Effect of different irrigation regimens on the evaluation of apical sealing ability of mineral trioxide aggregate

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Abstract
Introduction: This study aimed to evaluate and compare the apical sealing ability of different irrigation regimens (NaOCl, CHX, NS, and MTAD).
Methods: Seventy intact extracted single-rooted human teeth were selected. The roots were resected at 3 mm short of the apical foramen after access preparation. The canals were prepared with profile. This rotary file moved forward into the apical foramen so that the D16 section of the file passed the foramen of the resected part of the root. The teeth were randomly divided into 4 study groups. After placing them in humid sponge, the canals were irrigated with study irrigators for 5 minutes. The canals were dried and 5mm MTA was placed into the apex. After 24 hours, the teeth were placed in an ink solution. The teeth were cleaned and the microleakage was tested using stereomicroscope.
Results: The results showed no significant difference between the group of 2% Chlorhexidine and the other groups. 0.5% NaOCl group showed significantly less leakage than MTAD and normal saline groups.
Conclusions: The results of this study suggested that 0.5% NaOCl provided the best final irrigation before the apical placement of MTA.
Keywords: Irrigator, Microleakage, MTA.

Introduction
Severe caries or trauma can weaken the permanent teeth before the root completion. This situation could be worse in cases of open apexis or blunderbuss. Root canal treatment is inappropriate in these cases. In the past, the selective treatment for these cases was apexification, which meant the root canal debridement with files and irrigation following the use of calcium hydroxide. This treatment had many disadvantages such as poor patient compliance and cooperation, and possible damage to root dentine.

Moreover, the completion of this treatment would take between 6 to 24 months. During this period, the risk of tooth fracture is higher for the weak teeth (1, 2). Because of these complications, another method is used which includes initial debridement, treatment with calcium hydroxide for one week and placement of an apical barrier. It seems that MTA (Mineral Trioxide Aggregate) is the preferred material for apical sealing, as it has many advantages (3). This new method has reduced the number of follow-ups in long term and has considered the need for apical seal.

It had also decreased the treatment period, which would result in faster healing and protecting the growing teeth. The success of the new method for
creating a good apical seal in blunderbuss canals is associated with MTA adaptation to dentin walls. The different irrigation regimens such as 0.5% NaOCl, 2% CHX, MTA and normal saline are used for root canal irrigation. Considering the possible effects of these irrigation regimens on apical microleakage, we decided to compare the effect of these irrigators on apical sealing ability of MTA.

Methods

Seventy intact extracted single-rooted human teeth were used in this investigation with no or minimum curvature. All specimens were radiographed to assure canals configuration, patency and absence of intracanal calcifications. The single-rooted tooth with two or more canals, curved canals, calcified canals, tooth with absorptions and cracks were excluded and replaced with other appropriate teeth. The samples were maintained in sterile normal saline solution.

The conventional method of cleansing and shaping was used for each tooth. After access preparation, the canal orifices were located. Specimens were instrumented with file #10 up to apex, to assure that the canals were open. An accurate gauge was used to measure 3mm of apex. Apical ends were cut 3 mm from the dental apex using a diamond disc under continuous air/water spray to eliminate the apical ramifications and retrieve the apical foramen in the center of root structure. File #10 was moved forward into the canal.

As soon as it was visible from the root tip, the file stop was adjusted with the reference point and the working length was measured. The measured number was written on the facial plan of each tooth to be used as reference point in the next steps.

After the establishment of working length, coronal instrumentation was performed, using Gates Glidden burs # 2,3,4. The root canal was instrumented within the actual working length with 0.04 profile rotary file, series 29, # 7. The same file was entered through the apical foramen and moved forward. During the procedure, the root canals were constantly irrigated with at least 3 ml 0.5% sodium hypochlorite. To stimulate routine clinical procedure, after obturation, the teeth were maintained in 100% humidity for one week.

They were decalcified with file # 70 under irrigation with 0.5% sodium hypochlorite.

Teeth were randomly divided into four experimental groups:A: Chlorhexidine 2% B: Normal Saline C: MTA D: Naocl 5% 15 teeth were put in each group the other to teeth were put in positive and negative control groups.

Positive and negative control groups were considered as following:

Final irrigator for both groups was distilled water.

In negative control groups, the entire external root surfaces and the apical tips were made impermeable with two layers of nail polish. In positive control groups, the apex was maintained open and the canal was not filled in order to evaluate the dye penetration.

The roots of the study teeth were put in 5.2 cm cube-shaped sponge foam that was already moistened in water for 5 minutes. A Mailleferplugger # 2 was used at the length of W.L to make sure the sponge did not penetrate into the roots. The roots were dried with paper cone # 80 placed at 1mm short of the W.L. (moving forward the paper cone through the W.L absorbs the water from sponge.) 1ml of the study irrigant was injected into the canal with irrigation syringe. The apical stop was adjusted at 1mm short of the W.L. After 5-minute irrigation, the root canal was irrigated with 4ml of the same irrigator, and then dried with paper cone # 80 placed at 1mm short of the W.L. The cone was completely dry after removal.

The proper plugger for each tooth was selected among the Mailleferpluggers # 2 or # 3. Apical stop was placed at 1mm short of the W.L. MTA was applied into the access cavity according to the manufacturer instructions, using an MTA carrier, and a Mailleferplugger # 4 was used to move the material into the coronal part of the canal. Then the selected Mailleferplugger moved the material into the apex. MTA was condensed with paper cone # 80. The additional MTA was removed from the root canal walls. This procedure was repeated until an apical plaque with the length of 5mm was achieved. If the length was more than 5mm, the additional MTA was removed with file #70. The apical barrier was measured through radiography. If a bubble was observed, the filling was removed with file # 70 and apexification process was repeated.

A moistened cotton pellet was placed into the coronal part of the root, and a temporary restorative material was used for sealing the access cavity. Then the teeth were stored in 100% humidity for 24 hours to provide the primary setting of MTA.
The entire external root surfaces were made impermeable with two layers of nail polish to provide a better coronal seal. Then the teeth were immersed in Indian ink for 48 hours. Afterwards, the nail polish was removed with acetone. The tooth were maintained in 5% Nitric Acid for 72 hours. Acid was replaced after 24 and 48 hours. To observe the complete demineralization, several teeth were radiographed.

The teeth were irrigated with tap water for 1 hour. For the purpose of dehydration, the teeth were left for 24 hours in 70% ethyl alcohol, 24 hours in 80% ethyl alcohol, 1 hour in 90% ethyl alcohol, and 3 times (each time for 1 hour) in pure alcohol. The samples were then rendered transparent by soaking in methyl salicylate for 3 hours, and then the Indian ink became visible beyond the apex.

All the samples were maintained in methyl salicylate until the final analysis under stereomicroscope. Microleakage was observed with an image-capture system connected to a stereoscopic microscope at 10× magnification. If the microleakage was more than the whole length of the apical plaque, the measurement of the most coronal point of the plaque was done. The positive and negative control groups acted as we anticipated. The normality of the transformed data sets was confirmed by using the Kolmogorov-Smirnov test. The data sets were analyzed with analysis of variance (One-way ANOVA) to compare the effect of each group. Tukey's HSD test was used to explore the group with the most effects (p<0.05).

**Results**

The results of the study showed that the 0.5% NaOCl group showed significantly less leakage than MTAD and Normal saline groups (p<0.05) (table 1). Moreover, in 40% (6 teeth) of teeth in MTAD group and 46.6% (7 teeth) of teeth in normal saline group, dye penetration was observed in the whole length of apical plaque. This occurrence was not seen in any tooth in chlorhexidine and sodium hypochlorite groups.

**Table 1. The mean microleakage values in the experimental groups (in millimeter)**

<table>
<thead>
<tr>
<th>Experimental irrigating solution</th>
<th>Number of samples</th>
<th>Min-Max</th>
<th>Mean (±SD)</th>
<th>Standard error</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOCl</td>
<td>15</td>
<td>0.359-4.056</td>
<td>1.987±1.247</td>
<td>0/322017</td>
<td></td>
</tr>
<tr>
<td>CHX</td>
<td>15</td>
<td>0.557-4.279</td>
<td>2.674±1.170</td>
<td>0/302226</td>
<td>&lt;0/0001</td>
</tr>
<tr>
<td>MTAD</td>
<td>15</td>
<td>1.589-5.528</td>
<td>3.915±1.406</td>
<td>0/363139</td>
<td>&lt;0/0001</td>
</tr>
<tr>
<td>NS</td>
<td>15</td>
<td>1.030-5.501</td>
<td>3.988±1.594</td>
<td>0/411806</td>
<td></td>
</tr>
</tbody>
</table>

### MTAD in comparison with p<0.01  
### Normal salin in comparison with p<0.001

**Discussion**

MTA is a proper logical substitute for the traditional method of apexification with calcium hydroxide in endodontic application. MTA has many favorable properties, such as biocompatibility, sealing ability, and exacerbation of tissue repair (3). The use of MTA as an apical barrier, has reduced the number of patients’ follow-up visits, and has accelerated the repair procedure of immature tooth. We lack the studies about MTA as an apical barrier.

Available studies emphasized on MTA placement and optimum thickness of MTA apical barrier. There are controversies about proper method of MTA placement. In 2004, Lawley et al. (4) demonstrated that better sealing ability was seen after 45 days in the placement of MTA barrier using an ultrasonic instrument, but there was no significant difference after 90 days. In 2003, Aminoshariae et al. (5). Showed that Manual method of condensing MTA had better adaptation than ultrasonic method. In the study performed by De Leimburg et al. (6) in 2004, there was no statistical difference among MTA plaque with 1mm, 2mm, and 3mm thickness.

In 2004, Matt et al. (7) described that MTA with 5mm thickness had better results in sealing ability compared to 2mm thickness. According to Al-kahtani et al. (8) in 2005, the comparison of 5mm and 2mm MTA plaque had similar results to Martin et al. (9).

In 2010, Yildirim et al. (10) showed no significant difference in bacterial leakage between the 5mm and 2mm thickness of MTA plaque. On the other hand, the divergent anatomy of apical area in blunderbuss root makes the adaptation of MTA or any other material...
difficult. When the root is filled through the apex, the root structure becomes divergent and the access to apical area would not be easy. Since the most apical area of MTA has microleakage, more MTA should be condensed to make better adaptations. In the present study, the anatomy of immature root was reconstructed as blunderbuss root. Because of that, the thicker barrier of MTA was necessary.

In the study performed by Hachmeister et al. (11) in 2002, different thickness of MTA barrier was evaluated and all the apical barriers had microleakage after 70 days. They described that this issue was related to the placement method of MTA in root canal and the leakage has no involvement with MTA.

In the present study, microleakage was observed in all samples. This study suggests that sodium hypochlorite is a proper final irrigator, before MTA barrier placement. This result is based on the mechanism of function of sodium hypochlorite and its penetration to dentine tubules. Sodium Hypochlorite is a fat solvent and breaks down the fatty acids into fatty acid salts (soap) and glycerol (alcohol). This reaction reduces the surface adhesion of remaining solution (12).

This hypothesis is based on Buck et al. (13) in 2001. They described that sodium hypochlorite had the deepest penetration into dentine tubules and normal saline had the shallowest. The deep penetration of sodium hypochlorite into dentine tubules, and its ability to solve the fat and organic material from the dentine surface, results in better adaptation of MTA to dentine and also reduction of microleakage of MTA apical barrier. It seems that smear layer removal with the use of MTAD, before MTA placement as an apical barrier, reduces the MTA sealing ability. These results have similar to Kubo et al. in (14) 2003. They showed that decalcifying materials have negative effects on sealing ability of retrograde inserted MTA.

The results of this study were also similar to the study performed by Yan et al. (15) in 2006 which demonstrated that bond strength between MTA and dentine was reduced by the use of Glyde Prep gel (including EDTA) in comparison with NaOCl and CHX. Besides that, Yildirim et al. (10) showed that 4 weeks irrigation with NaOCl/EDTA increased the microleakage of MTA apical barrier.

The mean microleakage (1.247±1.987) observed in sodium hypochlorite group had been expected, since the adaptation of MTA in the most divergent area of apex was not possible. Since the 5mm plaque was placed, more than 3mm of the plaque remained impermeable. Not only the mean microleakage in MTAD and normal saline groups was not satisfactory, but also in 6 teeth of the first group and 7 teeth of the second group, dye had penetrated in the whole length of plaque, which could be very pleasant. Dye penetration in the whole length of plaque did not occur in any sample of NaOCl and CHX groups.

The amount of microleakage in CHX group was significantly less than NS and MTAD groups and it was not more than NaOCl either. This could be relevant to the number of samples, which was less than the other groups. It has been shown that a good apical and coronal seal is very important in the success of endodontic treatments. There are different methods for analyzing microleakage in dental material, but the evidence shows no objective finding that can prove the superiority of one over the other. The most common methods are: Fluid filtration, bacterial leakage, and dye penetration. In 2001, Pommel et al. (16) showed that there was no statistically solidarity among these 3 methods. Further investigations with different techniques should be conducted to compare the sealing ability of different root obturation methods. Dye penetration is the most common method in microleakage studies (17).

The advantages of this method are: low cost, low toxicity, availability, easy handling, and high accuracy (18). According to Torabinejad (19) in 1993, if a root filling material prevents the penetration of small portions like dye molecules, it is very possible that it can prevent the bacterial microleakage too.

In the present study, linear measurement of Indian ink penetration was used as sealing ability evaluation criteria. This study should be repeated with fluid filtration and bacterial leakage methods, and the results should be compared to avoid any error in clinical practice.

**Conclusion**

This experimental study with extracted human teeth samples demonstrated that final irrigation with 0.5% sodium hypochlorite resulted in better apical sealing ability of MTA in comparison with normal saline and MTAD. The results showed no significant difference between the group of 2% chlorhexidine and other groups.
Effect of different irrigation regimens on the evaluation


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