the Er:YAG laser and the control groups, while no significant difference was found between 17%EDTA and 6% phosphoric acid.
EDTA showed equal ability of smear layer removal in coronal and middle area, followed by apical area. While phosphoric acid had the same behavior in all three regions. On the other hand SEM photomicrographs revealed that Er:YAG laser is also able to remove smear layer, but the comparison of the data showed that this method is not as effective as in group 1 and 2.

Takeda et al compared the smear layer removal with either 17% EDTA, 6% phosphoric acid or Er:YAG laser. Although they did not find any differences between 17% EDTA and 6% phosphoric acid solution, they reported that Er:YAG laser (1W,100mJ,10Hz) was more effective in smear layer removal compared to EDTA and phosphoric acid (25). This may be due to different laser parameters, method of using laser and different scale system. In addition, since they used 25 gauge needles for irrigation which has less penetration in the root canal than the one (30 gauge) in our study, therefore most smear layer removal was seen in coronal region.

Pérez et al showed in a study comparing 15% EDTA, 5% phosphoric acid and 15% citric acid that 15% EDTA is more effective than two other solutions in smear layer removal, which supports the findings of our study on effectiveness of 17% EDTA and 6% phosphoric acid on smear layer removal. They also reported that 15% EDTA has more ability to remove the smear layer in the middle and coronal third, again this result is consistent with ours in the present study (16).

Theodoro et al examined the rate of smear layer removal by 24% EDTA gel and Er:YAG laser with two doses of 5.8 j/cm² and 10.3 j/cm². They found that the irradiation of Er:YAG laser with the later energy density is more effective in removing the smear layer compared to the other two techniques (26).

Kivanç et al compared the amount of debris, smear layer and recrystallization after irradiation of the root canals by either Er:YAG or Nd:YAG lasers. According to the results of their study, although there were no statistical difference found between the two methods, but Er:YAG laser showed better result compared to Nd:YAG laser. They concluded that neither of those lasers was effective in removing debris and smear layer while in the present study, more than %50 of the smear layer was removed from the root canal (27).

Although Er:YAG laser has been shown to be effective as a disinfecting and sterilizing method for root canal system (28-30), but the authors may recommend to use EDTA or Phosphoric acid to achieve the optimum clean up and removal of the smear layer inside the root canals.

Further studies is recommended to study other materials, solutions and laser wavelengths, specifically newer endodontic laser tips which are able to deliver the energy deep into the canal, and also a newer generation of radial firing tips.

**Conclusion**

Based on the results of this study, 17% EDTA and/or 6% Phosphoric acid are significantly more effective in removing smear layer and remaining debris from the root canal and although Er:YAG laser has shown a limited ability to remove smear layer, the effectiveness of this technique is not significantly different than the control group.

**References**

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