

# In-Vitro Evaluation of Microleakage of an Orthograde Apical Plug of Mineral Trioxide Aggregate in Permanent Teeth with Simulated Immature Apices

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## Abstract

This in vitro study evaluated the seal created by varying depths of mineral trioxide aggregate (MTA) plugs placed in an orthograde fashion in five groups of 10 teeth. One group received a 2 mm thick orthograde apical plug of MTA, the second group a 5 mm apical MTA plug, and the third group a 2 mm apical MTA plug with a second 2 mm increment, 24 h later. The remaining portion of the canal in these groups was left unfilled. Group four received a 2 mm MTA plug that set for 24 h and the canal was then back-filled with gutta-percha and eugenol based sealer. Group five was a positive control without an MTA plug. The apical seal was tested using a bacterial leakage model of *Actinomyces viscosus*. Results showed a statistically significant difference in only the 5 mm apical plug, which completely prevented bacterial leakage.

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Incomplete root development caused by trauma, caries, or other pulpal pathosis requires special attention and treatment. The aim is to seal a sizeable communication between the root canal system and the periradicular tissue and provide a barrier against which obturation material can be compacted. Calcium hydroxide apexification has been used successfully for years (1) but requires patient compliance and multiple appointments. The potential for coronal leakage or tooth fracture exists.

A single visit apexification technique using an osteoconductive, biocompatible material such as mineral trioxide aggregate (MTA) has shown promise in recent clinical cases presentations (2). However, in an in vitro study (3), severe leakage has been reported when MTA was used as an apical plug for root end induction or apexification. This leakage was attributed by the author to the inferior technique of delivery of MTA in the apical 3 to 4 mm.

The purpose of this study was to compare two techniques of MTA placement in the apical 3 to 5 mm of teeth with open apices. The hypothesis is that an MTA apical plug will provide for a better seal if delivered in two stages. The first stage would provide the foundation for the second, which would withstand greater forces of condensation resulting in better seal.

## Materials and Methods

Fifty single rooted extracted teeth were stored in a solution of 50% tap water and 50% Listerine (Warner Lambert Corporation, Morris Plains, NJ) to prevent growth of mold and bacteria. The inclusion criteria for the teeth were as follows: teeth exhibited one canal, confirmed by X-ray, had mature apices, no cracks, the roots were free from resorption, caries, and restorations, and were not dilacerated. Before randomization and group assignment, a 2.0 mm resection of the root tip was performed using a straight fissure carbide bur, and a divergent open apex was created by retrograde use of #50 Profile .04 and #3 Gates Glidden drill. An access cavity was prepared with a #4 round bur. Working length was established visually. Each tooth was cleaned and shaped using Profile .04 taper and Gates Glidden drills in a crown down fashion. Irrigation was carried out using 2.5% NaOCl. All instrumentation was carried out by the same operator.

Teeth were randomly divided into five groups. Group 1 received a 2 mm apical plug of MTA (Dentsply, Tulsa, OK). Group 2 received a 5 mm apical MTA plug. Group 3 received a 2 mm apical MTA plug followed by a second 2 mm increment 24 h later. The remaining portion of the canal in these three groups was left unfilled. Group 4 received a 2 mm MTA plug, allowed to set for 24 h and then back-filled with gutta-percha and a eugenol based sealer (Kerr EWT). Group 5 served as a positive control with no MTA plug.

A saline moistened Gel foam matrix was used to simulate periapical tissue. MTA was used according to the manufacturer's recommendations. The material was placed through the access opening and condensed to the apical area using a messing gun and hand pluggers. Finger nail polish was applied to the external tooth surface, excluding the resected root surface, to prevent side leakage through the dentinal tubules. Glass tubes of 13 mm × 100 mm equipped with microcaps were used to suspend the prepared teeth in Brain Heart Infusion (BHI) broth. A hole was made through the center of each cap and the tooth was placed into the hole to the cemento-enamel junction. The

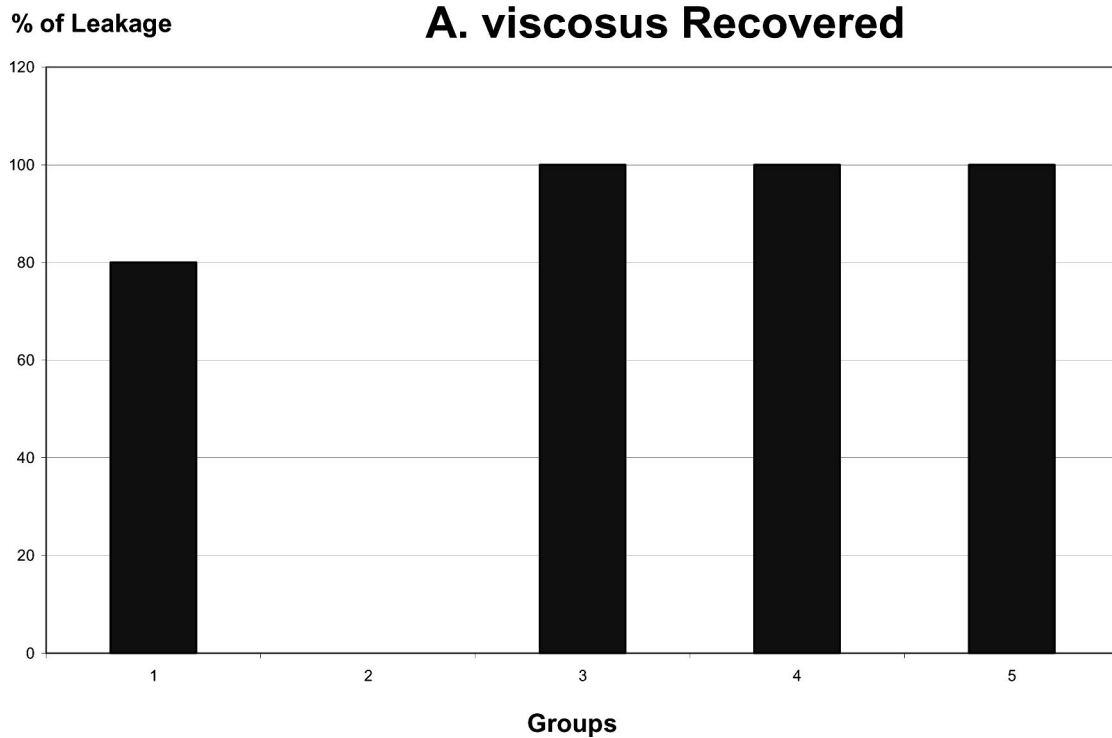


Fig 1. Percent of *A. viscosus* leakage among all groups.

gap between the tooth and the hole was filled with sticky wax. Aluminum foil was placed over the apparatus to protect against air contaminants entering the open canal during prolonged incubation. The completed apparatus was then sterilized by autoclaving at 15 lbs. pressure for 20 min.

The effectiveness of the MTA apical seal was tested using a bacterial leakage model with a culture of *Actinomyces viscosus*. A 24-h broth culture of the bacterium was placed inside the canal and the tooth was suspended in sterile BHI broth to a level sufficient to cover the apical 3 mm of the root tip. Tubes were incubated at 37°C in an environment of 5% CO<sub>2</sub> in air until the BHI broth became turbid, indicating bacterial growth. If no growth was observed, the inoculated tubes were allowed to incubate for a period of up to 70 days. Fresh 24-h broth cultures of *A. viscosus* were added on a weekly basis to all culture negative teeth throughout the study.

An aliquot was taken from all culture positive (turbid) tubes and plated onto Enriched Tryptic Soy Agar (ETSA) 120, incubated at 37 (0) C in 5% CO<sub>2</sub> in air for up to 72 h. Representative colonies of each recognizable morphotype were then subcultured for identification. Isolates were gram-stained and cellular morphology was examined using a phase-contrast microscope (Carl Zeiss Inc., Thornwood, NY) at a magnification of 1250×. Coccal strains were tested for catalase production and positive strains were plated onto Mannitol-Salt Agar (BD Diagnostic Systems, Franklin Lakes, NJ) to differentiate *Staphylococcus* species. Strains exhibiting a rod-like morphology were grown in BHI broth with 1% glucose for 48 h and volatile fatty acids resulting from the fermentation of the broth were extracted with ether. A gas chromatograph (Varian Associates, Inc., Walnut Dreek, CA) equipped with a thermal conductivity detector and a 6 × 4 mm stainless-steel column packed with SP-1220/1% H<sub>3</sub>PO<sub>4</sub> on Chromosorb WAW (Supelco Inc., Bellefonte, PA) was used to examine the acid end products for the production of propionic acid (VPI Anaerobe Laboratory Manual, Fourth Edition, Blacksburg, VA). The data was analyzed using Chi Square test.

Results

Only group 2 with the 5 mm MTA plug had no bacterial leakage (Fig. 1). There is a statistically significant difference between this group and the positive control, p < 0.0001 (χ<sup>2</sup> test).

In group 1 (2 mm MTA plug), nine samples were turbid and one clear. Eight of 10 samples were positive for both *A. viscosus* and *Staphylococcus*. One sample was positive for *Staphylococcus* and one sample did not demonstrate any cultivable bacteria. In group 3, six of 10 samples were positive for both *A. viscosus* and *Staphylococcus*. Four samples were positive for only *A. viscosus*. In group 4, all samples were positive for *A. viscosus*. There is no statistically significant difference for groups 1, 3, and 4 compared to the positive control, p < 0.0001 using a proportional test (χ<sup>2</sup> test).

Discussion

This study evaluated the sealing ability of MTA under specific conditions. This in vitro experimental model consisted of mature teeth instrumented to simulate immature roots. The model used to instrument and create an open apex was done in the study of Hachmeister et al. (3). This model produces standardized and reproducible anatomy that duplicates the essential features of a blunderbuss apex.

The manufacturer of MTA recommends a 3 to 5 mm thickness of MTA for the apexification procedure. We chose to investigate the sealing ability of the material at different thickness for the following reasons. First, placement through an access cavity with minimal resistance at the apex may make the thickness difficult to control. Second, the amount placed as a plug will determine the maximum depth of the post that could be used to reinforce the tooth. The greater the plug thickness, the less root length available for bonding, which could impact the strength attainable on a short, immature root. Finally, to evaluate the sealing effect of a minimum depth of MTA (2 mm) with gutta-percha obturation to help simulate the clinical situation.

The results of this experiment showed that there is a statistically significant difference only in group 2 compared to the control. The data in this study indicates that a 5 mm plug of MTA at the apex provides an absolute seal against microleakage of *A. viscosus*. This is in agreement with Lawley's (4) study where they found that 4 mm of MTA placed ultrasonically provided a good seal. Another study showed that hand condensation provided a better seal than ultrasonic condensation (5).

The two-step application failed to provide adequate sealing. The hypothesis was that two applications would enhance the seal, since the first application would act as a foundation thus allowing greater forces of condensation whereas reducing the chance of material extrusion. All samples in this group leaked. It is likely that this material lacks cohesion and does not set flat or sets with irregularities.

The results of this study are in disagreement with the Hachmeister et al. study (3). They used 1 and 4 mm plugs of MTA and tested them in a bacterial leakage model using *S. epidermidis* and found that all samples leaked by day 70. They attributed this to the material delivery. *S. epidermidis* is a major contaminant of the skin, air and external surfaces and possibly this contributed to this result. Our study also showed some contamination by *Staphylococcus* and that is why we used *A. viscosus*. Their study showed that 4 mm of MTA resisted displacing forces better than 2 mm. Our study indicated that 5 mm provided an excellent seal in immature permanent teeth with open apices, and provided enough depth to resist displacement.

Before placement of MTA for apexification, the manufacturer recommends that the canals be medicated with calcium hydroxide for 1 wk, with subsequent removal using sodium hypochlorite and instrumentation as needed. The rationale is to enhance the debridement of the canal. Porkaew et al. (6) investigated the effects of calcium hydroxide remnants along the canal walls on the sealing ability of gutta-percha and sealer, and found a significant decrease in dye leakage in canals medicated with calcium hydroxide. We do not know whether or not this has an effect on the sealability of MTA, because we did not use calcium hydroxide in this study. The Hachmeister et al. study showed that calcium hydroxide has no effects on MTA sealability (3). Studies have shown that MTA by itself is alkaline in nature as the main component is calcium (7).

The need for a complete apical seal in apexification cases is a controversial issue. Although the calcium hydroxide callus bridge formed in apexification cases is porous, it still carries a success rate of up to 90% (8). In this study, we believe that it is important to evaluate the sealing ability of an MTA apical barrier as part of an apical coronal seal. The sealing ability of MTA has been tested as a root-end filling (9–12) and as a perforation repair material (13) and as an obturation material (14).

MTA is condensed into root-end preparation against a base or physical barrier for support. Orthograde delivery is more technique sensitive. Placement must be verified with radiographs and condensation is limited because of minimal resistance of the open apex. In addition to the difficulty in delivering the material to the apex, the irregularities and divergent nature of the anatomy may limit adaptation to the dentinal walls, creating marginal gaps at the dentinal interface.

The idea of single visit apexification is not new and has been discussed and tested for many years (15–18). The success rate varies for different materials. A material like MTA with a high biocompatibility is a viable option, but still needs further testing. This material is osteoconductive, which may help the periapical tissue to adapt and heal. Its effectiveness has been shown in recent case reports (19).

The goal of this study is to test this materials sealing ability, in the hope that a more favorable long-term prognosis can be achieved with an MTA apexification procedure. This could potentially eliminate the current lengthy apexification procedure and allow earlier restoration of the tooth. Future studies focusing on a perfect placement technique to enhance the sealing ability of MTA as an apical plug would help to support this treatment modality. Our study model showed that 5 mm of MTA totally prevented leakage of *A. viscosus*.

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