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Root-end filling materials: apical microleakage and marginal adaptation

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Abstract
The purpose of this study was to evaluate the root-end sealing ability through dye leakage evaluation and the marginal adaptation through scanning electron microscopy (SEM) of some root-end filling materials. Thirty human uniradicular teeth were used. Teeth were divided into three groups: (1) retrofilled with MTA-Angelus, (2) with SuperEBA, and (3) with Vitremer. The root surfaces were isolated with nail polish and teeth were immersed in silver nitrate. Roots were sectioned transversally at each millimeter, in three sections and evaluated at a stereomicroscope to observe dye penetration. Using SEM the distance between the tested root-end filling materials and the surrounding dentin was measured at four points. The statistical analysis showed significant differences among the three materials in relation to the sealing ability (p < 0.05). Concerning marginal adaptation, MTA-Angelus presented the best results (p < 0.01). Absence of correlation between the two methodologies was clearly observed.

Key Words: Root-end filling materials; apical microleakage; marginal adaptation; scanning electron microscopy
Introduction

When nonsurgical root canal treatment fails to treat periradicular lesions of endodontic origin or retreatment is not indicated, endodontic surgery may be indicated (1). The quality of the apical sealing obtained by root-end filling materials has been assessed in different ways such as degrees of dye penetration (2–4), bacterial penetration (5–7) electromechanical ways (8) and fluid filtration technique (9, 10). Studies on dye penetration were considered an easy method to evaluate root-end filling materials (11). Scanning electron microscopy (SEM) has also been used to assess the adaptation and the sealing capacity of commonly used root-end filling materials (12–14). Several features of an ideal apical root-end filling material have been studied. The objective of most researchers is to find a material that presents ideal characteristics and is also a substitute to amalgam in root-end fillings (15, 16). A commonly used alternative is the silicon-reinforced zinc oxide eugenol-based cement containing ethoxybenzoic acid, SuperEBA (15). Various advantages are mentioned such as good apical sealing capacity, marginal adaptation, biocompatibility and low citotoxicity (17, 18). The mineral trioxide aggregate (MTA) was recently reported as a possible sealant to communications between the root canal system and the external tooth surface (11). Its superior apical sealing capacity has been proved by several studies when compared to other root-end filling materials. According to the manufacturer, the chemical composition of MTA-Angelus is: SiO2, K2O, Al2O3, Na2O, Fe2O3, SO3, CaO, Bi2O3 MgO, and some insoluble residues. Duarte (19) states that MTA-Angelus, manufactured in Brazil, is the equivalent product to Pro-Root (7, 20).

Vitremer light curing glass ionomer cement was proved to be an effective sealant to be used in moist environment such as root resections. This material presents no water sensitivity and was proved biocompatible (21). However, there are only a few studies that evaluate this material as a root-end filling material. The purpose of this study was to evaluate the root-end sealing capacity and the marginal adaptation of MTA-Angelus, SuperEBA, and Vitremer as root-end filling materials as well as determine the existence of a correlation between apical microleakage and marginal adaptation in tested materials.

Materials and Methods

The study was approved by the Scientific and Ethics Commission of the Faculty of Dentistry-PUCRS. Thirty extracted human single-root teeth were collected and stored in saline solution for not more than 4 months before the root-end filling procedures. Clinical crowns were sectioned at the cement-enamel junction with a low-speed diamond saw (KG Sorensen Ltd., Brazil) under continuous water spray to create a standardized length of about 16 mm. Teeth were radiographed to evaluate root and apical morphology. All specimens fit the requirements of the study.
A 25 mm #15 Flexofile (Moyco Union Broach, USA) was placed into each canal so that its tip could be seen at the foramen. All teeth were instrumented up to #40 Flexofile using the step-back technique. The irrigant solution was 20 ml of 0.5% NaOCl (Rioquimica Ltd., Brazil) for each tooth. After being cleaned and shaped, canals were dried with paper points (Endopoints Ind. E Co., Ltd., Brazil) and obturated with laterally condensed gutta-percha and Endofill (Dentsply, Brazil). Specimens were stored in saline solution for about 2 months until the moment of apical preparation.

**Figure 1**

(a) Scanning electron micrograph, using BSE, of MTA slice a, evidencing a dye leakage in dentin and inside the retrofilling material ([black small square]) (original magnification ×600).  
(b) Scanning electron micrograph, using BSE, of CIV slice b, evidencing a dye leakage in the dentin tubules ([black small square]), and interface retrofilling material/tooth (+) (original magnification ×1800).  
(c) Scanning electron micrograph using SE, of EBA slice a, showing gaps and their value, between the retrofilling material ([black right pointing small triangle]) and dentin ([black small square]) (original magnification ×1800).  
(d) Scanning electron micrograph using SE, of MTA slice a, showing no gaps between the retrofilling material ([black right pointing small triangle]) and dentin ([black small square]) (original magnification ×1800).

An apical resection at 90° to the long axis of the tooth was made at 3 mm from the end of the root. Root-end cavities of 3 mm were prepared with an ultrasonic tip (S12/90D-Gnatus, Brazil).

Once prepared, teeth were randomly divided in three groups of 10. Root-end fillings were performed in group 1 with MTA Angelus (Odonto-Logika Ltd., Brazil), in group 2 with Vitremer (3M Ltd., Brazil) and in group 3 with SuperEBA (Harry J Bosworth Co, Skokie, IL). Root-end filling materials were mixed and handled according to the manufacturer’s instructions, except for Super EBA that was prepared using a powder to liquid ratio of 2:1 to reach a putty-like consistency. Vitremer was placed after primer with the use of a Mark IIIp syringe (Centrix Inc., USA) and light-cured for 40 s. Immediately after that, the microleakage test was performed.

**Apical Microleakage**

Two coats of nail polish with different colors were applied to the whole surface of each root except for the apex. Teeth were placed in a 50% weight silver
nitrate solution for 1 h and kept in absence of light. Afterward, these were rinsed in running distilled water for 1 min to remove the silver ions of the surface. Thereafter, they were immersed in a photo-developing solution (Dektol, Kodak, Brazil) and exposed to light for 12 h. Teeth were then washed in distilled water and roots were transversally sectioned at each millimeter of the root-end filling with a slow-speed diamond saw. Sections resulted in three slices which were called A, B, and C. Sections were considered first, second, and third according to their distance from the apex. Each slice was mentally divided in four equal parts and measured by one examiner with a stereomicroscope at ×30 magnification. Dye penetration was recorded and scored 0, 1, 2, 3, or 4 according to the amount of microleakage.

With the use of silver nitrate, it was possible to confirm the results of dye penetration tests through the analysis of the images with a back-scattering detector (BSE) and a spectroscopy for energy dispersion (EDS) using SEM. These detectors confirmed the presence of silver in dentinal tubules and tooth/material interface.

Statistical analysis of the results was performed using the Kruskall-Wallis test.

**Marginal Adaptation**

The marginal adaptation was only evaluated in slices A and B, once slice C represented the union of the root-end filling material to gutta-percha and the endodontic cement.

Each section was mounted on an aluminum mounting stub (Electron Microscopic Science, USA) and was placed in a dissector with silica gel, for at least 1 wk. Stubs were sputter-coated with gold and examined under a Phillips XL30 SEM (Phillips, Netherland). The distance between the root-end filling materials and the cavity walls was measured at four points of each slice of the specimen using SEM with 1800× magnification. The means and standard deviation were calculated. All data were submitted to t test.

**Results**

The mean microleakage scores and standard deviations for each root-end filling material are summarized in Table 1. Kruskall- Wallis test (p < 0.05) showed significant differences between SuperEBA and Vitremer in all slices and MTA in slice A. In slices B and C there were statistically significant differences between MTA-Angelus and SuperEBA.

<table>
<thead>
<tr>
<th>Materials</th>
<th>No (teeth)</th>
<th>Mean ± SD (scores)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA-Angelus (slice a)</td>
<td>10</td>
<td>1.6 ± 1.5576</td>
</tr>
<tr>
<td>MTA-Angelus (slice b)</td>
<td>10</td>
<td>0.1 ± 0.3162</td>
</tr>
<tr>
<td>MTA-Angelus (slice c)</td>
<td>10</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Vitremer (slice a)</td>
<td>10</td>
<td>1.3 ± 1.1595</td>
</tr>
<tr>
<td>Vitremer (slice b)</td>
<td>10</td>
<td>2.1 ± 1.5951</td>
</tr>
<tr>
<td>Vitremer (slice c)</td>
<td>10</td>
<td>1.9 ± 1.8529</td>
</tr>
<tr>
<td>SuperEBA (slice a)</td>
<td>10</td>
<td>0.1 ± 0.3162</td>
</tr>
<tr>
<td>SuperEBA (slice b)</td>
<td>10</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>SuperEBA (slice c)</td>
<td>10</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>
Vitremer presented higher microleakage than other groups in all slices. MTA group leaked significantly less than Vitremer in slices B and C. SEM examination of the samples demonstrated variable gaps between materials and dentin walls. MTA presented the smallest gaps. t test showed there were statistical differences between MTA-Angelus and the other root end filling materials (p < 0.01). MTA - Angelus was also the best root-end filling material considering marginal adaptation. There were no statistical differences between SuperEBA and Vitremer (Table 2).

**TABLE 2.** Comparison of mean gap (µm) and standard deviation (SD) of retrofilling materials in experimental group.

<table>
<thead>
<tr>
<th>Materials</th>
<th>No (teeth)</th>
<th>Mean ± SD (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTA-Angelus (slice a)</td>
<td>10</td>
<td>0.812 ± 0.550</td>
</tr>
<tr>
<td>MTA-Angelus (slice b)</td>
<td>10</td>
<td>1.051 ± 3.346</td>
</tr>
<tr>
<td>Vitremer (slice a)</td>
<td>10</td>
<td>2.858 ± 1.837</td>
</tr>
<tr>
<td>Vitremer (slice b)</td>
<td>10</td>
<td>4.632 ± 3.024</td>
</tr>
<tr>
<td>SuperEBA (slice a)</td>
<td>10</td>
<td>2.384 ± 2.123</td>
</tr>
<tr>
<td>SuperEBA (slice b)</td>
<td>10</td>
<td>3.778 ± 2.904</td>
</tr>
</tbody>
</table>

The absence of correlation between the two methodologies was clearly observed through the Linear Correlation of Pearson (p < 0.05). This could be explained because the T values calculated for A and B slices and for their interaction according to Pearson Linear Correlation Coefficient were lower than the T0.05(28) value, showing the absence of correlation between apical microleakage and marginal adaptation in this study.

**Discussion**

Several methodologies can be used to evaluate apical microleakage. Among these we could mention filtration fluid (10) and bacterial leakage tests (22). However, we agree with Aqrawabi (11) who stated that if root-end filling materials were able to prevent the leakage of small particles such as dye, they would possibly prevent the penetration of bacteria and their sub-products. Various substances have been used to delineate apical microleakage. In this study, silver nitrate was used as a dye, because according to Wu et al. (23) it presents greater clearness and contrast and its use allows evaluations by SEM. Resin replicas were not made because they could hinder the silver nitrate visualization in the samples where dye leakage occurred. In the study of Torabinejad et al. (1), resin replicas were prepared and it was observed that the gap rates were similar between teeth and replicas. This emphasizes that the direct analysis of teeth could be carried out with no alterations of the results by the introduction of artifacts in the samples. SuperEBA is widely studied for root-end filling procedures and it has presented favorable characteristics. In 1999, Yaccino et al. (24), concluded that the thicker the mixture, the better the capacity of apical sealing of the material. In the dye leakage tests it was observed that SuperEBA was better than MTA-Angelus and Vitremer. These results can be compared to those by O’Connor et al. (25); Suntimuntanakul et al. (26); and Greer et al. (27), who observed that SuperEBA
presented an excellent sealing capacity when compared to some other root-end filling materials. MTA-Angelus manufactured in Brazil was chosen because it presents a similar composition to Pro-Root, according to the manufacturer. Duarte et al. (19) have demonstrated that both materials release calcium and provide alkaline environment. Besides, when used in direct pulp capping or pulpotomy both materials were biocompatible and effective to produce complete pulp healing. Menezes et al. (28) also showed that the tissue reactions were identical for Pro-Root and MTA-Angelus. Various studies reported that MTA presented excellent apical sealing, and demonstrated its superiority in comparison to other commonly used materials (6, 7, 9, 11, 16, 29, 30). Nevertheless, many of these studies were made with the use of methylene blue as a dye. The doubt that remains in relation to the validity of results is the fact, proven by Wu et al. (31), that MTA causes methylene blue discoloration.

In this study, MTA-Angelus demonstrated an intermediate behavior in the prevention of dye leakage, differently from most results observed in literature. The results of MTA group showed a very important characteristic, which was the silver nitrate penetration visualized both inside the root-end filling material and in the adjacent dentin in practically all the samples of slice A. Perhaps this fact could be explained by the acid pH of the dye, by a possible chemical interaction between the material and the silver nitrate, or even by the possibility of the material not being completely solidified at the moment of the dye immersion.

The findings in MTA group suggested that new studies using this methodology with different dyes, varied pH solutions, and distinct moments of material insertion in the retrocavity and in the dye may be needed to find the explanation for these results. MTA-Angelus marginal adaptation was better than the other tested materials. These results were similar the ones found by Torabinejad et al. (1); Peters & Peters (32) and Gondim et al. (14).

Vitremer presented the highest rates of dye penetration and in some samples it showed total penetration of silver nitrate. Vitremer was tested by Pretorius & Van Heerden (21) and presented excellent results. On the other hand, our results are in accordance with other studies in which glass ionomer cement was not good in preventing apical microleakage (5, 33).

In relation to the two types of variables that were analyzed in this study, we can evidence a clear lack of correlation between apical microleakage rates and gaps in the interface tooth/root-end filling material. These findings are contrary to Stabholz et al. (13). However, our results were similar those found by Abdal & Retief (12) and Yoshimura et al. (34) that also reported lack of correlation between microleakage and marginal adaptation of the materials. Based on our results and the researched literature, we were able to observe that the lack of gaps in the root-end filling material/tooth interface did not hinder the dye penetration. According to Gilheany et al. (35) that demonstrated leakage through dentinal tubules sectioned during apicoectomy, apical leakage can occur through other ways.

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References


