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Hard-Tissue Healing After Application of Fresh or Set MTA as Root–End-Filling Material

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The purpose of this study was to compare the effect of fresh mineral trioxide aggregate (MTA) with set MTA on hard-tissue healing after periradicular surgery. The root canals of 24 mandibular premolars in four 2-yr-old beagle dogs were filled with MTA. Two weeks later the root ends of half of the samples were surgically exposed and resected to the set MTA within the canals. After exposing and resecting the other 12 root ends, they were prepared with ultrasonic instrumentation and preparations were filled with fresh MTA. After 4 months, the animals were killed. Hard-tissue healing was analyzed histomorphometrically.

The results indicated that although freshly placed MTA resulted in a significantly higher incidence of cementum formation (12 of 12 versus 8 of 12, $p = 0.028$), there is no significant difference in the quantity of cementum or osseous healing associated with freshly placed or set MTA when used as root–end-filling material.

Resection of the root end during periradicular surgery results in an exposed apical dentin surface bounded by cementum with a root canal at its center. After ultrasonic root-end preparation, a root–end-filling material is usually placed to seal the root-end cavity preparation.

Healing after periradicular surgery necessitates regeneration of the apical attachment apparatus (dentoalveolar healing), as well as osseous repair of medullary and cortical bone (alveolar healing). Deposition of cementum over the resected root end is an essential step in dentoalveolar healing (1). The mechanism of dentoalveolar healing is not fully understood. It is thought that undifferentiated mesenchymal cells, fibroblasts, and fibroblast-like cells arise from the periodontal ligament and bone and surround the root end to begin the healing process. The undifferentiated cells transform into mature fibroblasts, cementoblasts, and osteoblasts and begin to reform the apical dentoalveolar apparatus (2). Cementum is laid down from the outside edges of the root toward the center of the resected root end. With most root–end-filling (1).

Alveolar healing is the regeneration of osseous tissues surrounding the root end. Harrison and Jurosky (3) in 1992 created osseous wounds in the mandibles and maxillas of rhesus monkeys and evaluated the alveolar healing histologically. They found that in these "excisional" wounds, the initial blood clot was replaced by granulation tissue emanating from the endosteal tissues. The alveolar healing progressed from the endosteal surfaces toward the external surfaces of the bony wound, resulting in formation of woven bone at 14 days and a functioning periosteum at 28 days.

Torabinejad et al. found that dentoalveolar healing adjacent to mineral trioxide aggregate (MTA) root-end fillings is unique because it results in regeneration of the periapical tissues, including apical cementogenesis (4). The characteristic of apical cementogenesis adjacent to MTA has been studied in cats (5), dogs (4), and monkeys (6). In these studies, fresh (unset) MTA was placed into the root-end cavities during surgery and allowed to set in contact with the blood in the osteotomy site. Previous studies have not revealed whether placement of unset MTA is required for optimal apical cementogenesis, or local osseous healing. It is not known whether orthograde-placed set MTA that is resected during surgery will achieve the same result. The purpose of this study was to examine hard-tissue healing adjacent to fresh or set MTA as root–end-filling material in dogs.

MATERIALS AND METHODS

A total of 24 roots of mandibular second, third, and fourth premolars from four 2-yr-old beagle dogs were used in this experiment. All endodontic procedures were performed under general anesthesia. Anesthesia was provided by an initial intramuscular injection of 10 mg/kg of tiletamine HCl and zolazepam HCl (Telazol®, Fort Dodge Animal Health, Overland Park, KS) and 0.04 mg/kg of atropine sulfate (Abbot Labs, Abbot Park, IL). The animals were then intubated and maintained on inhalation anesthesia with 1% to 3% isoflurane (Forane®, Ohmeda, Liberty Corner, NJ) and 1 to 2 l/min of oxygen for the remainder of the procedure. Local anesthesia was provided with a buccal infiltration of 1.8 ml of 2% lidocaine (Xylocaine®, Astra Pharmaceuticals, Wilmington, DE) with 1:50,000 epinephrine.

Mesial and distal roots were assigned to have either retrograde MTA (ProRoot®, Dentsply Tulsa Dental, Tulsa, OK) root-end fillings or MTA previously placed in an orthograde manner. After gaining occlusal access to the pulp chambers of each tooth, the

pulps were extirpated and the root canals were cleaned and shaped using Flex-o-Files® (Dentsply Maillefer, Tulsa, OK) to the apical stop of the canal. A uniform apical flare was accomplished with step-back filing. Through systematic sampling, half of the roots of the mandibular second, third, and fourth premolars were obturated entirely with MTA. The remaining roots were obturated to 5 mm from the apical stop with warm vertical compaction of gutta-percha. The coronal portion of each root in this group was obturated with MTA. The gutta-percha was placed in the apical segment of the canal in this group to facilitate easy ultrasonic apical preparation during planned periradicular surgery.

Two weeks after the root-canal procedures, the dogs underwent periradicular surgery on the mandibular right or left quadrants. After obtaining anesthesia, a full-thickness mucoperiosteal buccal flap with two releasing incisions (mesial of the first and distal of the fourth premolars) was reflected. This allowed access to the periradicular tissues in the mandibular second, third, and fourth premolars. The cortical bone over the root ends was removed using a #6 round bur in a high-speed handpiece using copious saline irrigation. The root ends in both groups were resected with a fissure bur approximately 3 mm from the apex at an angle approximately 60 degrees to the long axis of the root. In the roots with gutta-percha in the apical portion of the canal, root-end preparations were made to a depth of 3 mm with a Vista P5 ultrasonic unit (Vista Dental, Racine, WI) and S12/90 ultrasonic tip. Root-end cavities were filled with MTA mixed according to the manufacturer's recommendation. In the remaining roots, after root-end resection to the level of set MTA, no root-end cavity preparations were made. The mucoperiosteal flaps were sutured with 4-0 silk sutures. All animals were then given 0.01 mg/kg of buprenorphine (Buprenex®, Reckitt and Coleman Pharmaceuticals, Richmond, VA) subcutaneous for pain control and 300,000 units of penicillin (Bicillin C-R®, Wyeth-Ayerst Laboratories, Philadelphia, PA) to prevent infection. After surgery, the animals were placed on a soft diet.

The animals were killed by barbiturate overdose 16 weeks after the second surgical procedure (Euthasol®, Western Medical Supplies, Arcadia, CA). After perfusion with 10% buffered formalin, mandibular block sections containing the premolar teeth and surrounding tissues were resected. These specimens were demineralized in 5% formic acid and then dehydrated in 30%, 70%, and 100% alcohol. After embedding in paraffin, serial buccolingual sections of 7-μm thickness were cut through the center of the apical foramen along the long axis of the teeth. Selected sections were stained with hematoxylin and eosin and evaluated under a light microscope.

The dentoalveolar healing was assessed by measuring the area of cementum in the section adjacent to the root-end filling and dividing this area by the apical diameter of the root-end filling. This measurement, termed the dentoalveolar-healing quotient, was used to compare the two groups. This method was used to compensate for any discrepancies in the size of root–end-cavity preparations.

Digital photomicrographs at a magnification of 111× were made of the areas of interest in each slide. ImagePro Plus® software (version 1.0, Media Cybergenics, Silver Spring, MD) was used to measure the data directly from the photomicrographs. Images were made to document the apical diameter of the root-end fillings and the dimension was recorded in micrometers. Cementum adjacent to the MTA was photographed with the digital system. After digitally removing all parts of the image except the cementum, the area of the cementum adjacent to the MTA was measured in square micrometers (Fig. 1, area is depicted to the left of arrow *A*).

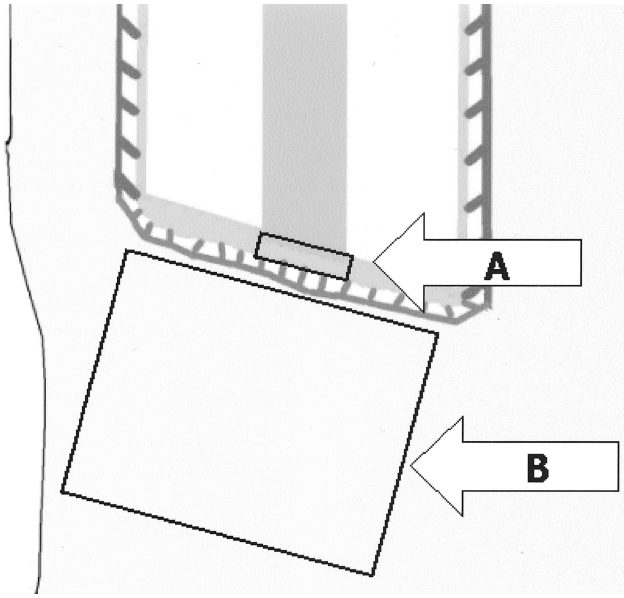

FIG 1. Measurement sites for dentoalveolar (*A*) and alveolar (*B*) healing.

The healing of bone adjacent to the root end was measured as follows. A rectangular field within the section measuring 1620 × 2164 μm was selected. Its longest side was parallel to, centered on, and slightly buccal to the resected root end (Fig. 1, area is depicted to the left of arrow *B*). In this field, the area occupied by bony trabeculae was calculated using ImagePro Plus® software, similar to the area of cementum. The percentage of the entire field occupied by bone was then calculated. This percentage was used as a measure of bone density. The alveolar healing was measured as the percentage area of the designated portion of the section adjacent to the root-end filling that was occupied by bony trabeculae. A single examiner without knowledge of the designation of the groups made all measurements.

RESULTS

Healing was uneventful in both groups. During histologic analysis, we noted cementum formation adjacent to the MTA in 8 of 12 set-MTA samples and in all freshly placed MTA samples. Inflammation was noted in the vicinity of three freshly placed MTA root-end fillings, although not directly adjacent to the MTA. In all of these samples, cementum had formed adjacent to the MTA.

The root-end fillings in the set group were consistent in contour, whereas the freshly placed MTA root-end fillings were characterized by more surface irregularity. The cementum adjacent to the set MTA was somewhat more uniform in thickness, whereas that adjacent to the fresh MTA seemed to be of varying thickness. Representative photomicrographs depicting the dentoalveolar healing observed in the fresh and set MTA groups can be found in Figs. 2 and 3, respectively.

Bone density was relatively comparable for most samples in both groups. Representative photomicrographs depicting the alveolar healing observed in the fresh and set MTA groups can be found in Figs. 4 and 5, respectively.

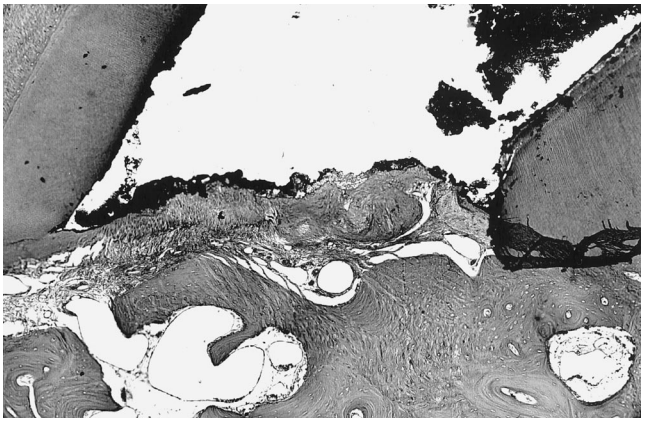

FIG 2. Dentoalveolar healing in fresh-MTA group. Cementum is noted directly adjacent to MTA (original magnification ×80).

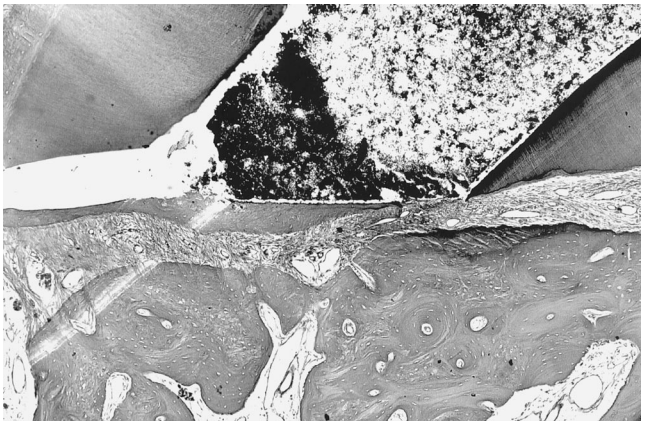

FIG 3. Dentoalveolar healing in set-MTA group. Cementum is noted directly adjacent to MTA (original magnification ×80).

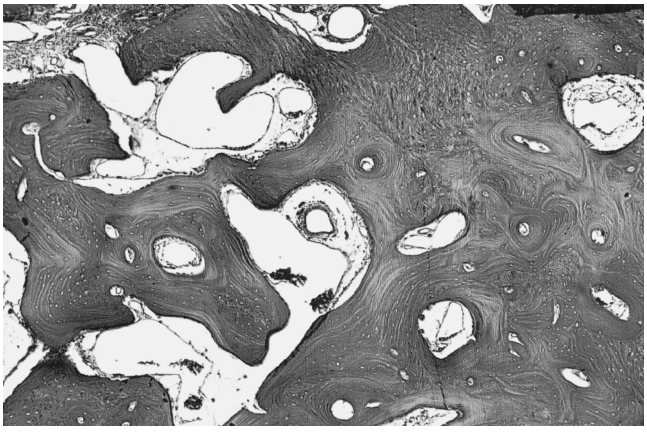

FIG 4. Alveolar healing of osteotomy site in fresh-MTA group. Note presence of new bone (original magnification ×80).

Four of 12 roots with set MTA did not form cementum adjacent to the root-end fillings. In these four roots, a fibrous capsule surrounded the MTA. The absence of cementum was not restricted to one animal, rather it occurred in three of four dogs.

The Mann-Whitney *U* test was used to compare the dentoalveolar healing quotients and the bone densities of the two groups at significance level alpha = 0.05. The mean healing quotients of the two treatments as expressed in the area of cementum (μm^2) in the section adjacent to the root-end filling divided by the apical diameter (μm) were 49.54 μm^2 cementum/μm apical diameter for the retrograde-MTA filling and 32.83 μm^2 cementum/μm apical diameter for the orthograde-set MTA. No statistically significant difference was found between the two groups ($p = 0.242$).

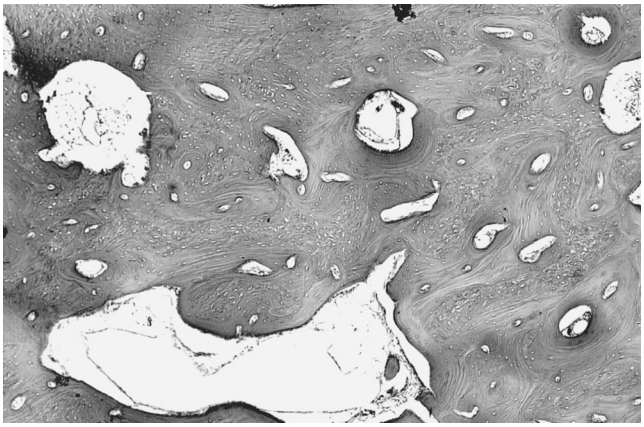

FIG 5. Alveolar healing of osteotomy site in set-MTA group. Note presence of new bone (original magnification ×80).

The proportions of each group that formed cementum adjacent to the MTA also were compared by a two-sample test for binomial proportions. The difference between these proportions, 66% in the set-MTA group and 100% in the fresh-MTA group, was found to be statistically significant ($p = 0.028$). The proportion of samples in the fresh-MTA group, which produced complete dentoalveolar healing, was significantly higher than that in the set-MTA group.

The results for mean bone density opposite the root-end fillings were 53.08% for the retrograde–MTA-filling group and 61.73% for the orthograde–set-MTA group. No significant statistical difference was found between the two groups ($p = 0.443$).

DISCUSSION

An examination of the raw data shows a trend for more cementum formation with freshly placed MTA root-end fillings. Also, the frequency of cementum formation adjacent to fresh MTA (100%) was higher than that adjacent to set MTA (66.67%). Statistical analysis shows that this difference in frequency of complete dentoalveolar healing between groups is significant. No statistically significant difference was found in the amount of cementum formed adjacent to set and fresh MTA when used as root–end-filling material. Also, no significant differences in alveolar healing were noted.

Our findings show that resecting a root end containing the set material does not significantly alter the biocompatibility of MTA, which seems to support the production of hard tissue. Torabinejad et al. (7) evaluated the cytotoxicity of MTA and found no difference in the zone of lysis between set and freshly mixed material. Osorio et al. (8) in 1998 used set-MTA samples in their examination of the cytotoxicity of endodontic materials on human-gingival fibroblasts. Their findings that the samples of set MTA were not cytotoxic support the fact that the resected surface of set MTA does not seem to hinder hard-tissue formation. Zhu et al. (9) in 1999 also used set MTA in their study of the adhesion of human osteoblasts, finding that the osteoblasts spread and made intimate contact with the set material.

Koh et al. (10) in 1997 used set MTA in their study of the biological response of human osteoblasts to the material. They found that MTA caused an increase in the production of interleukin (IL)-1α, IL-1β, IL-6, and osteocalcin. In another study Koh et al., in 1998, reported similar results (11). IL-1α and IL-1β interact with receptors on osteoblasts, which in turn activate osteoclasts. Second only to collagen, osteocalcin is an abundant protein, which is present in bone and may be an indicator of bone-matrix production.

Mitchell et al. (12) in 1999 found that set MTA was biocompatible when tested with a culture of human osteosarcoma cells. They further reported that MTA induced the production of IL-6, IL-8, and macrophage–colony-stimulating factor. IL-6 is a powerful factor produced by osteoblasts to induce bone resorption. IL-8 promotes the development of new blood vessels and activates the precursors of osteoclasts. Macrophage-stimulating factor may have a significant function in osteoclast development and maturation.

The results of this study show that both fresh and set MTA are biocompatible. They both produce substances that promote dentoalveolar and bone regeneration.

In addition to biocompatibility, hard-tissue formation could occur as a result of the superior sealing ability of MTA. Numerous studies have measured the sealing ability of MTA. They demonstrate that MTA has a superior seal as measured by dye leakage (13, 14), fluid filtration (15), bacterial leakage (16, 17), and leakage to endotoxin (18).

The process of placing MTA in an orthograde manner and then resecting the set material with a high-speed handpiece apparently does not significantly disturb the apical seal of MTA and has no significant bearing on the subsequent apical tissue regeneration. Hachmeister et al. (19) recently found in an in vitro study that MTA apical barriers placed orthograde in teeth with open apexes exhibit somewhat more bacterial leakage than those placed in retrograde manner. This may be related to the packing technique used and the resultant density of the MTA.

Histologic studies, such as this investigation, cannot identify whether the sealing ability or the biocompatibility of MTA is responsible for hard-tissue formation. Further in vitro and in vivo studies are needed to elucidate the answer to this question. The difference in the frequency of hard-tissue formation adjacent to set versus fresh MTA root-end fillings may be because of a lower rate of tissue regeneration adjacent to set MTA. Further studies may determine whether this is the case.

Based on our findings, it seems that the potential of set MTA to induce the regeneration of apical hard tissue is not significantly degraded as a root–end-filling material. Therefore in selected cases, orthograde placement of MTA can be used as an obturation material before surgery. After root-end resection, there would be no need for root-end preparation and filling procedures. The advantages of this method would include: less need for vasoconstrictors, greater ease of surgery, no root-end preparation and resultant microcracks, and no significant difference in resultant apical hard-tissue regeneration. The indications might include patients with medical contraindications for vasoconstrictors and those cases where surgical access for retropreparation and root-end filling is anticipated to be difficult and time consuming.

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