The effect of different final irrigation solutions on apical impermeability

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Abstract

Aim: This study aimed to compare ethylenediaminetetraacetic acid (EDTA), phytic acid, and citric acid as chelation agents in final irrigation procedures on teeth with apical impermeability.

Methodology: We used 66 mandibular premolar teeth with a single root and single canal extracted for periodontal or orthodontic reasons. All teeth crowns were removed to achieve a 14-mm root length. Mechanical preparation of the root canal was completed with a Reciproc R25 (VDW, Munich, Germany) file system. Teeth were randomly separated into three groups: Group 1: 5 ml 17% EDTA, Group 2: 5 ml 1% phytic acid, and Group 3: 5 ml 20% citric acid. We used 6 ml 5.25% sodium hypochlorite (NaOCl) in the mechanical preparation of all groups. Three teeth were selected for both the positive and negative control groups. All teeth in the experimental groups were filled by the lateral condensation method using AH Plus canal sealer and gutta-percha. For hardening of the canal sealer, samples were kept at 37 °C and at 100% humidity in a drying oven for 7 days. Later, following removal from the drying oven, two layers of nail polish were used to cover all but the apical 2 mm of each tooth. The teeth were then kept in 2% methylene blue solution at 37 °C for 7 days. The root was divided by cutting in the buccolingual direction and gutta-percha was removed. The dye leakage was measured linearly in millimeters (mm) under a stereomicroscope.

Results: As a result of the apical leakage assessment, the lowest apical leakage is observed in the phytic acid group. There was a statistically significant difference between the phytic acid group and citric acid group. There was no statistically significant difference between other groups.

Conclusion: While phytic acid showed similar results with EDTA on apical impermeability, it was more effective than citric acid.

Keywords: microleakage, EDTA, phytic acid, dye penetration test

Introduction

The success in an endodontic treatment can be achieved with a mechanical preparation of the root canal. This involves removing all vital or necrotic tissues, microorganisms, and side products from the root canal system with irrigation and disinfection. A three-dimensional impermeable filling should then be used on the tooth (1).

Following the endodontic treatment, an impermeable apical blockage in the dentine tubules is desired to prevent bacteria and toxins from reaching the periapical tissues. Apical leakage is the most
common problem seen in endodontic treatment failures (2).

Apical microleakage can be defined as having tissue fluid, bacteria, or molecule or ion transfers between the cavity walls and the applied filling material. The microleakage at the root canal can cause the transfer of bacteria, tissue fluid, and chemical materials between the canal sealer and dentin wall, canal sealer, and gutta-percha, or inside the canal sealer cavities. It is believed that gutta-percha is impermeable (3). Various factors may lead to apical leakage. Some of these factors include the existence or absence of a smear layer, the cavity between the dentin wall and filling material, volumetric changes in the filling material, physical properties of the canal sealers, root canal filling techniques, and anatomic variations in the tooth (2).

The smear layer created during the mechanical preparation and closing of the entrance of dentin tubules consists of organic and inorganic contents that include microorganisms, necrotic material, and odontoblasts (4). The smear layer settles inside the irregularities of the dentin tubules and root canal system and prevents the full adaptation of medicaments and canal filling sealers to the root canal wall (5). Therefore, removal of the smear layer is considered a method of improving apical impermeability (6).

Due to the cytotoxic effects of the irrigation agent EDTA on the periradicular tissues, alternative agents with a higher biocompatibility, which influence removing the smear layer, were explored. In recent years, phytic acid has been preferred as a chelation agent in endodontic treatments to remove the smear layer (7). Studies have shown that phytic acid has a greater biocompatibility and a lower cytotoxicity than EDTA (8, 9).

Citric acid is also a commonly used chelation agent to remove the smear layer from the root canal system in endodontic treatments (10).

In our review of the literature, no dye leakage studies with phytic acids were found. Therefore, the main purpose of this study was to assess microleakage amounts at the apical section following the root canal filling of teeth with phytic acid, EDTA, or citric acid. These were applied as the final irrigation solution after the mechanical preparation of the root canal using the dye penetration method under a stereomicroscope.

**Materials and Methods**

In this study, we used 66 mandibular premolar teeth with single roots and single straight canals that had completed apical root development and were extracted for orthodontic purposes. The ethical approval was taken from the review board of Dicle University (ethical board number: 2020-39). As a result of the assessment, those teeth containing cavities, fractures, or cracks on the root surface were not included in the study. The buccolingual and mesiodistal radiographic assessments of the teeth were performed, and teeth with multiple root canals, calcified canals, internal or external resorption, and an incomplete apex were also not included.

The samples were kept in a 10% formalin solution before the laboratory procedures began. The crown was then removed with a water-cooled diamond separator to keep the root length of each tooth at 14 mm. The roots were kept in sterile saline solutions until the mechanical preparation of the canals were completed.

The working length was calculated as 1 mm shorter than the length of a no. 15 K type file (Dentsply/Maillefer, Ballaigues, Switzerland), as seen from the apex. In preparation, a Reciproc R25 file and the Reciproc program of the endodontic engine (Eighteenth, E-Connect S) were used. All teeth irrigations during the mechanical preparations were completed with 6 ml of 5.25% NaOCl. The teeth were then randomly divided into three experimental groups with 20 teeth in each group for the final irrigation solutions. The remaining six teeth were divided into two groups as the positive and negative control groups. All operations were conducted by one researcher.

**Experimental Groups**

**Group 1**: 5 ml of 17% EDTA (Saver®, Prime Dental Products Pvt Ltd., Maharashtra, India) inside the canal for 1 minute and irrigation with 5 ml of distilled water.

**Group 2**: 5 ml of 1% phytic acid (Sigma, Aldrich, USA) inside the canal for 1 minute and irrigation with 5 ml of distilled water.

**Group 3**: 5 ml of 20% citric acid (Cerkamed Medical Company, Poland) inside the canal for 20 minutes and irrigation with 5 ml of distilled water.

During the chemo-mechanical preparation, an irrigation needle with a side hole (Endo Top, Cerkamed, Poland) was placed inside the canal by keeping it 1 mm shorter than the working length during irrigation. The canal was then dried with sterile paper points (VDW, Munich, Germany).

The root canal fillings of the teeth in the experimental group were completed with AH Plus (Dentsply DeTrey GmbH, Konstanz, Germany) root canal filling pads and gutta-percha cones with the cold lateral condensation technique after the preparation. The canal entrance was then closed with a glass ionomer cement (3M, ESPE Ketac, St. Paul, MN, USA).

**Control Groups**

Positive Control Group: Six teeth were filled with only gutta-percha without a canal sealer.

Negative Control Group: Six teeth were filled with gutta-percha and AH Plus canal sealer.

All samples with a canal filling were maintained in the drying oven for 7 days at 37 °C and 100% humidity to complete the canal sealer polymerization. Later, two layers of nail polish were applied to all surfaces other than the apical 2 mm. All surfaces of the positive control group teeth, except for the apical 2 mm, and all surfaces of the negative control group teeth, were coated with two layers of nail polish. All teeth were kept in the drying oven inside a 2% methylene blue dye solution for 7 days at 37 °C. Before removing the
methylene blue dye, the samples were washed under running water for 5 minutes and dried.

**Statistical analysis**

All roots were sectioned in the buccolingual direction using a diamond disk. At this stage, contact with canal filling materials was avoided. After cutting the roots, the gutta-percha was removed from the canal. Two cross sections were then collected from each tooth with a 12.5X stereomicroscope zoom for examining dye leakage inside the root from the apical to coronal regions. The farthest point the dye migrated from the apical to coronal region was measured with a millimetric ruler placed next to the root during measurements. Dye penetration measurements were completed from the two cross-sections from one root and the highest value was used for the statistical assessments (Fig. 1).

![Figure 1](image)

Figure 1. Dye leakage amounts in the root canal in the experimental and control groups. (a) Image from the negative control group; (b) Image from the positive control group; (c) A cross-sectional image from the EDTA group; (d) A cross-sectional image from the phytic acid group; and (e) A cross-sectional image from citric acid group.

**Results**

Data were analyzed using IBM SPSS V23. Normal distributions were analyzed with Shapiro-Wilk tests. Kruskal-Wallis H tests were used for comparing microleakage values without a normal distribution, according to groups. Dunn tests were applied for multivariate comparisons. The quantitative data for the analysis results are given as mean ± standard deviation and median (min-max). The significance level was selected as p<0.05.

**Table 1. Microleakage value (mm) comparisons for the experimental groups.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean ± Standard Deviation</th>
<th>Median (Min-Max)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDTA</td>
<td>4.42 ± 2.94</td>
<td>3.95 (1.06-9.62)</td>
<td>b</td>
</tr>
<tr>
<td>Phytic Acid</td>
<td>2.94 ± 4.59</td>
<td>2.32 (0.3-8.11)</td>
<td>a</td>
</tr>
<tr>
<td>Citric Acid</td>
<td>4.59 ± 3.98</td>
<td>4.52 (2.41-7.43)</td>
<td>b</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis H test, a-b: There was no difference between groups with the same letters.
During group comparisons, the lowest microleakage was observed in the phytic acid group and the highest in the citric acid group. The statistical comparison of the groups revealed there was a significant difference only between the phytic acid and citric acid groups (p<0.05).

**Discussion**

Apical leakage is one of the most common reasons for an endodontic treatment failure. Apical leakage might occur due to various factors, such as the existence or absence of accessory canals, periodontal problems, a smear layer, physical or chemical properties of the canals, the root canal filling technique, and a filling shorter than the working length (2).

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Of all failures in root canal treatments, 60% are due to the inability to provide an apical filler. This could lead to a bacterial transfer to the root canal with periradicular exudence leakage and this will then decrease the success rate of the treatment (11). Irrigation, which can be defined as being complementary to the mechanical preparation, must be applied to the root canal. Irrigation operations help increase the canal filling sealer penetration to the tubule by opening the dentin tubule entrance by dissolving the organic-inorganic materials and removing the infected tissues from the root canal (12). In this study, the AH Plus canal filling sealer was preferred due to its low dissolution property, good apical coverage, and its ability to attach to root dentin (13).

In our study, microleakage amounts at the apical section following root canal filling with phytic acid, EDTA, and citric acid, which were applied as final irrigation solutions, were assessed using the dye penetration method. Since EDTA is a chelation agent, it is independent of elevated hydrogen ion concentrations during decalcification and can show effects at around pH 7 levels (14). Citric acid, which is a weak organic acid, can be used in varying concentrations between 1%-50% in endodontia (15). Phytic acid is a new irrigation solution used in endodontia as a recent alternative to EDTA. A 1% concentration of phytic acid is suitable for endodontia (7). The literature review showed no studies that have evaluated the effects of phytic acid on apical leakage.

There are various methods used to assess apical leakage in endodontia. One of these methods is the dye penetration method. The most preferred dye solution is 2% methylene blue (16, 17). The main reasons for its use in dye penetration tests are that this dye enables quantitative measurements with linear leakage, has a smaller molecular size than bacteria, it penetrates deeper than other dyes, and it penetrates to irritate periapical tissues along the infected root canal due to its acidic nature (18, 19). When these advantages are considered, a 2% methylene blue solution was preferred for our study.

Studies that have assessed apical leakage using the dye penetration method report that the results may be influenced by differences in samples, the preparation method, the sealer, the time the dye is kept in, the leakage method, and the leakage interpretation (20). The methylene blue solution used in dye penetration tests is acidic. It demineralizes the dentin and may increase the leakage amount (20). Tamse et al. conducted a study that compared the leakage levels of drawing ink, methylene blue, eosin, and Procion brilliant blue dye solutions. The apical leakage was assessed with the transparentizing and cross-sectional methods, and the researchers reported similar dye leakage levels (21). In apical leakage studies, dye leakages have been measured spectrophotometrically or linearly (22). It has been reported that the spectrophotometric method gives volumetric measurements rather than linear measurements of the microleakage. These studies have shown a good fit between the results obtained from linear and spectrophotometric methods (23, 24). In our study, the leakages in both the experimental and control groups were measured with the linear (dimensional) dye leakage measurement technique after the dye penetration test.

According to findings obtained from our study, the lowest microleakage occurred in the phytic acid group, followed by the EDTA and citric acid groups. While there was a significant difference between the phytic acid and citric acid groups (p<0.05), there was no difference between the phytic acid and EDTA or the EDTA and citric acid groups (p>0.05). It is believed that phytic acid produces less apical microleakage because this acid opens dentin tubules more and increases canal sealer penetration to the tubules.

Balasubramanian et al. (6) assessed the effects of 17% EDTA, 10% citric acid, and a mixture of doxycycline, citric acid, and detergent (MTAD) used as final irrigation solutions on apical leakage and found that there were no intergroup significant differences. This study supports our finding in terms of the EDTA and citric acid groups.

Farhad et al. (25) assessed the effect of three different final irrigation protocols to remove the smear layer on apical leakage with a methylene blue dye penetration test and linear measurement techniques. They used 17% EDTA, 5.25% NaOCl, 7% citric acid + 5.25% NaOCl, and 20% citric acid + 5.25% NaOCl irrigation solution combinations. The highest dye penetration was observed in the EDTA group and the lowest was in the 20% citric acid group. In our study, there was no significant difference between the groups that used EDTA and citric acid as final irrigation solutions. The reason for the difference in findings may be due to using NaOCl in the final irrigation after EDTA and citric acid in the previous study while we applied a different final irrigation protocol. In our study, NaOCl was not preferred in the final irrigation operation since the direct effect of chelation agents on the apical leakage was assessed.

Another study linearly measured the effect of different irrigation solutions with various canal sealers on the apical leakage with the dye penetration method using 3% NaOCl, 2% chlorhexidine (CHX), and 3% hydrogen peroxide (H2O2) as irrigation solutions. They then completed the final irrigation of all samples with EDTA and distilled water. The researchers used MTA Fillapex, AH Plus, and Realseal SE canal sealers in root canal fillings. This group reported lower apical leakages in all canal sealers with the H2O2 group and the highest apical leakages in the NaOCl and CHX groups (26).

Conclusions

According to the results of this study, apical leakage occurred in all EDTA, citric acid, and phytic acid groups. A significant difference between the groups was only found between the phytic acid and citric acid groups. Phytic acid was the agent with the lowest dye leakage. More comparative studies are needed to evaluate the effect of phytic acid on apical leakage.
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Ethical Approval: Ethics committee approval was received for this study from Human Ethics Research Committee at Dicle University (Decision No: 2020-39).

Peer-review: Externally peer-reviewed.


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