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The Sealing Ability and Retention Characteristics of Mineral Trioxide Aggregate in a Model of Apexification

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Treatment of the immature pulpless tooth presents both an endodontic and restorative challenge. A more favorable long-term prognosis may be achieved with a mineral trioxide aggregate (MTA) apexification procedure followed by an internal bonding technique. We investigated the efficacy of this treatment option by testing the sealing ability and retention characteristics of MTA when placed as an apical barrier in a standardized in vitro open apex model. MTA was placed as an apical barrier at a thickness of 1 mm or 4 mm, with and without prior calcium hydroxide medication. The barriers were challenged with bacteria exposure within a leakage model and displacement forces on an Instron machine. In the leakage study, 100% of the MTA apical barriers showed bacterial penetration by day 70, compared with 20% of MTA root-end fillings used as controls. The displacement study demonstrated a statistically significant greater resistance to force with a 4-mm thickness of MTA, regardless of calcium hydroxide use. We concluded that it was the intracanal delivery technique and not the MTA that contributed to the leakage observed. MTA shows promise in our proposed treatment option of immature pulpless teeth if the sealing ability can be enhanced by improving the delivery technique.

Cessation of root development caused by trauma or pulpal disease presents both an endodontic and restorative challenge. The divergent apical architecture makes complete debridement and control of the obturation material nearly impossible (1). Fragile root canal walls may be too weak to withstand the normal forces of mastication, becoming more prone to fracture (2). Apexification procedures using calcium hydroxide have been historically used to establish apical closure and avoid surgery (3). To address the fracture potential, internal bonding procedures using a bonded composite technique have been described to strengthen the weakened root (4).

Despite the clinical success of the calcium hydroxide apexification technique, there are some disadvantages. The treatment requires high patient compliance and multiple appointments extending over a long period of time. In addition to the unpredictability of apical closure, other negative factors include conflicts in patient follow-up, financial concerns, esthetic demands, and susceptibility to coronal microleakage or fracture (5, 6). An alternative for the multiappointment apexification procedure has been a single-step technique using an apical barrier. Several materials have been proposed for use as an apical barrier, and their biocompatibility and osteogenic potential have been demonstrated (5–10). One such material, mineral trioxide aggregate (MTA) (ProRoot, Dentsply Tulsa Dental, Tulsa, OK), has been advocated as an apexification material by Shabahang et al. (7), because it permits an adequate seal of the canal and prevents bacterial leakage. When MTA is used for apexification, it is placed as the apical barrier and allowed to set for 3 to 4 h, and obturation can then proceed.

Although apexification and apical barriers alleviate some problems in the immature pulpless tooth, the possibility of root fracture often clouds the prognosis. A fracture, especially in the cervical area, can force many practitioners to choose extraction (11), necessitating costly restorative procedures, such as implants or fixed partial dentures. In the younger patient, where continued bone growth and development is paramount, saving the fractured tooth may require elaborate orthodontic and periodontic techniques (2). Composite resin materials, capable of bonding to dentin, create the potential to internally rebuild the root, providing dimensional and structural reinforcement (12). Pene et al. (4) demonstrated that the strength of an immature tooth could be improved significantly by using a bonded composite technique to fill the canal space. Several authors have described a technique using the Luminex Post System (Dentatus USA, Ltd., New York, NY), which is a clear plastic light-transmitting post used to aid in polymerization of composite resins placed deeply within the canal (12–14).

The combination of apexification and subsequent internal bonding may eliminate treatment problems that accompany the pulpless immature tooth. However, it continues to require high patient compliance with multiple appointments, perhaps extending over many months. The goal of our investigation was to propose an alternative technique that may decrease treatment time and possi-

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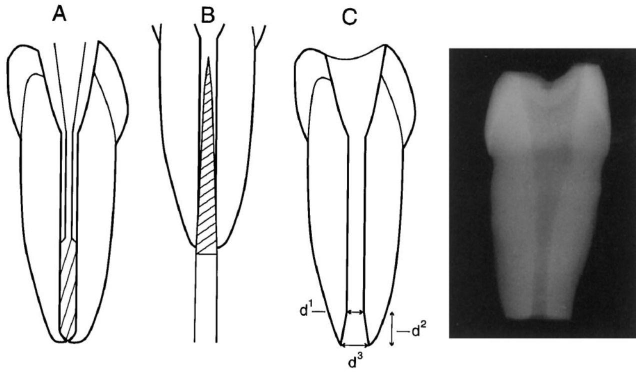

FIG. 1. An open apex model. (d[1]) represents the preparation size within the canal (0.90 mm), (d[2]) represents the divergent apical area, and (d[3]) represents the preparation size at the foramen (1.14 mm).

bly improve the long-term prognosis. The new treatment concept involves eliminating the gutta-percha obturation step after apexification. MTA, placed in an apexification technique, could become the sole obturation material, preserving and restoring the open apex. Subsequent internal bonding could be accomplished directly against this barrier.

Therefore, the purpose of this study was to investigate the validity of this new technique by testing the sealing properties and retention characteristics of MTA when placed as an apical barrier by using a newly developed, reproducible, standardized, in vitro model to simulate the structurally weakened root and divergent apex of the immature tooth.

MATERIALS AND METHODS

One hundred forty-six extracted, human, single-rooted, mandibular bicuspids were used for this study. The teeth were radiographed and examined under ×4.5 magnification for fractures and stored in 10% formalin. A coronal access preparation was made with a #4 round bur (Caulk Division, Dentsply International, Milford, DE) in a high-speed handpiece. The remaining pulp tissue was removed with barbed broaches (Dentsply Maillefer, Tulsa, OK). Approximately 2 mm of the apical tip of the root was resected with a #57 bur (Caulk Division, Dentsply International; Milford, DE), to remove any apical deltas and standardize the canal exit to the center of the tooth. Working length was determined visually with stainless steel hand files (Moyco Union Broach; York, PA) through the canal until the tip was flush with the apical surface. The canals were instrumented with a #2 Peeso reamer (Dentsply Maillefer; Tulsa, OK) to the working length. A divergent open apex was prepared by retrograde apical preparation with a #50 Profile .04 taper (Dentsply Tulsa Dental) inserted to the length of the cutting blade (D = 16) (Fig. 1). The teeth were kept in moist gauze during preparation and 5.25% NaOCl was used for irrigation. The prepared teeth were radiographed and stored in saline.

Leakage Study

Seventy-four teeth were randomly divided into six experimental groups. In group 1 ($n = 14$), a 1-mm apical barrier of MTA was placed into the canals without prior calcium hydroxide use. In group 2 ($n = 14$), the canals were medicated with calcium hydroxide before placement of a 1-mm apical barrier of MTA. In group 3 ($n = 14$), a 4-mm apical barrier of MTA was placed into the canals without prior calcium hydroxide use. In group 4 ($n = 14$), the canals were medicated with calcium hydroxide before placement of a 4-mm apical barrier of MTA. In group 5 ($n = 8$), a 4-mm apical barrier of MTA was placed into the canals without prior calcium hydroxide use but group was not exposed to ethylene dioxide sterilization before bacteria placement. In group 6 ($n = 10$), the MTA was placed as a surgical root-end filling.

When used, calcium hydroxide (Pulpdent, Pulpdent Corp., Watertown, MA) was delivered into the canal with a Lentulo Spiral (Moyco Union Broach, York, PA). The teeth were then stored at 37°C and 100% humidity for 7 days. The calcium hydroxide was removed with stainless steel files (Moyco Union Broach) and 5.25% NaOCl irrigation. Teeth in groups 1 to 5 were inserted into wet, flower-arrangement foam (Foliage Fresh, FloraCraft, Ludington, MI) to the CEJ. A #70 hand condenser was placed into the canal to length to ensure that no foam material was wedged into the canal space. The MTA was mixed according to manufacturer's directions. A Messing gun (EndoGun, Medidenta, Woodside, NY) provided with the ProRoot kit (Dentsply Tulsa Dental) was used to place the material as close to the apex as possible. A #70 hand condenser and the thick end of moistened paper points were used to condense the material to the apex. Radiographs were taken to ensure proper placement and increment thickness. If adjustments were needed, the material was irrigated out with saline and new material was placed. Moistened paper points were placed in the canals and the access openings were covered with moistened cotton pellets. For teeth in group 6, receiving root-end fills, a flattened gutta-percha cone was inserted to a snug fit, leaving a root end void of 3 to 4 mm. The retrofill approach was used to deliver and condense the MTA against the gutta-percha cone, and density and location were verified with radiographs. The cone was removed and replaced with a moistened paper point. The access openings were covered with moist cotton pellets, and the entire tooth was wrapped in moist gauze. All specimens were stored at 37°C and 100% humidity for 7 days. The material was then tested with paper point pressure to assure an adequate set.

Twelve teeth served as the controls. Half of these were obturated with a single cone of gutta-percha without sealer. The other half had the entire root sealed with sticky wax (Kerr, Emeryville, CA). To prevent bacterial leakage through the root surfaces, the roots of all teeth were coated with two coats of nail varnish, except over the apex. The apices of the negative control teeth were also sealed with nail varnish.

The upper portion of the leakage apparatus was assembled by cutting 2 cm off the end of a microcentrifuge tube (Intermountain Scientific Corporation, Kaysville, UT). The tooth was inserted into the tube with the root protruding. The tube end was heat softened to closely adapt it to each root. The microcentrifuge tube and root interface and the remaining root, except for the apical 1 mm, were then sealed with sticky wax. An 11-mm circular opening was prepared into the plastic lid of a scintillation vial. The tooth/tube assembly was inserted into the lid hole and sealed with sticky wax, and teeth in groups 1 through 4 were sterilized overnight with ethylene dioxide. All specimens were then attached to sterile 20-ml scintillation vials (Kimble Glass Inc, Vineland, NJ) containing sterile brain-heart infusion broth (Difco/Benton Dickinson, Sparks, MD), so that 1 mm of the apex was immersed in the broth.

Using a micropipette, a bacterial suspension was placed into the canals of all of the experimental and eight of the control teeth. The canals of two teeth from each of the control groups were filled with sterile saline to test the sterility of the model. Fresh bacterial

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TABLE 1. Number of leaking samples per group

DAY	Group 1: 1-mm MTA No CaOH (n = 14)	Group 2: 1-mm MTA with CaOH (n = 14)	Group 3: 4-mm MTA No CaOH (n = 14)	Group 4: 4-mm MTA with CaOH (n = 14)	Group 5: 4-mm MTA No sterilization (n = 8)	Group 6: Root-end fillings (n = 10)
1–10	14	11	13	13	7	0
11–20	0	1	0	0	1	0
21–30	0	0	0	0	0	0
31–40	0	0	0	0	0	0
41–50	0	0	0	0	0	0
51–60	0	2	0	0	0	2
61–70	0	0	1	1	0	0

suspension was added to the canals every 3 to 4 days. Periodic plating of the bacteria that was being replaced demonstrated continued viability. Turbidity of the brain-heart infusion broth in the scintillation vial indicated leakage through the apical barrier or root-end filling. Cultures taken from the turbid broth were streaked on brain-heart infusion agar plates and incubated at 37°C. The colonies were identified by morphology and Gram reaction.

The bacteria used were *Enterobacter aerogenes* (ATCC 13048), *Enterococcus faecalis* (ATCC 19433), and *Staphylococcus epidermidis* (ATCC 49741). The bacteria used were chosen for their viability together within the brain-heart infusion medium and because they could easily be differentiated after culture by staining. The bacteria were reconstituted and grown overnight in brain-heart infusion broth. The optical densities of the bacterial suspensions were determined by using a Beckman DU 640 spectrophotometer (Beckman Instruments, Inc., Fullerton, CA). The individual bacteria suspensions were then diluted with sterile brain-heart infusion broth to yield concentrations of approximately 2.0×10^6 bacterial per ml. Each species was individually plated on brain-heart broth agar plates as a reference for future identification. The three species were mixed and frozen at −83°C to provide a uniform bacterial suspension throughout the experiment.

The experimental data were analyzed by using a survival curve analysis.

Displacement Study

Sixty extracted, human, single-rooted, mandibular bicuspids were used for this study. Half of the teeth were randomly assigned to a calcium hydroxide medicated group. The canals were treated by spinning calcium hydroxide (Pulpdent) into the canal with a Lentulo Spiral. They were stored at 37°C and 100% humidity for 7 days and then mounted in orthodontic acrylic (GAC Dentsply, Tulsa, OK) to the CEJ coronally and within 1 mm of the apex. The tooth/acrylic block was then stabilized into the wet flower arrangement foam for placement of the MTA. The teeth, which were not medicated with calcium hydroxide, were randomly divided into two groups. Group A (n = 15) received a 1-mm apical barrier of MTA and group B (n = 15) received a 4-mm apical barrier of MTA. The teeth medicated with calcium hydroxide were also randomly divided into two groups. Group C (n = 15) received a 1-mm apical barrier of MTA and group D (n = 15) received a 4-mm apical barrier of MTA. After the MTA placement, moistened paper points were placed into the canals and the access was covered with moistened cotton pellets. The material was allowed to set in the canals for 8 days at 37°C and 100% humidity. Each tooth was tested with paper point pressure to ensure the set of the MTA material before the displacement test. One sample from group C and one sample from group D were discarded because of displacement of the MTA with paper point pressure.

An aluminum jig was fabricated to fit each tooth/acrylic block individually. The #7 end of a 5/7 hand plugger (Hu-Friedy, Chicago, IL) was mounted on the moving head of the Instron Universal Testing Machine (Instron Corp., Canto, MA). The plugger acted as the force probe and was inserted into each canal applying vertical pressure to the MTA. The maximum force applied to the material before dislodgement was recorded in pounds. A pilot study revealed that the #7 hand plugger began to bend at forces greater than 20 lb. Therefore, the Instron was stopped at, or slightly above, 20 lb if the material was not dislodged. The mean dislodgement force and standard deviation were calculated, and a two-way analysis of variance (ANOVA) was used to test for an overall mean strength difference between the medicated and nonmedicated teeth, an overall difference due to barrier thickness, and a thickness by medication interaction.

RESULTS

Leakage Study

The bacteria placed in the root canals of the positive control group caused turbidity in the brain-heart infusion broth by 24 h. Culture and plating of the turbid broth identified the three species of bacteria placed into the sterile canals. No specimens in the negative control group showed turbidity by experiment end at 70 days. The broth did not change in turbidity in those samples in which sterile saline was placed into the canals.

In groups 1 to 5, those teeth which had the MTA placed as an apical barrier, 91% showed turbidity by day 10. The remaining samples showed turbidity by day 61. Culture and plating of the turbid broth identified only one of the original three species of bacteria placed into canals, *E. aerogenes.*

In group 6, those teeth which had MTA placed as a root-end filling, 20% showed turbidity by day 60. Culture and plating of the turbid broth identified the *E. aerogenes* species only. Leakage times are illustrated in Table 1.

Displacement Study

The peak load required for the displacement of the MTA in the 1-mm no calcium hydroxide group was 2.82 lb, the 1 mm with calcium hydroxide group was 2.80 lb, the 4-mm no calcium hy-

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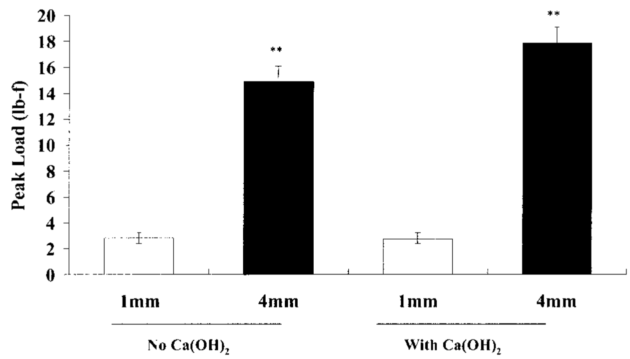

FIG. 2. The mean lb-force required to displace the MTA apical barrier. One millimeter and 4-mm groups represent the thickness of MTA placed with or without prior calcium hydroxide medication. **Indicates statistically significant data.

droxide group was 14.93 lb, and the 4 mm with calcium hydroxide group was 17.86 lb (Fig. 2).

There was a significant difference when comparing the variable of barrier thickness. Those specimens having 4 mm of MTA resisted displacement at significantly greater force loads ($p < 0.001$). No significant difference was noted when calcium hydroxide was used at any thickness of MTA apical barrier.

DISCUSSION

We evaluated several factors associated with a new treatment concept for the pulpless tooth with an immature root. To test the validity of using MTA as a stand-alone obturating material, its sealing ability and displacement resistance were evaluated after intracanal delivery. Our in vitro experimental model consisted of mature teeth instrumented to simulate immature roots. The #2 Peeso reamer instrumentation created relatively thin dentin walls, while opening the canal to a 0.90-mm diameter near the apex. The #50, .04 tapered Profile, instrumenting from a retrograde approach, created a funnel-like apex, diverging to 1.14 mm at the root tip. This model produced a standardized and reproducible anatomy that duplicated the essential radicular features of a pulpless tooth. It allows for parametric and quantitative evaluation of treatment modalities for managing this challenging type of clinical case.

Under our experimental conditions, 91% of the MTA barriers demonstrated bacterial leakage by day 10. Interestingly, only one of the bacterial species, *E. aerogenes,* was identified in the turbid broth of the experimental samples. Motility of this species may be one possible explanation, because it possesses several flagella (15), although a prior in vitro leakage study showed mobility was not a factor (16). In the displacement study, simulated condensation against the MTA barrier revealed a significant greater resistance to displacement forces when a 4-mm barrier was placed versus a 1-mm barrier.

The manufacturer of MTA recommends a 3 to 5-mm thickness of MTA be placed at the apex for the apexification procedure. We chose to investigate the properties of the material at two different thicknesses, one within the guidelines (4 mm) and one considerably smaller (1 mm), for two reasons. First, placement through an access cavity with minimal resistance at the apex may make the thickness challenging to control. And second, the amount placed as a barrier will determine the maximum depth of bonding that could be achieved when internally reinforcing the root (12–14). The greater the thickness of barrier present, the less root length available for bonding. It is possible that this factor could impact the strength attainable on a short immature root. Our results showed that MTA thickness had no impact on leakage but did exert a significant impact on displacement resistance. The actual forces placed on an MTA barrier when internally restoring the root with bonded resin materials requires further investigation.

Before placement of MTA for apexification, the manufacturer recommends that the canals be medicated with calcium hydroxide for 1 week, with subsequent removal using sodium hypochlorite and instruments as needed. The rationale is to enhance the difficult task of debriding the canal system with an open apex. Porkaew et al. (17) investigated the effects of calcium hydroxide remnants along the canal walls on the sealing ability of gutta-percha and sealer. They found a significant decrease in dye leakage in canals medicated with calcium hydroxide. They inferred that the calcium hydroxide reacts to form calcium carbonate, providing an initial decrease in permeability. However, with time, the resorbable calcium carbonate may create voids at the material dentin interface. It is likely in the open apex situation that calcium hydroxide remnants will be left on the canal walls. However, in this experimental model, medication with calcium hydroxide had no significant effect on MTA leakage times or displacement resistance. We can assume that remnants of calcium hydroxide on the walls in an open apex do not affect the properties of MTA.

Within the leakage study, one group was not exposed to ethylene dioxide sterilization. This was to determine if the 24-h dry time during the sterilization process affected the sealing properties of the MTA. Leakage results showed no difference in the materials sealing ability when sterilization was avoided. Thus, the leakage of MTA delivered via an intracanal approach was not due to the sterilization process.

Testing the sealing ability of an apical barrier may be considered controversial. However, in this study, we believe that it is important to evaluate the sealing ability of an MTA barrier because the proposed treatment technique, in which internal bonding is performed directly against the apical barrier, may require an adequate seal for success. The sealing ability of MTA has been tested when this material is used as a root-end filling and as a perforation repair material with great success (18–20). However, no studies demonstrate its sealing ability when placed using an orthograde approach. As such, we chose to incorporate an MTA root-end filling group as an experimental control. When we placed the MTA into our in vitro model via a retrofill approach, an 80% resistance to leakage was noticed over a 60-day observation period. Thus, we concluded that it was the intracanal delivery technique and not the MTA that contributed to leakage observed in this study. During retrograde placement, MTA is condensed into the root-end preparation against a blunted file, a gutta-percha cone, or a matrix. This allows for direct visualization as the material is packed against a base or physical barrier for support. Orthograde delivery can be considered more technique sensitive. Placement must be verified with radiographs versus direct visualization, and condensation is limited due to minimal resistance of the open apex. In addition to the difficulty in manipulating the material to the apex, the inherent irregularities and divergent nature of this anatomy may affect its adaptation to the dentin walls, predisposing the material to marginal gaps at the dentin interface.

It might be thought that a control material to compare with MTA should have been incorporated into this study. However, no parallel control material was tested because our proposed technique is based on the very unique properties of MTA when used in an

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apical barrier/apexification situation. It was not felt that there is a comparable material that could be used in the same manner—placement as a barrier with internal bonding (composite) placed directly behind it. Instead, we chose to test the properties of the MTA itself by varying the thickness of the MTA and by treating with or without calcium hydroxide before placement of the MTA. Therefore, the experimental groups constituted a complete factorial design based on selected amounts of MTA and calcium hydroxide.

The goal of this study was not to test the MTA for clinical effectiveness specifically but to test the feasibility of using this material in a new treatment technique. It is hoped that a more favorable long-term prognosis for pulpless immature teeth can be achieved with an MTA apexification procedure followed by internal bonding with resin materials. Saupe et al. (13), in comparing fracture resistance, found that the Luminex resin-reinforced dowel system could offer up to 50% more resistance to fracture than a conventional cast post and core. The MTA apexification technique can potentially eliminate the lengthy apexification procedure and allow internal bonding to be performed much earlier in the treatment process. Future studies focused on perfecting a technique to enhance the sealing ability of the MTA as an apical barrier would lend further support to this treatment option.

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