An investigation of microleakage from root-end fillings in ultrasonic retrograde cavities with or without finishing: A quantitative analysis

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Objective. The aim of this study was to make a quantitative assessment of the sealing ability of Super-EBA, IRM, and Pro Root MTA root-end fillings subjected to 3 different finishing techniques.

Study design. Eighty-one ultrasonically prepared root-end cavities in human canines were separated randomly into 3 test groups of 27 roots each. The cavities were filled with Super-EBA, IRM, or Pro Root MTA and finished by ball burnishing. Sequentially, 18 roots from each group received a final smoothing with either a 30-fluted tungsten carbide finishing bur or a Zekrya carbide 28-mm bur. Samples were prepared and immersed in 2% methylene blue dye neutral solution for 12 hours. Roots were ground into a powder and prepared for analysis in an absorbency spectrophotometer.

Results. The results revealed that Pro Root MTA displayed significantly less mean dye microleakage \((P < .05)\) than Super-EBA and IRM root-end fillings. The Super-EBA root-end fillings, although presenting a greater mean dye microleakage, did not differ significantly \((P > .05)\) from IRM. The finishing technique did not significantly \((P > .05)\) affect the incidence of microleakage among the materials tested.

Conclusions. The favorable results obtained with MTA in leakage studies may be related to its good marginal adaptation. Spectrophotometric analysis may provide valuable information about the sealing capacity of root-end fillings. None of the procedures tested were able to avoid leakage, a finding that stresses the importance of the eradication of irritants within the root canal system.


Conventional root canal treatment does not always result in clinical success. Some irritants may remain after cleaning and shaping the root canal system, which could gain access to the periapical tissues through the interface between the filling materials and the root canal wall dentine. The major objective of periradicular surgery, with placement of an adequate root-end filling, is to provide an apical seal that inhibits this migration of irritants.\(^1\)

A number of materials have been evaluated for use as root-end fillings. They include amalgam, gutta-percha, zinc oxide eugenol cements, composite resins, glass ionomer, polycarboxylate cements, ethoxybenzoic acid (EBA) cement, and mineral trioxide aggregate (MTA). According to Arens et al,\(^2\) an ideal root-end filling material should be easy to manipulate, radiopaque, dimensionally stable, nonabsorbable, nontoxic, promote healing, and not be affected by the presence of moisture. No material has yet been found to meet all these requirements. Apparently some of these materials have been derived from their use in conservative dentistry, where the criterion for success is, in some ways, different.\(^3\) Amalgam is still used, even though there have been claims regarding its disadvantages, such as irritant effects from its corrosion products, galvanic
interaction, tissue discoloration, and introducing mercury into the body. Zinc oxide-eugenol—based cements are sensitive to moisture, are partially soluble in oral fluids, and may cause irritation of periapical tissues. On the other hand, good clinical results were reported with Super-EBA and intermediate restorative material (IRM) compared to amalgam root-end fillings. In a histological assessment, Trope et al also described that in an overall periapical condition, Super-EBA and IRM were statistically superior to composite resin, glass ionomer, and amalgam when used as root-end filling materials in dogs.

MTA seems to be equal or superior to the other materials used as root-end fillings, with respect to leakage, cytotoxicity, and marginal adaptation. Furthermore, the formation of cement and periodontal ligament fibers adjacent to MTA was shown in animals.

It is assumed that the better the marginal adaptation of root-end fillings, the fewer irritants would pass through the interface between the filling materials and the root canal wall. The burning of Super-EBA and IRM with ball burnishers or curettes was recommended to ensure marginal adaptation and prevent the open margins that could occur when these materials are wiped with wet cotton pellets.

In a scanning electron microscope study, the marginal interfaces between tooth structure and root-end filling material was evaluated, following 3 IRM and EBA finishing techniques. The root-end fillings were finished by ball burnishing, burnishing with a moistened cotton pellet, or a carbide finishing bur; the latter displayed significantly better marginal adaptation.

The degree of apical seal adaptation and quality achieved by root-end filling materials has been evaluated by the use of dyes, radioisotopes, bacterial penetration measurements, scanning electron microscopy, electrochemical means, and fluid filtration techniques.

The purpose of this study was to make a quantitative assessment of the sealing ability of Super-EBA, IRM, and Pro Root MTA root-end fillings submitted to 3 different finishing techniques.

METHODS

The study was approved by the Piracicaba Dental School Ethics Committee for Human Research. Eighty-one freshly extracted, periodontically affected canines were selected for this study. After careful extraction, all teeth were stored in a 2.0% formaldehyde solution in distilled water for no longer than 3 months prior to the root-end filling procedures.

Crowns were cut off at the cemento-enamel junction with a low-speed diamond wafer saw (Isomet, Buhler Ltd, Lake Bluff, Ill) under continuous water spray. The integrity of the apical third was determined using an operating microscope (M900, DF Vasconcellos; São Paulo, SP, Brazil) at ×20 magnification. In an attempt to avoid further damage, none of the teeth were submitted to endodontic treatment or were allowed to desiccate.

Root-end cavities and their replicas were made in the same manner as described in previous studies. The 81 roots were randomly assigned into 3 test groups of 27 each. The root-end cavities were dried with paper points (Tariman Ltda; Manaus, AM, Brazil) and a P-40 plugger (Dentsply-Maillefer; Ballaigues, Switzerland) was placed 3 mm from the apical opening of each root to provide for adequate root-end filling material condensation. Group 1 samples were filled apically using fast-set Super-EBA cement (Harry J. Bosworth Co, Skokie, Ill) placed in the cavities and condensed thoroughly with a P-1 double-ended plug (Analytic, SybronEndo; Orange, Calif), followed by additional material until a surplus was seen to emerge from the cavity margins. The root-end surface was burnished with a B-3 condenser/ball burner (Analytic, SybronEndo) to remove excess and improve the adaptation of the material. Roots in group 2 were filled with Pro Root MTA (Dentsply-Tulsa Dental; Tulsa, Okla) placed in the cavities and burnished in the same manner as for group 1. Group 3 received the IRM (L. D. Caulk Co; Milford, Del) also placed in the cavities as performed for group 1. All materials were mixed according to the manufacturer’s directions. Immediately following burnishing, the restored roots, except for the MTA group, were stored in water at 37°C for 24 hours to ensure setting of the filling materials. MTA root-end fillings were allowed to set in moist gauze for 2 hours before the final immersion in water for 24 hours.

Specimens from each experimental group were randomly divided into 3 subgroups of 9 roots each. Two of these subgroups received a final smoothing with either a 30-fluted tungsten-carbide tapered finishing bur (Jet-Sybron; Morrisburg, Ontario, Canada) or a Zekrya carbide 28-mm bur (Dentsply-Maillefer) in a high-speed air rotor handpiece with water spray. Final smoothing was applied to these roots by directing the bur across the root-end surface in a forward direction, as described by Weston et al. A new bur was used for every 3 roots, and an attempt was made to produce the smoothest possible surface in all specimens. The remaining 3 subgroups (1 filled with MTA,
The root-end cavities, root-end fillings, and the final smoothing were performed by 1 operator (E.G.J.) using an operating microscope (M900, DF Vasconcellos) at 13 magnification. After all procedures the root-ends were relabeled by another individual, so that the operator would not perceive what kind of material or finishing technique was used during the leakage evaluation. Radiographs of all root-end fillings were taken to ensure that there were no imperfections in the apical third.

The method used to evaluate leakage in this study was adapted and modified from that reported by Aguiar et al. The apical 4 mm of each specimen were cut perpendicularly to the long axes of the roots with a low-speed diamond wafer saw (Isomet) under a continuous water spray. These apical sections were embedded in epoxy resin to facilitate handling and to allow them to be immersed in the methylene blue solution (Fig 1).

A plastic matrix was placed over a wax tablet and the root-end was positioned in the center of the matrix with the resected root-end surface toward the wax tablet. A 3-mm2 piece of tracing paper was placed between the root-end surface and the wax tablet to avoid possible contamination or penetration of wax into the root-end filling/root canal wall interface. The epoxy resin was poured into the matrix, and after 8 hours the epoxy resin cylinders were removed from the matrix and stored in distilled water at 37°C for 24 hours prior to immersion in the dye solution.

Next, the interface between the apical portion and the epoxy resin of all samples was protected by an instantaneous cyanocrylate adhesive Superbond (Henkel Loctite Adhesives Ltda; Itatiaia, SP, Brazil). The root ends were immersed in a neutral aqueous 2% methylene blue solution (Fórma e Ação; São Paulo, SP, Brazil) for 12 hours at 37°C; samples were rinsed in tap water and dried. The surface layer of the root-end fillings was abraded with aluminum oxide disks Sof-Lex Pop-on (3M Dental Products; St Paul, Minn) to remove possible superficial dye absorption by the restorative material.

The 4-mm apical portions were removed from the epoxy resin cylinders and each one was weighed prior to and after being ground into a powder in a mill for hard tissues (Marconi Equip Ltda; Piracicaba, SP, Brazil). If the difference between the initial and the final weight was greater than 10%, the sample was discarded. The powder of each block was individually immersed in a glass tube containing 4 mL of absolute alcohol PA (Merck KGaA; Darmstadt, Germany) for 24 hours in order to dilute the methylene blue dye. The solutions were centrifuged (Tomy-IC-15AN, Tomy Seiko Co; Tokyo, Japan) at 3000 rpm for 3 minutes, and the supernatant was analyzed using a spectrophotometer DU 65 (Beckman Instruments Inc; Fullerton, Calif) adjusted to a 668-nm wavelength.

In order to determine absorbance, the spectrophotometer was adjusted to a wavelength appropriate for measuring methylene blue, corresponding to the maximum absorbency of the dye. To calibrate the spectrophotometer, the absorbency of the standard solutions (0.1, 0.2, 0.3, 0.5, 1, 2, 4, 6 μg/mL) was determined at peak absorbency at 668 nm. A coefficient of linear correlation (r = 0.9998) and a straight-line equation (y = a + bx) were determined. The following relationship was obtained: Absorbency = 0.2716 × (Dye concentration) – 0.0075. To quantify the dye concentration (μg/mL) leaked between the tooth and the restoration, the “y” was substituted for the absorbency value of each sample.

Analysis of variance and Tukey tests (with the level of significance set at 95%) were used to compare the microleakage related to the root-end filling materials after final smoothing with burs.

RESULTS

Radiographs revealed that all roots were prepared and filled to the appropriate depth. No samples were replaced or excluded from the study because of an improper root-end filling technique or excessive loss of weight after being ground to a powder.

No test material was able to prevent microleakage. Considering root-end filling materials, the Tukey test (Table I) revealed that MTA displayed a significantly lower mean dye microleakage (P < .05) than EBA and IRM retrofillings. The Super-EBA retrofillings, although presenting a greater mean dye microleakage, did not differ significantly (P > .05) from IRM. Considering the finishing technique, the comparisons between averages by simple statistics are listed in Table II.

Finally, root-ends finished with a ball burnisher, although not differing significantly from those finished with Zekrya or 30-fluted carbide burs, displayed the greatest mean dye leakage.
DISCUSSION

In an attempt to avoid tooth damage, vital teeth were obtained by careful extraction. In an effort to avoid root dehydration and extensive manipulation, root canals were not treated. These factors may be responsible for the cracking patterns obtained in in vitro studies.20 Furthermore, any modification in the original root canal diameter would influence the ultrasonic root-end instrumentation time, jeopardizing the root-end diameter similarity achieved after controlled instrumentation. Gutta-percha removal during cavity preparation would also make it difficult to standardize the timing of instrumentation.

A large number of in vitro studies dealing with the marginal adaptation and sealing ability of retrograde materials have been published. The methodology of these leakage studies and their results, which are often contradictory, is being questioned, with regard to its clinical relevance. Factors such as the choice of storage solution, pH, molecular weight of the dye, the use of conventionally filled or unfilled root canals, and whether the crown and the root (except the cut surface) were isolated from the dye, as well as a great deal of other variables, can crucially influence the outcome in these in vitro studies. The most popular method for leakage assessment, namely, linear measurement of tracer, should be considered as a semiquantitative technique, since it does not provide any information about the volume of tracer that penetrates a root filling.21 Furthermore, this kind of leakage study is difficult to reproduce and compare, since the linear measurements were made after distinct procedures (longitudinal splitting, cross sectioning, clearing of the specimens).21 In spite of these dissimilar procedures and the uncertain relationship between in vitro and in vivo studies, root dehydration and inappropriate storage may play a decisive role in the outcome of the results. It should also be taken into account that common procedures for sterilizing specimens used in bacterial microleakage models (autoclaving and ethylene oxide) may alter the structure of dentine.22

The use of a fluid transport system23 may avoid the problems caused by entrapped air or fluid hindering the diffusion of tracer through the restoration, however the use of positive pressure may have no clinical relevance. Whether entrapped air can be eliminated from small voids along a root canal filling by applying reduced pressure still remains questionable.24

Methylene blue solution penetrating through the root-end filling does not indicate the passage of bacteria, however the leakage of bacterial by-products, like metabolites, toxins, and degradation products smaller than bacteria but of high molecular weight, may occur and play a decisive role in periapical disease.25

In this study, several modifications of the methodology used in previous studies were made.26,27 Including optical wavelength, immersion time, and the use of a 4-mm apical portion of the root. The most significant modification, however, was the use of a hard tissue mill for grinding the apical portions into a powder rather than using nitric acid to dissolve the roots. Nitric acid might not only lead to complete dissolution of the dye present in dentin and cement, but would probably also make it difficult to make accurate recovery of tracer present in the root-end filling material interface, since the materials would behave differently in the presence of acid. Furthermore, the dissolution in acid would be another variable to be taken into account, since the methylene blue solution may be significantly discolored in the presence of acid.28

One problem that may be encountered by using the method is that sample weight invariably changes following root grinding. However, thanks to careful gathering of fine particles, none of the samples were modified by more than 5% of their original weight.

It could be expected that the amount of dye recovered from the root would be determined from the size of the tooth.27 In this study, the use of a standardized 4-mm root-end cavity and limitation of immersion time, not allowing the tracer to penetrate the total volume of the tooth, may have promoted a more accurate assessment of relative leakage.

The use of a short immersion period can also be justified by the possible discoloration of the dye following contact with the test material. Wu et al.29 studied the stability of methylene blue color following

### Table I. Means and standard deviations of calculated marginal dye leakage for retrofilling materials

<table>
<thead>
<tr>
<th>Retrofilling Material</th>
<th>n</th>
<th>Marginal leakage values (µg/mL)</th>
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<tbody>
<tr>
<td>Super EBA</td>
<td>27</td>
<td>0.0813 ± 0.1280*</td>
</tr>
<tr>
<td>IRM</td>
<td>27</td>
<td>0.0519 ± 0.0528*</td>
</tr>
<tr>
<td>Pro Root MTA</td>
<td>27</td>
<td>0.0309 ± 0.0048</td>
</tr>
</tbody>
</table>

Means followed by different labels are statistical different by Tukey’s test (.05).

*P > .05.

### Table II. Means and standard deviations of calculated marginal dye leakage for finishing techniques

<table>
<thead>
<tr>
<th>Finishing technique</th>
<th>n</th>
<th>Marginal leakage values (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30-fluted bur</td>
<td>27</td>
<td>0.0593 ± 0.1180</td>
</tr>
<tr>
<td>Zekrya bur</td>
<td>27</td>
<td>0.0345 ± 0.0073</td>
</tr>
<tr>
<td>Ball burnisher</td>
<td>27</td>
<td>0.0703 ± 0.0766</td>
</tr>
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contact with 6 dental filling materials, including MTA and zinc oxide-eugenol. The authors measured the optical density of a 1% methylene blue solution spectrophotometrically prior to and after 24, 48, and 72 hours of immersion. Results showed that the optical density value of methylene blue decreased for the MTA group after all periods of immersion. The rate of discoloration obtained in the above-mentioned research cannot be related to that obtained in this study because of the different methylene blue solution concentration used in the latter, and because a 12-hour period of immersion was not tested in the former. It may be expected that a more concentrated solution kept in contact with the dental filling materials for only 12 hours would have shown only a minimal change of its color stability.

In a number of leakage studies in vitro, EBA cement has been shown to lead to less leakage when compared with many other retrograde filling materials.\(^{30,31}\) Clinically, the use of EBA also produced acceptable results.\(^{3}\) However, under the experimental conditions of this study in vitro, the EBA and IRM cements presented a significantly worse apical seal than MTA, as also reported by other studies.\(^{8}\)

It would seem that MTA, as a root-end filling material, provides an apical seal at least comparable to most root-end filling materials described in the literature.\(^{8,32,33}\) This greater sealing capability of MTA is probably related to its superior marginal adaptation\(^{17}\) and to the possible expansion of the material while setting in a moist environment.

There is a constant search for materials and restorative techniques to overcome the disadvantages of current materials. However, clinicians should be aware of the many factors that interfere with the success of a root-end filling being carried out, such as the configuration of the cavity, the amount and quality of the remaining dental structure, and, predominantly, the elimination of potential causes of persistent periapical inflammation. It also should be considered that it requires practice to handle MTA, and that care must be taken not to wash out or disturb the material after placement.

The favorable results obtained with MTA in leakage studies may be related to its good marginal adaptation. Spectrophotometric analysis may provide valuable information about the sealing capacity of root-end fillings. All the procedures currently in use were unable to prevent leakage, demonstrating the importance of the eradicating irritants coming from the root canal system.

The authors very much appreciate the help and advice given by Dr Flavio Henrique Aguiar. We are also thankful to Dr Jose Roberto Lovadino, Assistant Professor, Department of Restorative Dentistry; Jose Ranali, Professor and Chairman; and Francisco Carlos Groppo, Assistant Professor, Department of Pharmacology, School of Dentistry of Piracicaba, State University of Campinas-UNICAMP, for generously providing part of the equipment used in this study. This publication was based on a thesis submitted by the first author to the Faculty of Dentistry of Piracicaba, University of Campinas, in partial fulfillment on the requirements for a PhD degree in Endodontics.

**REFERENCES**


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