Healing after Root-end Microsurgery by Using Mineral Trioxide Aggregate and a New Calcium Silicate–based Bioceramic Material as Root-end Filling Materials in Dogs

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Abstract

Introduction: The purpose of this study was to compare healing after root-end surgery by using grey mineral trioxide aggregate (MTA) and EndoSequence Root Repair Material (RRM) as root-end filling material in an animal model. **Methods:** Apical periodontitis was induced in 55 mandibular premolars of 4 healthy beagle dogs. After 6 weeks, root-end surgeries were performed by using modern microsurgical techniques. Two different root-end filling materials were used, grey MTA and RRM. Six months after surgery, healing of the periapical area was assessed by periapical radiographs, cone-beam computed tomography (CBCT), micro computed tomography (CT), and histology. **Results:** Minimal or no inflammatory response was observed in the majority of periapical areas regardless of the material. The degree of inflammatory infiltration and cortical plate healing were not significantly different between the 2 materials. However, a significantly greater root-end surface area was covered by cementum-like, periodontal ligament–like tissue, and bone in RRM group than in MTA group. When evaluating with periapical radiographs, complete healing rate in RRM and MTA groups was 92.6% and 75%, respectively, and the difference was not statistically significant (P = .073). However, on CBCT and micro CT images, RRM group demonstrated significantly superior healing on the resected root-end surface and in the periapical area (P = .000 to .027). **Conclusions:** Like MTA, RRM is a biocompatible material with good sealing ability. However, in this animal model RRM achieved a better tissue healing response adjacent to the resected root-end surface histologically. The superior healing tendency associated with RRM could be detected by CBCT and micro CT but not periapical radiography. *(J Endod 2015;41:389–399)*

Key Words

Biocompatibility, CBCT, micro CT

Numerous materials have been used for root-end filling, such as amalgam, glass ionomer, composite resin, intermediate restorative material, Super EBA, and mineral trioxide aggregate (MTA). However, none of these materials are ideal. Currently, MTA is the material of choice, and its use is supported by the superior results in several published studies. *In vitro* studies have shown MTA to have both excellent biocompatibility and sealing ability, and these results have been corroborated in *in vivo* studies as well (1, 2). In several animal experiments that used MTA as a root-end filling material, cementum formation on the surfaces of MTA with no or minimal inflammation has been reported (3–8). Even though MTA is the root-end filling material of choice on the basis of biological principles, the cost and handling properties remain practical obstacles to its use (2).

Recently, other calcium silicate cements such as EndoSequence Root Repair Material (RRM) (Brasseler USA, Savannah, GA) have been introduced to the endodontic field to overcome these limitations. RRM is dispensed in premixed, ready-to-use injectable or putty form. According to the manufacturer, the main compositions of both RRM formulations are the same (calcium silicates, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic, and filler agents), differing only in particle size. *In vitro* studies have shown that the cytotoxicity and sealing ability are comparable to MTA (9–12); however, there are no *in vivo* or clinical studies to test its efficacy. Therefore, the purpose of the present comparative study was to evaluate healing after root-end surgery by using RRM putty and grey MTA (ProRoot MTA; Dentsply Tulsa Dental, Tulsa, OK) as root-end filling materials. Healing was assessed with different imaging modalities: periapical radiography, cone-beam computed tomography (CBCT), and micro computed tomography (CT). Tissue responses adjacent to RRM and grey MTA were also assessed.

Materials and Methods

This experiment was conducted in accordance with the Fourth Military Medical University, Xian, China Research Committee approved animal protocol. Four healthy, adult (19–22 months) beagle dogs were used. The experiment was conducted on bilateral mandibular first to fourth premolars. The general principles of surgery are summarized, and all procedures were performed under general anesthesia in an operating room. Intramuscular injection of xylazine hydrochloride (2 mg/kg) followed by intravenous injection of 3% pentobarbital (20 mg/kg) was used for sedation and general anesthesia. Butorphanol tartrate (0.4 mg/kg) was used as an analgesic and was given subcutaneously before surgery and then twice a day for 7 days after the surgery.

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.11.005

Penicillin G benzathine and penicillin G procaine 120,000 IU were administrated subcutaneously every day for 7 days.

Induction of Apical Periodontitis

After the animals were anesthetized, preoperative periapical radiographs were taken of all mandibular first to fourth premolars by using the parallel technique with phosphor plates (Gendex, Hatfield, PA) and XCP film holders (Dentsply Rinn, Elgin, IL). The teeth were accessed, and the pulp tissue was extirpated by using barbed broaches and Hedstrom files (Dentsply Tulsa Dental) without rubber dam isolation. The root canal system was infected by placing small gutta-percha points contaminated by dental plaque from the maxillary molar region of the same animal. A cotton pellet soaked in a mixture of dental plaque and normal saline was placed in the chamber, and the access cavity was sealed by using glass ionomer cement (Fuji IX; GC Corporation, Tokyo, Japan). The formation of periapical lesions was confirmed with periapical x-rays 6 weeks later in all teeth.

Root-end Surgery Phase

After confirmation of periapical lesions, the animals were anesthetized by using the previously described protocol. The surgical sites were wiped with 0.12% chlorhexidine. Two percent lidocaine with 1:50,000 epinephrine (Dentsply Pharmaceutical, York, PA) was used to achieve optimal hemostasis. All first premolars (8 teeth) were designated as the material control group. For these teeth, nonsurgical root canal treatment was completed. The procedure involved biomechanical preparation to apical size #40, passive ultrasonic irrigation (IrriSafe; Acteon North America, Mt Laurel, NJ) with 2.5% NaOCl, followed by 17% EDTA and 2% chlorhexidine and warm vertical obturation with gutta-percha and sealer (AH Plus Jet; Dentsply Maillefer, Tulsa, OK). The access cavities were sealed with composite resin. Among the material control group (8 teeth), 4 root-end cavities were filled with MTA and 4 with RRM putty. Root-end surgeries were performed as described below.

The experiment groups consisted of the mandibular second to fourth premolars (total of 24 teeth), 6 roots in each quadrant, in which root-end surgeries were completed without disinfecting and treating the canals. Three roots of each quadrant were randomly assigned to the MTA experiment group and the other 3 to the RRM experiment group. Both materials were used in 3 root-end cavities on each side of mandible except in 1 animal because of root fusion of a second premolar. In total, 27 root ends were filled with MTA, which included 4 in MTA control group. Twenty-eight root ends received RRM including 4 in the RRM control group. In addition, a positive control group was also included, in which the canals remained infected without root-end fillings (see Table 1 for summary of grouping).

Root-end surgeries were performed on all mandibular premolars by using modern microsurgical techniques described by Kim and Kratchman (13). Full-thickness mucoperiosteal flaps were elevated, and osteotomies were made at the root apices. The apical 3 mm of each root was resected, followed by complete curettage of the granulomatous tissue. Three-millimeter root-end cavities were prepared with ultrasonic tips (JETip; B&L Biotech, Bala Cynwyd, PA). All surgical procedures and root-end preparations were performed under an operating microscope at $\times 8$ to $\times 24$ magnifications.

After the root-end filling procedure, periapical radiographs were taken to serve as a baseline of radiographic healing and to confirm the quality of the root-end filling. The flap was repositioned and sutured tightly with nylon suture (Ethilon, Somerville, NJ), which was removed after 7 days under sedation.

Animal Death and Specimen Preparation

Six months after the root-end surgeries, all animals were killed by injecting an overdose of pentobarbital (80 mg/kg) intravenously. Follow-up periapical radiographs were taken. Subsequently, the jaws were perfused with normal saline, followed by 10% buffered formalin. The mandibles were harvested and sectioned in half with a saw. Specimens were immersed in formalin for fixation.

Radiographic Examinations

In addition to periapical radiographs, further radiographic examinations were also acquired on the mandible specimens, including CBCT (CS 9000 3D; Carestream Health, Rochester, NY) and micro CT (vivaCT 40; Scanco Medical, Bruttisellen, Switzerland). The exposure parameters for the CBCT scans were 75 kV, 10 mA, and 10.8 seconds with a voxel size of 76 μm, and for the micro CT scans the parameters were 55 kV and 142 μA with a voxel size of 30 μm.

Histologic Processes

After fixation in 10% buffered formalin for 1 month, specimens were decalcified in 4% formic acid (ImmunoCal; Decal Chemical Cooperate, Tallman, NY). After 4 weeks, the specimens were further cut into small sections containing 1–2 roots. Decalcification was monitored every week, and the end point was determined by x-ray examination and a physical test, which was 7–10 weeks after the start date. After adequate decalcification, samples were dehydrated in serial concentrations of ethanol and embedded in paraffin wax. Serial sections of 5-μm thickness were made in the buccolingual direction. Three consecutive sections were collected from every 10 sections and stained with hematoxylin-eosin stain, Masson trichrome stain, and Brown and Brenn stain, respectively.

Radiographic Healing Assessment

Three calibrated examiners, who were experienced endodontists familiar with root-end surgery, reviewed all the images (B.K., F.S., and M.K.). The examiners were blinded to the material used. A specific healing category was assigned for each specimen when all 3 examiners agreed or achieved a consensus after discussion.

Periapical radiographs were projected on a big screen in a dark room and were displayed in random order. Healing was determined as complete, incomplete, uncertain, or unsatisfactory according to the criteria established by Rud et al (14) and Molven et al (15).

CBCT and micro CT images were converted to DICOM format, and the scans were reviewed by using OsiriX (Pixmeo, Geneva, Switzerland) in multi-planar reconstruction mode. To evaluate the healing of an individual root, axes were aligned to obtain ideal mesiodistal and buccolingual sections. The sagittal plane was parallel to mesiodistal long axis of the tooth, and the coronal plane was aligned along with the root canal; both planes passed through the middle of the resected root-end surface. The slice thickness was set to 1 mm, which is the approximate diameter of the root-end filling. After proper alignment, semiquantified and quantified analyses were performed to evaluate several healing parameters. The parameters and the scoring criteria are listed in Table 2 and Figure 1.

Histologic Evaluation

Slides were assessed in random order with light microscopes (MVX10 and BH-2; Olympus America, Center Valley, PA) at $\times 1$ to $\times 40$ magnification. The parameters and grading system used for histometric analysis are listed in Table 3.