

Influence of the thickness of mineral trioxide aggregate on sealing ability of root-end fillings in vitro

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The purpose of this study was to compare the ability of different thicknesses of mineral trioxide aggregate (MTA) to prevent apical leakage through the use of a protein-dye complex with Coomassie Brilliant Blue G. Sixty-four teeth were divided into 4 groups, then filled with MTA to depths of 1, 2, 3, or 4 mm. Two teeth served as the positive controls. Another 2 teeth served as the negative controls. Root-filled teeth were mounted in an apparatus and then challenged with protein solution. The evaluation was conducted at 24-hour intervals for 60 days. The 1-mm-thick MTA was the least effective in preventing apical leakage ($P < .05$). No significance difference was found between 2- and 3-mm-thick MTA ($P > .05$). Four-millimeter-thick MTA was significantly more effective than the other thicknesses tested ($P < .05$). The results of this study suggest that the thickness of 4 mm is most adequate for the use of MTA as a root-end filling material. (**Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:108-11**)

Surgical endodontic treatment may be necessary if healing fails to occur after conventional endodontic treatment or if conventional treatment cannot be performed. This treatment usually consists of raising a mucoperiosteal flap, ostectomy, root-end resection, root-end preparation, and root-end filling. An effective root-end filling material must provide a complete apical seal, preventing the movement of tissue fluids into the root canal system and the egress of microorganisms and their by-products from the root canal system.¹

Mineral trioxide aggregate (MTA) has been proposed as a root-end filling material. Researchers have reported that the sealing ability of MTA is superior to that of amalgam and gutta-percha and equal or better than intermediate restorative material and Super-EBA.²⁻⁴ However, in most previous studies, the thickness was an uncontrolled variable; therefore, the results obtained in these studies may be considered inconclusive. There are no studies that correlate marginal leakage with the thickness of the retrograde MTA.

An evaluation of root-end filling materials to provide an apical seal has been assessed in terms of the penetration of radioisotope, dye, and bacteria. Each technique has significant limitations that can result in errors.⁵⁻⁷ As a result, the validity of the data obtained has been questioned.⁸ We believe that there is currently no definitive in vitro method for the analysis of root-end microleakage analysis. The purpose of this study was to

compare the ability of different thicknesses of MTA to prevent leakage through the use of a protein-dye complex with Coomassie Brilliant Blue G.

MATERIAL AND METHODS

Protein solution

The protein solution used in this experiment was bovine serum albumin at 22% obtained from Prothemo Produtos Hemoterápicos Ltda (São Paulo, Brazil) and maintained at 4°C until use.

Preparation of protein reagent

The reagent was prepared as described by Bradford.⁹ Coomassie Brilliant Blue G (B5133; 100 mg; Sigma Chemical Co, St Louis, Mo) was dissolved in 50 mL of 95% ethanol. In this solution, 100 mL of 85% phosphoric acid was added. The resultant solution was diluted with distilled water to a final volume of 1 L. The final concentrations in the reagent were 0.01% (wt/vol) Coomassie Brilliant Blue G, 4.7% (wt/vol) ethanol, and 8.5% (wt/vol) phosphoric acid. The reagent was filtered and stored in a dark glass vial maintained at 4°C.

Tooth preparation

Sixty-four single-rooted, caries-free, human maxillary teeth with straight canals were selected for this experiment. The teeth were kept in a 1% sodium hypochlorite (NaOCl) solution overnight for surface disinfection and were later stored in a phosphate-buffered saline solution until used. Sixty-two teeth were prepared as follows: standard access cavities were made with a No. 1012 diamond bur, and the coronal portions of the canals were flared with Gates-Glidden burs (Nos. 3 and 4). The apical foramen was enlarged with a No. 30 file to standardize the diameter. The preparation was completed by using a step-back of 1-mm increments.

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Two milliliters of 2% NaOCl solution was used as an irrigant between the use of each file to eliminate debris. Under a continuous water spray, the apical 3 mm of roots were cut at 90° to the long axis of the teeth, with a No. 3097 diamond bur in a high-speed handpiece. Two layers of nail polish were applied to the external surfaces of all roots to prevent leakage through the root surface.

Experimental groups

After root-end resection, 60 teeth were randomly distributed into 4 equal test groups containing 15 teeth each. Class I cavities were then prepared in root ends with a high-speed No. 330 carbide bur under water-cooling conditions as follows: group I, the cavities were prepared to a depth of 1 mm; group II, the cavities were prepared to a depth of 2 mm; group III, the cavities were prepared to a depth of 3 mm; group IV, the cavities were prepared to a depth of 4 mm. Saline solution was used to clean the root-end cavities. The root-end cavities were dried with paper points and filled with MTA (Dentsply Tulsa Dental, Tulsa, Okla) according to the manufacturer's recommendations. Pluggers with a snug fit were used as a matrix against which the root-end materials were condensed. Radiographs were made to evaluate the quality of the retro-end fillings. Two teeth with the apical cavity prepared and an open access served as the positive controls. Another 2 teeth with intact crowns served as the negative controls.

Protein leakage test

The apparatus used to evaluate protein leakage was prepared by using a glass vial with a rubber stopper and a cylinder prepared from a 20-mL plastic syringe (Fig 1). Initially, a heated instrument was used to make a hole through the center of every rubber stopper. Immediately after the root-end filling, each tooth was inserted under pressure in the hole so that its root-end was outside of the vial and its crown within the vial. Syringe cylinders were then adapted to the external surface of the stoppers to create a reservoir around the root-end of the tooth. Rapid-setting cyanoacrylate was applied in the interfaces tooth/stopper and cylinder plastic/stopper.

The glass vials were filled with distilled water so that the coronal surface of each tooth was immersed in the solution. The reservoir of the apparatus was filled with 3 mL of protein solution and coated with aluminium foil. The whole apparatus was maintained in 100% humidity at 37°C during the 60-day experiment. At the same time each day, the water in the glass vial was refreshed and the protein solution of the reservoir was

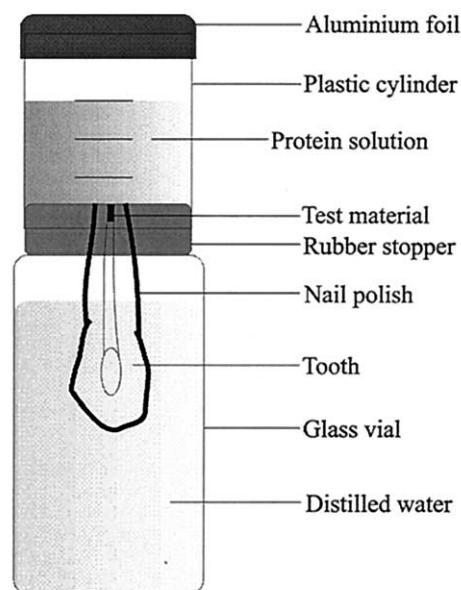


Fig 1. Apparatus used to test protein leakage.

Table. Distribution of teeth retrofilled with MTA of different thicknesses that exhibited protein leakage after 60 days

Group	Total (n)	Leakage	%	Range (d)
Positive controls	2	2	100	1
Negative controls	2	0	0	–
1-mm-deep cavity	15	15	100	6-33
2-mm-deep cavity	15	7	47	10-53
3-mm-deep cavity	15	6	40	14-49
4-mm-deep cavity	15	1	7	31

replenished. The water of the glass vials (test solution) was transferred into test tubes.

Protein assay

The protein reagent (1 mL) was pipetted into a test tube. One hundred microliters of the test solution was added to the test tube, and the contents were mixed. This was repeated each day for 60 days or until color conversion was observed. The number of days it took until color conversion of the protein reagent was taken to be indicative of apical leakage.

Statistical analysis of the data was carried out through 1-way analysis of variance and the Tukey test with the significance level established at 5% ($P < .05$).

RESULTS

The specimens in the positive-control group exhibited color conversion of the protein reagent to the blue

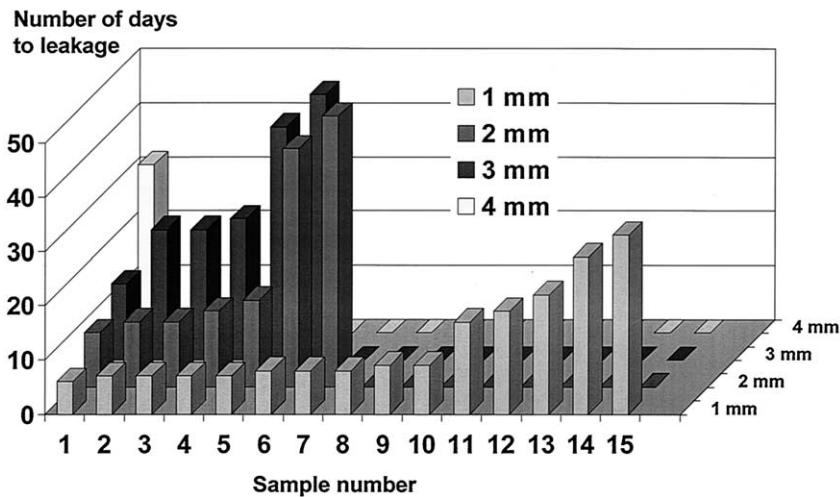


Fig 2. Number of days until leakage was detected for each of the 15 samples in each group.

color within 1 day of the beginning of the experiment. By contrast, in the negative-control group, there was no evidence of color alteration throughout the experiment. All samples in the 1-mm-deep cavity group leaked within 6 to 33 days. After 60 days of evaluation, protein leakage was observed in 47% (7/15 specimens) of the teeth with 2-mm-deep cavities and in 40% (6/15 specimens) of the teeth with 3-mm-deep cavities. Seven percent (1/15 teeth) of the teeth with 4-mm-deep cavities exhibited leakage. Data are shown in the Table and in Figure 2.

One-way analysis of variance was performed on the data. The analysis revealed statistically significant differences among the 4 test groups ($P < .05$). Multiple comparisons, according to the Tukey test, revealed significant statistical differences among the groups: the 4-mm-thick MTA was significantly more effective in preventing protein leakage than were the other MTA thicknesses tested ($P < .05$). The differences between 2- and 3-mm-thick MTA were not statistically significant ($P > .05$). The 1-mm-thick MTA was found to be less effective in preventing apical leakage ($P < .05$).

DISCUSSION

The most widely used methods to test the quality of root-end filling materials are the dye-penetration techniques, which make use of methylene blue and India ink. However, most dyes have a low molecular weight and can penetrate sites that protein and bacteria cannot. Most dye leakage studies have measured the degree of leakage in 1 plane, making it impossible to evaluate the total leakage.^{5-6,10} pH and chemical reactivity may also influence the degree of dye penetration.¹⁰

Because of the inherent inadequacies of tracer sub-

stances and dye for the evaluation of apical seal, leakage studies making use of saliva or bacterial culture have been recommended to test the suitability of potential root-end filling materials.^{2-4,10} However, the time needed for the bacteria to grow is within 24 to 48 hours from contamination, which can result in inaccurate data.⁷ In addition, normal saliva—which usually harbors several different bacterial species—may require different conditions of temperature, pH, and oxygen to grow in the laboratory.⁷

The use of protein-dye complex in this experiment provided the advantage of eliminating the problems involved with radioisotope, dye, and bacteria during leakage identification. This method is based on the observation that Coomassie Brilliant Blue G is converted to the blue color when in contact with the protein.^{9,11} Moreover, the protein-dye complex method had great sensitivity in protein identification and low sensitivity to interference from nonprotein compounds. In addition, the process is rapid (ie, it takes approximately 2 seconds) and reproducible and the protein-dye complex remains dispersed in the solution for a long period of time (weeks), not requiring critical timing for the assay; therefore, substantial numbers of samples can be evaluated at the same time.

The purpose of placing a root-end filling after root-end resection and preparation is to establish an effective barrier between the root canal and the periapical tissues. Investigators have suggested that insufficient apical seal is a major cause of endodontic surgical failure.¹ MTA as a root-end filling material provides a better apical seal than materials such as amalgam, intermediate restorative material, Super-EBA, and gutta-percha.²⁻⁴ Apart from selecting the sealing material, an-

other important factor that is under the direct control of the operator is the depth of the root-end filling. Some researchers have placed MTA into 2.5-mm-deep cavity preparations³; others have used 3-mm-deep cavity preparations^{2,4,12} or have suggested 4-mm-deep cavity preparations.¹²

The sealing ability of MTA is attributable to its hydrophilic nature and expansion when cured in a moist environment.¹² We have shown that the optimum depth of the retrograde cavity must be reached to achieve an adequate apical seal. Torabinejad and Chivian¹² have advocated 3- to 4-mm-thick MTA in endodontic surgical techniques with class I preparations, but our results here indicate that 4-mm-thick MTA has sealing efficacy that is significantly superior to that achieved with 3-mm-thick MTA. In addition, no significant difference was found between leakage patterns of the 2- and 3-mm-thick MTA groups. The 1-mm-deep cavities were shown to be less effective in preventing apical leakage. These findings suggest that of the thicknesses evaluated here, the 4-mm-thick MTA is the most adequate when used as root-end filling material.

Under the experimental conditions of this study, the depth increase of a root-end filling reduced apical leakage. However, one must remember that this method is a static model that does not represent clinical conditions found in the periradicular region, such as the dynamic interaction between the root canals and the periradicular tissues. Thus, direct extrapolation to a clinical situation must be exercised with caution.

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