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Perforation Repair Comparing Mineral Trioxide Aggregate and Amalgam Using an Anaerobic Bacterial Leakage Model

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The purpose of this study was to evaluate the ability of mineral trioxide aggregate (MTA) and amalgam to seal furcal perforations in extracted human molars using an anaerobic bacterial leakage model. Furcal perforations were made in 39 maxillary and mandibular human molars with a high-speed bur. These were randomly divided into two experimental groups of 18, with the remaining three teeth used as positive controls. Experimental group 1 was repaired with MTA and group 2 with amalgam. Three additional teeth without perforations served as negative controls. A dual chamber anaerobic bacterial leakage model was assembled. Brain heart infusion broth with yeast extract, hemin, menadione, and the chromogenic indicator bromcresol purple was used as the culture broth for *Fusobacterium nucleatum*. Eight of 18 amalgam samples leaked, whereas none of the 18 MTA samples leaked. MTA was significantly better than amalgam in preventing leakage of *F. nucleatum* past furcal perforation repairs.

Perforations are procedural accidents that can adversely affect the outcome of endodontic therapy. Sinai (1) found the prognosis for a tooth with a perforation depends on the location of the perforation, how long the perforation is exposed to contamination, and the feasibility of sealing the perforation.

In a study of perforated pulp chambers in monkey teeth, Seltzer (2) concluded that damage to the periodontium always occurred, but was minimized when the perforation was sealed immediately. Unsealed perforations are exposed to microorganisms and other contaminants, resulting in downward epithelial migration and destruction of the underlying bone (2).

Ideally, the material used to seal a perforation should be nontoxic, capable of providing an adequate seal, nonresorbable, radiopaque, and bacteriostatic (3). Many different materials have been suggested for their ability to seal perforations, including light-cured glass ionomer, calcium hydroxide, zinc oxide-eugenol, Super-EBA cement, amalgam, and tricalcium phosphate (4–8).

ElDeeb et al. (9) found amalgam to be superior to both Cavit and calcium hydroxide for perforation repair in dog teeth. Benenati et al. (5) found amalgam to be superior to gutta-percha for the repair of iatrogenic perforations by dental students. Balla et al. (6) found no complete healing 6 months after repairing furcal perforations in Rhesus monkeys with amalgam, Life, hydroxyapitite, or tricalcium phosphate. The authors suggested the inadequate sealing ability of the repair materials may have been responsible for the constant presence of inflammation (6).

Recently, the sealing ability of a new material, mineral trioxide aggregate (MTA), was compared with amalgam and Intermediate Restorative Material (IRM) for sealing a standardized lateral root perforation in mandibular molars (10, 11). Perforations repaired with MTA showed significantly less leakage of methylene blue, compared with perforations repaired with amalgam or IRM (11). A bacterial leakage study using *Streptococcus epidermidis* showed MTA to leak significantly less than amalgam, EBA, or IRM when used as root-end fillings (12).

The purpose of this study was to compare the ability of MTA and amalgam for sealing furcal perforations in extracted human molars using an anaerobic bacterial leakage model.

MATERIALS AND METHODS

Forty-two extracted human maxillary and mandibular molars were used in the study. The molars had minimal restorations or caries and roots that were not fused. All teeth were stored in physiological saline with 0.2% sodium azide (Sigma Chemical Co., St. Louis, MO).

The occlusal surface of the crowns and the apical 5 mm of the roots were removed using high-speed tapered diamonds. A standardized 5 × 5 mm endodontic access opening was made in each tooth with high-speed carbide burs to hold the tip of a 6 ml irrigation syringe (Sherwood Medical Co., St. Louis, MO). Pulp tissue and debris were removed and the pulp cavity irrigated with sterile saline. Cyanoacrylate cement (Zapit; MDS Products, Inc., Anaheim Hills, CA) was used to seal the root ends. Two coats of nail polish (Max Factor, Cincinnati, OH) was applied over the entire tooth surface and allowed to dry at room temperature. A mixture of three parts sawdust and one part plaster was mixed with water to make a matrix that simulated the bony socket. Teeth were placed into the unset matrix to make an impression. The matrix set in 10 to 15 min, and the teeth were then removed.

To ensure each perforation was centered between the roots, a #330 high-speed carbide bur was used to perforate the chamber floor from the external surface, whereas the tooth was hand held. Each perforation was enlarged with a #80 file passed 5 mm beyond the root surface. The teeth were then randomly divided into two groups of 18 teeth. MTA (Loma Linda University, Loma Linda, CA) was used to repair the perforations in group 1, and a high copper amalgam (Valiant PhD; L. D. Caulk, Milford, DE) was used to repair the perforations in group 2. Three teeth that were perforated but not repaired served as positive controls. Another three teeth that were not perforated served as negative controls. After treatment, the teeth were replaced in their individual matrices.

Paper points were used to remove excess moisture at the perforation site. Colla-plug (Calcitek, Carlsbad, CA) was packed into the perforation to provide a matrix to pack against. For group 1, MTA was mixed into a putty consistency and placed into the perforation with a Messing gun (Union Broach, Emigsville, PA). For group 2, amalgam was triturated according to the manufacturer's instructions and placed into the perforation using a Messing gun. Both materials were condensed with a double-ended endodontic plugger #5 to #7 (Hu-Friedy, Chicago, IL). The MTA and amalgam were placed using original magnification of ×3.5 (Surgitel Loupes; General Scientific Corp., Ann Arbor, MI). Both groups were left in the matrix with 100% humidity and allowed to set for 72 h at 37°C.

A dual chamber anaerobic bacterial model was assembled using a 6 ml irrigation syringe and tooth as the upper chamber and a 20 ml scintillation vial (Wheaton, Millville, NJ) as the lower chamber (12). The syringe was secured via a hole drilled through the cap of a 20 ml scintillation vial. The tooth was attached with cyanoacrylate cement to the tip of the syringe to complete the upper chamber and the joint sealed with two coats of nail polish. A plastic cap from a disposable syringe was used to cover the tube opening of the upper chamber.

The entire apparatus was sterilized using cobalt 60 gamma radiation. Brain heart infusion broth (bpBHI) supplemented with yeast extract (5 g/L), hemin (5 mg/L), menadione (10 mg/L) (BBL/VWR, Seattle, WA), and 20 μg/L bromcresol purple (Sigma) was aseptically placed into the lower chamber to a level above the tooth furcation in the lower chamber (13). Bromcresol purple is a chromogenic indicator that changes from a purple color at pH 6.8 and above to yellow as the pH decreases to 5.2 in the presence of acidic bacterial by products (13). The vials were placed in the anaerobic chamber (Bactron; Sheldon Man., Inc, Cornelius, OR) for 48 h to eliminate any oxygen in the system, reduce the media before inoculation, and check for sterility of the system. One hundred microliters of bpBHI broth turbid with *Fusobacterium nucleatum* was pipetted into the upper chamber syringe reservoirs along with 3 ml of sterile broth. Three milliliters of fresh sterile broth was added to each vial every 2 wk. The vials were incubated in the anaerobic chamber at 37°C and observed every 2 to 3 days for turbidity and/or color change of the broth, indicating bacterial growth from penetration of bacteria past the perforation repair. Once turbidity or color change was detected, a sample of the broth was Gram-stained to verify the presence of *F. nucleatum*. Leakage of *F. nucleatum* was compared between the two groups and statistically analyzed using the χ^2 test.

RESULTS

The positive controls showed turbidity and color change/turbidity 1 to 2 days after inoculation. The negative controls did not leak for the experimental duration of 45 days. None of the 18 samples in group 1 (MTA) showed any detectable leakage, whereas eight of 18 samples in group 2 (amalgam) leaked by 45 days. This difference was statistically significant ($p < 0.0005$). The experimental perforations that leaked were detected between 21 and 38 days. Gram stains of bpBHI broth from both the upper and lower chambers confirmed the presence of *F. nucleatum*.

DISCUSSION

Because anaerobes predominate in infections of endodontic origin, the use of an anaerobic leakage model is clinically relevant (14, 15). Bae et al. (13) found *F. nucleatum* produced turbidity and a color change in this model in one day and remained viable for 2 wk (13). The broth (bpBHI) used in this study containing the chromogenic substrate (bromcresol purple) is colored purple at pH 6.8 and above. It begins to change to a yellow color as the pH drops to 5.2 in the presence of acidic bacterial byproducts (13). In addition to this indicator aiding in leakage detection, it was helpful in determining the viability of the test bacteria in the upper chamber. After the addition of fresh bpBHI broth every 2 wk, all samples were observed for color change in the upper chamber as well as in the lower chamber. Lack of a color change would indicate loss of viability of the bacteria in the upper chamber. This occurred with two of the MTA samples. Consequently, 100 μl of a fresh inoculum of *F. nucleatum* was added to the upper chamber, and a color change was seen 1 day later.

The results obtained in this study were consistent with those found by Torabinejad et al. (12), where 11 of 12 amalgam samples leaked facultative bacteria after a median of 28.5 days and only one of 12 MTA samples leaked after a median of 90 days. The results of this study showed that 8 of 18 amalgam samples leaked strict anaerobic bacteria (*F. nucleatum*) after 45 days, whereas none of the 18 MTA samples leaked during the same period. The distinct physical and chemical properties of amalgam and MTA may be responsible for the results found in this study. Placement of both repair materials was standardized by using a Messing gun and the same double-ended pluggers. Perhaps amalgam may require a higher compaction force to achieve adequate marginal adaptation. Although Colla-plug was placed in the perforation as a matrix before placement of the test materials, overfills were commonly seen with the amalgam repairs. Benenati et al. (5) found that 70% of the failures in their study of perforation repairs were associated with extrusion of the repair material. Lee et al. (11) compacted MTA with a cotton pellet. It seems that MTA does not have to be compacted as firmly as amalgam to adapt adequately to the tooth surface.

It is doubtful that the lack of bacterial leakage seen in the MTA samples involves an antibacterial component in the material. Torabinejad et al. (16) looked at the antibacterial effects of MTA by measuring zones of inhibition on agarose gels and found that, whereas it did have some effects on facultative bacteria, it had no effect on strict anaerobes, including *F. nucleatum*.

When Torabinejad et al. (17) examined marginal adaption of root-end fillings using a scanning electron microscope, they found that 6 of 12 amalgam samples had no apparent gaps, whereas six had gaps up to 18.8 μm. The MTA samples had the smallest gap sizes, with a mean of 2.68 μm (17). However, SEM analysis evaluates only a two-dimensional plane. Although large gaps may be seen, it does not indicate how deep they are or if they are continuous along the interface. This shortcoming may explain why

some studies have found a lack of correlation between marginal gap sizes and leakage (18, 19).

Valiant PhD is a high-copper, zinc-free alloy made up of an admixture of two-thirds spherical particles and one-third lathe-cut particles. Nelson and Mahler (20) found this alloy to be one of the best in preventing leakage. They believe that the admixture conforms to the dentinal walls better than alloys containing only spherical particles. Depending on the environment (wet vs. dry) and the type of amalgam (spherical, admixture, or lathe-cut), varying degrees of contraction or expansion can occur that can increase or decrease as time goes on. Nelson and Mahler (20) showed that Valiant PhD contracts in a moist environment. In this study, the teeth were placed in a moist matrix to simulate the in vivo condition most often encountered when repairing perforations. Contraction of the alloy may have contributed to the leakage of *F. nucleatum* in this study. MTA, on the other hand, has been characterized as a powder consisting of hydrophilic particles, mostly calcium and phosphorous (10). This hydrophilic property, along with its similar mineral composition to dentin, may give MTA an advantage as a repair material over amalgam, as well as other materials. In this study, teeth with furcation perforations repaired using MTA allowed the passage of *F. nucleatum* significantly less than teeth repaired with Valiant PhD amalgam.

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