Sealing Properties of Mineral Trioxide Aggregate Orthograde Apical Plugs and Root Fillings in an In Vitro Apexification Model

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Abstract

One-visit mineral trioxide aggregate (MTA) apexification is gaining in popularity over the use of calcium hydroxide barriers. This study examined the sealing properties of two MTA apexification procedures using an in vitro apexification model. White MTA was introduced into instrumented single-rooted canals with standardized artificially created open apices to form 3- to 5-mm-thick orthograde apical plugs or complete orthograde fillings of the root segments. The remaining canal spaces in the former group were backfilled with thermoplasticized gutta-percha without a sealer. A Flodec fluid filtration device was used to record fluid flow across filled root canals at 48 hours and after 4 weeks of immersion in phosphate-buffered saline (PBS). Although MTA root fillings exhibited a better seal than MTA apical plugs at 48 hours, seals of these two groups were not significantly different after 4 weeks. Interaction of MTA with PBS may result in apatite deposition that improves the seal of MTA apical plugs with time. *(J Endod 2007;33:272–275)*

Key Words

Apexification, apical plug, fluid filtration, mineral trioxide aggregate, orthograde root filling

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Endodontic treatment of immature necrotic teeth with necrotic pulps and open apices involves induction of apical closure by apexification procedures to create more optimal conditions for conventional root canal fillings (1). The traditional use of calcium hydroxide [$Ca(OH)_2$] apical barriers has been associated with unpredictability of apical closure (2), risks of re-infection resulting from the difficulty in creating long-term seals with provisional restorations (3), and susceptibility to root fracture arising from the presence of thin roots (4, 5) or prolonged exposure of the root dentin to $Ca(OH)_2$ (6, 7). Thus, there is increasing popularity with one-visit apexification procedures that use mineral trioxide aggregate (MTA) as osteoconductive apical barriers (8–12). MTA is relatively non-cytotoxic (13) and stimulates cementogenesis (14). The Portland cement–based material generates a highly alkaline aqueous environment by leaching of calcium and hydroxyl ions (15), rendering it bioactive by forming hydroxyapatite in the presence of phosphate-containing fluids (16). Unlike the extended use of $Ca(OH)_2$ in immature roots, prolonged filling of these roots with MTA did not reduce their fracture resistance (17).

Although MTA provides a reasonable seal in root-end fillings and perforation repairs (18, 19), controversy exists with respect to its efficacy to provide apical seals in apexification procedures. Although a 5-mm-thick apical plug has been shown to completely eliminate bacterial leakage (20, 21), the susceptibility of MTA apical plugs to bacterial leakage increases with time (22). The inferior quality of orthograde MTA seals is related to the difficulties in delivering the material from an orthograde direction (23, 24), with equivocal reports on the superiority of hand (25), ultrasonic (26), or ultrasonic-assisted hand-delivery techniques (27) in eliminating voids and improving the adaptability of MTA to root canal walls. Although orthograde root fillings with MTA are seldom indicated in conventional root canal therapy (28), this technique has been used quite often in apexification procedures (29, 30). Although there is no compelling evidence to suggest that orthograde MTA obturations increase the fracture resistance of immature root canals (17), it is uncertain whether complete filling of immature roots with MTA provides a better seal than orthograde MTA apical plugs. Thus, the objective of this study was to compare the changes in sealing properties of the two MTA apexification procedures over time using an in vitro apexification model. The null hypotheses tested were: (1) there are no differences in the ability of orthograde MTA plugs or root fillings to seal open root apices; and (2) the sealing properties of these two apexification procedures do not change with time.

Materials and Methods

Thirty-two single-rooted, extracted anterior human teeth were used under a protocol approved by the IRB of the Medical College of Georgia. The crowns were sectioned so that all root segments were about 20 mm long. Three millimeters of each root tip were removed with a high-speed burr under water cooling. Each root segment was pressed into moistened floral foam (Smithers-Oasis, Kent, OH, USA) to create a channel for adaptation of the root segment during the obturation procedures, with the moistened foam simulating soft periapical tissues.

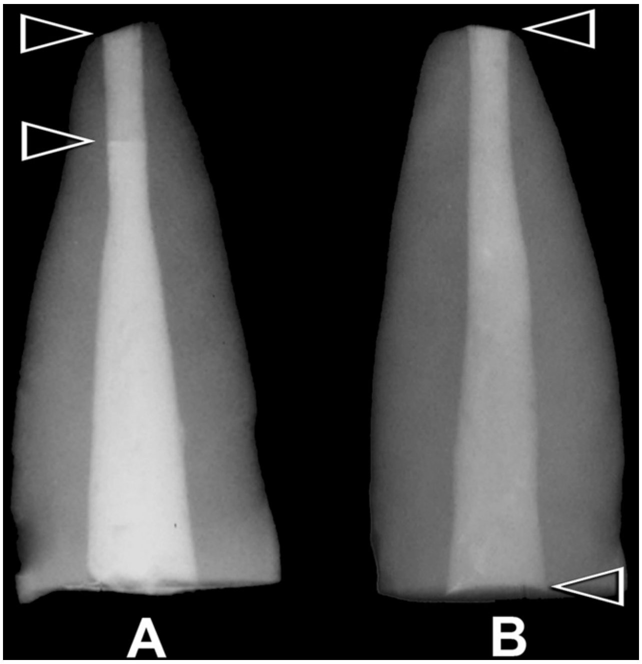

Figure 1. Radiographs of root segments with artificially created open apices that were closed with (*A*) An orthograde, 3- to 5-mm-long white MTA apical plug, followed by backfilling with gutta percha only without the use of a root canal sealer. (*B*) An orthograde white MTA root canal filling that extended from the open apex to the root canal orifice. The root canal space between the open arrowheads in each respective root segment represents the region occupied by the white MTA in this one-visit apexification model.

Cleaning, shaping, and canal obturation were performed under an operating microscope (Global Surgical Corp., St. Louis, MO, USA). Instrumentation was performed to 2 mm beyond the severed apical exit of each root using Gates-Glidden drills and 0.06 taper nickel–titanium rotary instruments (Profile, Dentsply Tulsa Dental, Tulsa, OK) to ISO #40. A standardized open apex was then created by retrograde preparation of the canal with the ISO #40 rotary instrument to the coronal length of its cutting blade (D16; 1.36 mm diameter). The canals were alternately irrigated with 6.15% sodium hypochlorite (NaOCl) (Clorox, Oakland, CA, USA) and 17% EDTA (Pulpdent Corp., Watertown, MA, USA) between instrumentation, with the latter used as the final rinse. The root segments were kept in moist gauze until obturation.

Apexification Procedures

Group I: MTA orthograde apical plug ($n = 12$)

Each root segment was inserted into the channel previously created in the moistened floral foam and dried with multiple paper points. White MTA (ProRoot, Dentsply Tulsa Dental) was mixed with a liquid/powder ratio of 0.35 and introduced into the canal from an orthograde direction with a messing gun (EndoGun; Medidenta, Woodside, NY, USA). The MTA was initially condensed with the thick end of moistened paper points and subsequently compacted with a size #11 endodontic plugger (Hu-Friedy, Chicago, IL, USA) to create a 3- to 5-mm-thick apical plug. Digital radiographs were taken to ensure void-free MTA placement and plug thickness (Fig. 1*A*). The remaining canal space was backfilled using Obtura II (Spartan, Fenton, MO) without a root canal sealer. The gutta-percha was inserted only to occupy space without contributing to the seal of the root canal, so that the apical seal of the MTA plug could be evaluated. The thermoplasticized gutta-percha was condensed with endodontic pluggers to 2 mm beneath the coronal orifice, creating a space for insertion of the fluid filtration device. The space was filled with Cavit (3M ESPE, St. Paul, MN, USA) as a provisional restoration.

Group II: MTA orthograde root filling ($n = 12$)

The aforementioned procedures were repeated with sequential introduction, condensation, and compaction of the white MTA at 3-mm-thick increments to fill the entire root canal space (Fig. 1*B*).

Positive and Negative Controls

Four roots were used as positive controls by filling the entire canal space with thermoplasticized gutta-percha without the adjunctive use of a sealer. In the negative controls, the four roots were filled as in group I and dipped into molten sticky wax to seal the root surfaces and the MTA apical barriers. The sticky wax was subsequently removed from the cut coronal surface of each root to provide a clean surface for attachment of the fluid filtration assembly.

Fluid Filtration

All the filled roots were stored in 100% relative humidity at 37°C for 48 hours to allow the white MTA to harden. After removing the Cavit provisional restoration, each root was attached to a Plexiglas platform penetrated by an 18-gauge stainless steel tube. Sealing quality of the filled roots was assessed using a computerized version of a previously reported fluid filtration protocol (31, 32). Briefly, each mounted root was connected to a fluid-filled pressure system that measured water movement through the obturated root under a constant pressure of 69 kPa by monitoring the movement of an in-line bubble within the Flodec device (De Marco Engineering, Geneva, Switzerland) (33). Automated data collection was performed every 1.04 seconds. The system was allowed to run until the fluid flow became stable, as visualized by the graphic display in the Flodec software before commencement of the recording for a 10- to 12-minute period. After obtaining the baseline fluid filtration measurement, each Plexiglas platform containing the attached root segment was stored at 37°C for 4 weeks in phosphate-buffered saline (PBS) containing 0.1% NaN_3 to prevent bacterial growth. A second fluid filtration measurement was performed in the manner previously described.

Statistics

Mean fluid flow ($\mu L\ min^{-1}$) generated at 69 kPa (i.e., 703 cm of water pressure) was normalized and expressed as the hydraulic conductance ($\mu L\ min^{-1} cmH_2O^{-1}$). Because the pooled data from the four subgroups (two apexification procedures × two storage periods) were not normally distributed (Kolmogorov–Smirnoff test), data obtained with the two apexification procedures at each storage period were separately analyzed with Mann–Whitney rank-sum test. Likewise, for each apexification group, before and after data obtained from the two storage periods were analyzed using a Wilcoxon signed-rank test. Statistical significance for all analyses was set at $\alpha = 0.05$.

Results

The intrinsic permeability of the fluid filtration apparatus pressurized to 69 kPa, recorded with a hemostat closing the polyethylene tubing to the mounted roots, was between zero and $5.7 \times 10^{-6}\ \mu L\ min^{-1}\ cmH_2O^{-1}$. The mean hydraulic conductance in the positive control was $1.6 \pm 0.38 \times 10^{-2}\ \mu L\ min^{-1}\ cmH_2O^{-1}$ and the negative control approximated null permeability after subtraction for intrinsic leakage. Similar values were obtained from the control groups after 4 weeks. That is, the hydraulic conductance values of the four experimental subgroups were two orders of magnitude higher than the intrinsic permeability of the fluid filtration system, and values after adjustments for intrinsic permeability are presented in Table 1. These values are two

TABLE 1. Effect of MTA apexification techniques and storage time on the seal of roots with artificially created, open apices

Apexification procedures	Hydraulic conductance (mL min^{-1} cmH_2O^{-1})*	
	After storing at 100% RH for 48 hours†	After immersion in PBS for 4 weeks†
White MTA orthograde apical plug (3–5 mm)‡	$4.0 \pm 1.7 \times 10^{-4\ B,b}$	$1.3 \pm 0.8 \times 10^{-4\ A,a}$
White MTA orthograde root filling‡	$2.0 \pm 1.2 \times 10^{-4\ A,a}$	$2.2 \pm 1.8 \times 10^{-4\ A,a}$

*Values are means ± standard deviations ($n = 12$), expressed in units of microliters per minute per centimeter H_2O.

†For each technique, non-parametric before and after data obtained from the two storage periods were separately analyzed using a Wilcoxon signed-rank test. For each row, data with different lowercase superscripts are statistically significant ($p < 0.05$). RH, relative humidity; PBS, phosphate-buffered saline.

‡For each storage period, non-parametric data derived from the two techniques were separately analyzed using a Mann–Whitney rank-sum test. For each column, data with different uppercase superscripts are statistically significant ($p < 0.05$).

orders of magnitude lower than that of the positive control. A significant difference was observed between the MTA apical plugs and MTA fillings at 48 hours ($p = 0.007$) but not after 4 weeks ($p = 0.47$). MTA apical plugs exhibited an improved seal after storing in PBS for 4 weeks ($p < 0.001$). A representative example of the fluid flow measurements from a representative MTA apical plug specimen at 48 hours and after 4 weeks is depicted in Fig. 2. There was no difference between the hydraulic conductance of MTA orthograde fillings obtained from the two storage periods ($p = 0.90$).

Discussion

The fluid filtration results in this study were of the same order of magnitude as those obtained from obturation of roots with closed apices. The hydraulic conductance of root fillings performed with warm vertical compaction of gutta-percha and AH-Plus sealer (Dentsply Caulk, Milford, DE, USA) was $2.8 \pm 1.5 \times 10^{-4}$ μL min^{-1} cmH_2O^{-1} (31), which is similar to that obtained in the MTA orthograde filling group. In that study (31), the hydraulic conductance of root fillings performed with passive application of resin-coated gutta-percha cones and EndoREZ sealer (Ultradent, South Jordan, UT, USA) was $7.1 \pm 2.0 \times 10^{-4}$ μL min^{-1} cmH_2O^{-1}, which is higher than all the four subgroups examined in this study. It is pertinent to point out that in the present study, the root canal space beyond the MTA apical plug was obturated with gutta-percha only, without a sealer. Although this practice is far removed from what would have been done clinically, we adopted this design to ensure water from the fluid filtration system passed through similar "material lengths" in both experimental groups. Because this experimental parameter remained constant, we may reject both of the null hypotheses that there are no differences in the ability of orthograde MTA plugs or root fillings to seal open root apices, and that the sealing properties of these two apexification procedures do not change with time. We speculate that a better seal of the entire root canal would have been achieved for a non-experimental setting in which a root canal sealer is used with the gutta-percha backfill after performing the MTA apical plug. The seal is likely to be improved further with the adjunctive use of a material along the filled canal orifice that is capable of furnishing a reliable coronal seal.

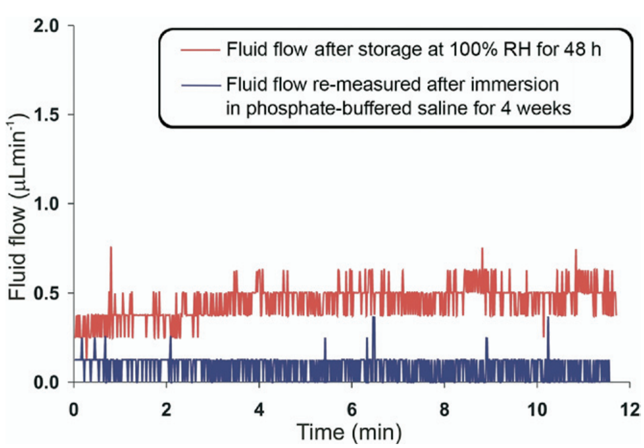

Figure 2. Flodec-derived, fluid flow recordings through the filled root segments from a representative specimen in the orthograde apical plug group. Fluid flow at 48 hours after obturation is shown in red and that re-measured after the same specimen was immersed for 4 weeks in phosphate-buffered saline is shown in blue. RH, relative humidity.

The observation that the seal of the MTA orthograde apical plug group improved after the specimens were immersed in PBS for 4 weeks merits further discussion. Continuous leaching of calcium and hydroxyl ions from the set white MTA (34) and their interaction with the PBS that diffused through the filled open apex could have resulted in hydroxyapatite deposition (16, 34) within potential spaces between the MTA and root dentin, partially obliterating these spaces with hydroxyapatite crystallites and reducing fluid flow. Because the MTA apical group exhibited more leakage at 48 hours than the orthograde MTA filling group, this could have permitted a better opportunity in the former group for the diffusion-controlled interaction between the PBS and white MTA. We also discovered that the so-called hydroxyapatite (16, 34) formed by MTA in the presence of phosphate-containing fluid is initially produced as amorphous calcium phosphates that eventually hydrolyze to poorly crystalline, B-type carbonated apatites (35) (Tay and Pashley, unpublished observations). These carbonated apatites are different from stoichiometric synthetic hydroxyapatites in that the latter do not exist in biological systems and do not contribute to the osteogenic and cementogenic potential of calcium phosphate–based materials (35). In the future, it may be possible to take advantage of the bioactivity of MTA by using PBS as the final rinse in instrumented root canals and leaving them slightly moist with PBS to encourage the deposition of carbonated apatites as a strategy to reduce leakage in root canals.

Conclusion

The results obtained with the fluid filtration technique cannot be used to predict clinical success of MTA apexification procedures because they represent only how well the MTA material adapts to the root canal. Whether that MTA apexification achieves clinical success depends on many additional variables. However, within the limits of the study, it may be concluded that a 3- to 5-mm-thick MTA orthograde apical plug that is recommended by the manufacturer for one-visit apexification produces a reasonable seal that improves over time in the presence of phosphate-containing fluids. Although MTA orthograde fillings produce better initial seals, the similarity in fracture resistance between thin roots filled with saline vs. those that were filled completely with MTA (17) suggest that there is no particular advantage in using this procedure for root reinforcement in apexification procedures.

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References

1. Rafter M. Apexification: a review. Dent Traumatol 2005;21:1–8.
2. Subay RK, Kayatas M. Dens invaginatus in an immature maxillary lateral incisor: a case report of complex endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:37–41.
3. El-Meligy OA, Avery DR. Comparison of apexification with mineral trioxide aggregate and calcium hydroxide. Pediatr Dent 2006;28:248–53.
4. Tait CM, Ricketts DN, Higgins AJ. Weakened anterior roots—intraradicular rehabilitation. Br Dent J 2005;198:609–17.
5. Al Ansary M, Day P, Duggal M, Brunton P. Interventions for treating traumatised non-vital immature permanent incisors. Int J Paediatr Dent 2006;16 Suppl 1:2.
6. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. Endod Dent Traumatol 1992;8:45–55.
7. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol 2002;18:134–7.
8. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999;25:197–203.
9. Steinig TH, Regan JD, Gutmann JL. The use and predictable placement of mineral trioxide aggregate in one-visit apexification cases. Aust Endod J 2003;29:34–42.
10. Kratchman SI. Perforation repair and one-step apexification procedures. Dent Clin North Am 2004;48:291–307.
11. Pradhan DP, Chawla HS, Gauba K, Goyal A. Comparative evaluation of endodontic management of teeth with unformed apices with mineral trioxide aggregate and calcium hydroxide. J Dent Child (Chic) 2006;73:79–85.
12. Felippe WT, Felippe MC, Rocha MJ. The effect of mineral trioxide aggregate on the apexification and periapical healing of teeth with incomplete root formation. Int Endod J 2006;39:2–9.
13. Keiser K, Johnson CC, Tipton DA. Cytotoxicity of mineral trioxide aggregate using human periodontal ligament fibroblasts. J Endod 2000;26:288–91.
14. Baek SH, Plenk H Jr, Kim S. Periapical tissue responses and cementum regeneration with amalgam, SuperEBA, and MTA as root-end filling materials. J Endod 2005;31:444–9.
15. Fridland M, Rosado R. Mineral trioxide aggregate (MTA) solubility and porosity with different water-to-powder ratios. J Endod 2003;29:814–7.
16. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. J Endod 2005;31:97–100.
17. Andreasen JO, Munksgaard EC, Bakland LK. Comparison of fracture resistance in root canals of immature sheep teeth after filling with calcium hydroxide or MTA. Dent Traumatol 2006;22:154–6.
18. Nakata TT, Bae KS, Baumgartner JC. Perforation repair comparing mineral trioxide aggregate and amalgam using an anaerobic bacterial leakage model. J Endod 1998;24:184–6.
19. Fischer EJ, Arens DE, Miller CH. Bacterial leakage of mineral trioxide aggregate as compared with zinc-free amalgam, intermediate restorative material, and Super-EBA as a root-end filling material. J Endod 1998;24:176–9.
20. Al-Kahtani A, Shostad S, Schifferle R, Bhambhani S. In vitro evaluation of microleakage of an orthograde apical plug of mineral trioxide aggregate in permanent teeth with simulated immature apices. J Endod 2005;31:117–9.
21. de Leimburg ML, Angeretti A, Ceruti P, Lendini M, Pasqualini D, Berutti E. MTA obturation of pulpless teeth with open apices: bacterial leakage as detected by polymerase chain reaction assay. J Endod 2004;30:833–6.
22. Matt GD, Thorpe JR, Strother JM, McClanahan SB. Comparative study of white and gray mineral trioxide aggregate (MTA) simulating a one- or two-step apical barrier technique. J Endod 2004;30:876–9.
23. Hachmeister DR, Schindler WG, Walker WA 3rd, Thomas DD. The sealing ability and retention characteristics of mineral trioxide aggregate in a model of apexification. J Endod 2002;28:386–90.
24. Vizgirda PJ, Liewehr FR, Patton WR, McPherson JC, Buxton TB. A comparison of laterally condensed gutta-percha, thermoplasticized gutta-percha, and mineral trioxide aggregate as root canal filling materials. J Endod 2004;30:103–6.
25. Aminoshariae A, Hartwell GR, Moon PC. Placement of mineral trioxide aggregate using two different techniques. J Endod 2003;29:679–82.
26. Lawley GR, Schindler WG, Walker WA 3rd, Kolodrubetz D. Evaluation of ultrasonically placed MTA and fracture resistance with intracanal composite resin in a model of apexification. J Endod 2004;30:167–72.
27. Yeung P, Liewehr FR, Moon PC. A quantitative comparison of the fill density of MTA produced by two placement techniques. J Endod 2006;32:456–9.
28. Al-Hezaimi K, Naghshbandi J, Oglesby S, Simon JH, Rotstein I. Human saliva penetration of root canals obturated with two types of mineral trioxide aggregate cements. J Endod 2005;31:453–6.
29. Hayashi M, Shimizu A, Ebisu S. MTA for obturation of mandibular central incisors with open apices: case report. J Endod 2004;30:120–2.
30. Karp J, Bryk J, Menke E, McTigue D. The complete endodontic obturation of an avulsed immature permanent incisor with mineral trioxide aggregate: a case report. Pediatr Dent 2006;28:273–8.
31. Gillespie WT, Loushine RJ, Weller RN, et al. Improving the performance of EndoREZ root canal sealer with a dual-cured two-step self-etch adhesive. II. Apical and coronal seal. J Endod 2006;32:771–5.
32. Brackett MG, Martin RL, Sword J, et al. Comparison of seal after obturation techniques using a polydimethylsiloxane-based root canal sealer. J Endod (in press).
33. Monticelli F, Sword J, Martin RL, et al. Sealing properties of two contemporary single-cone obturation systems. Int Endod J (in press).
34. Bozeman TB, Lemon RR, Eleazer PD. Elemental analysis of crystal precipitate from gray and white MTA. J Endod 2006;32:425–8.
35. LeGeros RZ. Calcium phosphates in oral biology and medicine. In: Meyers H, ed. Monographs in oral sciences, vol. 15.Basel, Switzerland: Karger, 1991.