Comparative evaluation of phosphoric acid, EDTA and saline, with and without ultrasonic irrigation for smear layer removal- a SEM study

Parag M Wani, Rajesh R Shetty1, Pradnya S Kale2, Pritesh G Jagtap1

Abstract

Background: Smear layer can be a source of infection in the root canal. However, different physical and chemical agents are available for smear layer removal.

Aim: The aim of this study was to compare the effectiveness of six different irrigation techniques for removing the smear layer in vitro using scanning electron microscopy (SEM).

Materials and Methods: Eighteen single rooted human maxillary anterior teeth were accessed, instrumented, and subjected to various irrigation techniques: 37% phosphoric acid, 17% EDTA and saline, alone and with ultrasonic irrigation for 30 seconds, respectively. The irrigated samples were sectioned and assessed using SEM. Three photomicrographs (1000X) were recorded for each sample (coronal, middle, and apical). Takeda et al., scoring system was used to evaluate the smear layer left as observed in the images. Kruskal-Wallis test was used to calculate the statistical difference at 5% level of significance.

Results: In 30 second, 37% phosphoric acid with ultrasonic irrigation showed the best results followed by 37% phosphoric acid alone and saline with ultrasonic irrigation. 17% EDTA with ultrasonic irrigation was least effective.

Conclusion: The results of the present study has shown that phosphoric acid solution may be considered as a favorable agent used for smear layer removal in endodontic therapy and its effectiveness can be enhanced using ultrasonics.

Key words: EDTA, Phosphoric acid, smear layer, ultrasonics

Introduction

Smear layer is debris retained on dentin after hand or rotary instrumentation. It is made up of two parts: A superficial one, which is 1-5 microns thick and loosely adhering to the underlying dentin and the smear plug that is about 40 microns is packed in the dentinal tubules. It has been found that the smear layer debris consists of ground dentin, predentine, pulpal remnants and odontoblast processes and in the case of infected teeth it contains bacteria.

Many researchers believe that the smear layer should be removed as this layer harbors the survival and multiplication of the bacteria and can result in them re-entering the dentinal tubules and re-infecting the canals. The antimicrobial action of the medications in the dentinal tubules can be altered in an undesirable fashion due to the smear layer. The ability of the sealer to penetrate the dentinal tubules and thereby the adaptation of the root canal filling to the root canal might also be compromised due to the presence of this smear layer.

Various chemical agents as well as some physical agents like ultrasonics and lasers in combination or alone has been tested for their ability to remove the smear layer. The predictability of smear layer removal decreases as we go from the coronal to the apical region of the root and this could be attributed to the wider canal orifice coronally that tapers apically. Various methods have been tested to increase the penetration of irrigating solutions into the apical third of the root canals like enhancing the irrigating solution by adding surfactants and using ultrasonics.

The various chemical agents used are sodium hypochlorite, chelating agents like EDTA solutions at concentrations ranging from 15-17%, and organic acids like citric acid (5-50%), and polyacrylic acid (5-40%) or other acids like phosphoric acid (5-37%). Phosphoric acid has been extensively used to remove the smear layer from coronal dentin as it is a commonly used etchant before a composite restorative material and its performance in root dentin has been evaluated in only few studies. Literature search reveals that there are only a few studies concerning the effect of the various agents on the smear layer removal. Also, differences exists in the methodologies, time intervals and concentrations of the solutions tested which limits the ability to make valid comparisons between...
these treatments. Hence, the aim of this \textit{in vitro} study was to compare the effectiveness of 37\% phosphoric acid, 17\% EDTA and saline, with and without ultrasonic irrigation in removing the smear layer evaluated by means of scanning electron microscopy (SEM).

**Materials and Methods**

**Smear layer production and irrigation protocols**

Eighteen single-rooted maxillary human anterior teeth, extracted for periodontal or prosthetic reasons, were used. The teeth with straight roots, mature root apex, and similar anatomic characteristics were selected for this study. Routine procedure for smear layer production was followed.\cite{20} The teeth were accessed by using round diamond burs (ManiInc., Japan). The teeth were shaped by using Protaper Ni Ti Hand System (Dentsply Maillefer, Ballaigues, Switzerland) The sequence used was as follows; #20-#50 (2\%) followed by a sequence of GatesGlidden drills (Dentsply Maillefer, Ballaigues, Switzerland) from 1–3 to prepare the middle-cervical third. The protaper sequence used in the apical third was S1, S2, F1, F2, F3, F4, F5 (Dentsply Maillefer, Ballaigues, Switzerland) till the apex. All the files were used for the entire working length till the apex. Between files, the canals were irrigated with 1 ml of 5.4\% sodium hypochlorite (Auvecholor, Dental Avenue Pvt. Ltd, India). After instrumentation, the teeth were irrigated with 5 ml of distilled water. 30-gauge needle was used for irrigation. Irrigation was done for 60 seconds. All the teeth had their apexes sealed with sticky wax (DPI, India) to prevent the flow through them. Three teeth each was subjected to the different irrigation techniques respectively [Table 1].

The substances used were 17\% EDTA (Canalarge, Amdent, India), 37\% phosphoric acid solution (Emcure pharmaceuticals Ltd, India), saline (Fresenius India Ltd.) and ultrasonics K-file #30 (Acteon, Satelec, France).

**Scanning electron microscopy**

After subjecting to the six different techniques, all teeth were irrigated again with 5 ml distilled water and dried with medium sized paper points (Dentsply Maillefer, Ballaigues, Switzerland). Finally, two longitudinal groves were prepared on both the buccal and lingual surfaces using a diamond disc without penetrating the canal. Hammer and chisel were used to split the root into two halves. The half portion containing the most visible part of the apex was used for study. The samples were analyzed using scanning electron microscope (Icon Analytical, Mumbai, India). They were all numbered and three photomicrographs of each root sample (coronal, middle and apical) was made. First a scan of all the samples was made at x30 magnification. Then, the most representative area of each third of the sample root was selected and magnified at x100. Each x100 image was scanned and the three most representative areas were magnified at x1000. This final photomicrograph was assessed by three independent blinded examiners. The scoring system described by Takeda \textit{et al.},\cite{21} was used: Score 1 = no smear layer, with all tubules cleaned and opened; Score 2 = few areas covered by smear layer, with most tubules cleaned and opened; Score 3 = smear layer covering almost all the surface, with few tubules opened; and Score 4 = smear layer covering all the surfaces. Out of the 3 examiner scores, two matching scores were considered as the final score for each assessed sample. Figure 1 is a composite picture of an individual photomicrograph of one root sample (coronal, middle, and apical).

**Data analysis**

Interexaminer reliability for the SEM evaluation was verified by Kappa test. Data was analyzed by calculating the median and Kruskal-Wallis test was used to calculate the statistical difference at 5\% level of significance. Statistical Package for the Social Sciences software (SPSS version 12) was used for statistical analysis.

**Results**

The Kappa test showed good agreement between observers (Kappa value = 0.83).

Overall, based on the median scores, 37\% phosphoric acid with ultrasonic irrigation showed the best results followed by 37\% phosphoric acid alone and saline with ultrasonic irrigation [Table 2]. The least effective was 17\% EDTA with ultrasonics in which smear layer covered all the surfaces. Based on the median scores, for the coronal and middle third it was evident

**Table 1: Six different irrigation techniques used in the study**

<table>
<thead>
<tr>
<th>Group</th>
<th>Substance used</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>17% EDTA</td>
</tr>
<tr>
<td>II</td>
<td>37% phosphoric acid</td>
</tr>
<tr>
<td>III</td>
<td>Saline</td>
</tr>
<tr>
<td>IV</td>
<td>17% EDTA+ultrasonic</td>
</tr>
<tr>
<td>V</td>
<td>37% phosphoric acid+ultrasonic</td>
</tr>
<tr>
<td>VI</td>
<td>Saline+ultrasonic</td>
</tr>
</tbody>
</table>

(Irrigation period just 30 seconds)

**Table 2: The smear layer scores based on the Takeda et al**

<table>
<thead>
<tr>
<th>Coronal</th>
<th>Middle</th>
<th>Apical</th>
<th>Overall median score</th>
</tr>
</thead>
<tbody>
<tr>
<td>37% phosphoric acid+ultrasonic</td>
<td>1;1;1</td>
<td>1;1;2</td>
<td>2;2;2</td>
</tr>
<tr>
<td>Saline+ultrasonic</td>
<td>1;1;3</td>
<td>1;1;3</td>
<td>2;4;4</td>
</tr>
<tr>
<td>37% phosphoric acid</td>
<td>2;2;2</td>
<td>2;2;2</td>
<td>3;3;4</td>
</tr>
<tr>
<td>Saline</td>
<td>3;3;3</td>
<td>3;4;4</td>
<td>3;4;4</td>
</tr>
<tr>
<td>17% EDTA</td>
<td>3;3;4</td>
<td>4;4;4</td>
<td>4;4;4</td>
</tr>
<tr>
<td>17% EDTA+ultrasonic</td>
<td>2;2;3</td>
<td>3;4;4</td>
<td>4;4;4</td>
</tr>
</tbody>
</table>

(Nine samples assessed per group)
Wani, et al.: Phosphoric acid and ultrasonic in smear layer removal

The smear layer scores of the six different techniques were compared, a significant difference was observed between 37% phosphoric acid with ultrasonics and the other techniques except for saline with ultrasonics [Table 3].

**Discussion**

The literature describes a variety of chemicals with a broad range of concentrations and regimens as well as numerous physical agents to remove the smear layer. This study compared EDTA, a well-known chelating agent, phosphoric acid, a strong acid routinely used in dentistry to remove the smear layer and smear plugs formed during coronal cavity preparations and ultrasonics that are known to enhance the action of the irrigant by causing acoustic streaming and cavitation. Phosphoric acid in the range of 10%, 24%, and 32% in removing smear layer from root canals has been reported in the literature. In addition, there are no studies that have used phosphoric acid at 37% concentration along with ultrasonic irrigation. Therefore, the present study is first to compare the action of 37% phosphoric acid with well-established solution of 17% EDTA for 30 seconds, with and without ultrasonic irrigation. However, in the present study, EDTA resulted in lower performance, which means that this solution was not able to remove the smear layer in 30 seconds. This finding is in accordance with other studies assessing the use of EDTA for 1 minute, showing that it did not work well in this period of time. Ultrasonic application with EDTA did not enhance its effect, this is in accordance with earlier studies by Ciucchi et al. and Abbott et al. This could be due to reduced demineralizing effect of the chelator by reducing the working time as EDTA only develops its full effectiveness after increased working time i.e. more than a minute.

One has to be careful while using the 37% phosphoric acid as an irrigant because it may carry a higher risk of cytotoxicity, especially when used in the apical third of the root canal if it extrudes beyond the apex, but there are still no studies to substantiate this in the literature. Gel with its higher viscosity might be better than the liquid to prevent extrusion but the disadvantage of using the gel is that some residual layer of gel is retained in the apical third even after washing with 5 ml of distilled water. Therefore, there seems to be no advantage of gel over the solution.

Regarding the dentinal erosion, it seems to be a time-dependent phenomenon. At one minute or longer, erosion is seen equally in the middle and coronal third, though not in the apical third. In light of these findings the exposure period of 30 seconds used in the study might not have that severe effect of dentinal erosion, though further studies with respect to 37% phosphoric acid solution are necessary before definitive conclusion can be arrived at.

Future directions for research could be to evaluate the depth of demineralization, its effect on adhesion and its cytotoxicity.
in the periapical region before it could be routinely used in clinical practice.

Conclusion

The findings of this study suggest the possibility that 37% phosphoric acid solution along with ultrasonic irrigation may be a favorable agent for smear layer removal.

Acknowledgement

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References


Table 3: Intergroup comparison using the Kruskal-Wallis test

<table>
<thead>
<tr>
<th></th>
<th>37% Phosphoric acid+Ultrasons</th>
<th>Saline+ultrasons</th>
<th>37% Phosphoric acid</th>
<th>17% EDTA+ultrasons</th>
<th>Saline</th>
<th>17% EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>37% Phosphoric acid+ultrasons</td>
<td>0.345</td>
<td>0.01*</td>
<td>0.001*</td>
<td>0.001*</td>
<td>0.001*</td>
<td></td>
</tr>
<tr>
<td>Saline+ultrasons</td>
<td>-</td>
<td>-</td>
<td>0.06</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>37% Phosphoric acid</td>
<td>0.549</td>
<td>0.03*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>17% EDTA+ultrasons</td>
<td>0.04*</td>
<td>0.007*</td>
<td>0.961</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>0.009*</td>
<td>0.002*</td>
<td>0.243</td>
<td>0.159</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*statistically significant

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