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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.

Keywords

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. Penicillin G benzathine and penicillin G procaine 120,000 IU were administered 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.

Data Analysis

All data were entered into IBM SPSS Statistics (IBM Corporation, Armonk, NY). Differences in healing between animals, tooth position, and left or right side of the jaw were assessed by using ordinal regression. To compare healing parameters between materials, Mann-Whitney test was used. Nonparametric statistics methods were used because the outcomes are ordinal data and the distance between scores are not equal. However, average scores of each parameter were also presented in the study for easy understanding. Type I error was set at 0.05, and scores are presented as average $\pm$ standard error.

Results

Four roots were excluded from the radiographic analysis, 3 (2 MTA and 1 RRM) because of severe concomitant periodontal involvement and one root (MTA) because of severe root resorption. As a result, 59 roots were analyzed, including 3 in the MTA material control group, 4 in the RRM material control group, 21 in the MTA experiment group, 23 in the RRM experiment group, and 8 in the positive control group. In addition, 5 more roots were excluded from histologic analysis because of histologic procedure errors (3 MTA and 2 RRM). At the end, the number of roots assessed histologically in each group was 3 in the MTA control group, 4 in the RRM control group, 18 in the MTA experiment group, 21 in the RRM experiment group, and 8 in the positive control group.

In all radiographic and histologic parameters, no association was found between healing tendency and specific animals, tooth position, or side of the jaw. No statistical difference in healing was found in the experiment groups (infected canal with root-end filling) and the material control group (disinfected canal with root-end filling). Hence these 2 groups were combined for the final analysis.

Histologic Assessment

RRM Is Biocompatible—In roots with RRM and MTA fillings, tissues adjacent to the root ends usually showed no signs of inflammation or minimal inflammatory infiltration regardless of the material. Furthermore, very few or no inflammatory cells were observed around material remnants accidentally left in the periapical area during the surgical procedure (Fig. 2A and B). Histometric analysis showed the degree of inflammatory infiltration adjacent to the root end was not significantly different between the 2 materials (average score was 1.36 ± 0.19 in MTA group and 1.10 ± 0.04 in RRM group) (Fig. 3I). These

findings suggest RRM is a biocompatible material, and its biocompatibility is comparable to that of MTA.

RRM Has Good Sealing Ability—Bacteria were found in all canals of the experiment groups (infected canal with root-end filling) and the positive control group (infected canal without root-end filling) (Fig. 2C and D). Bacteria were also found in 2 of 7 material control canals (disinfected canal with root-end filling). All roots in the positive control group were associated with severe inflammation, and no signs of healing were observed. In contrast, healing was observed in the majority of roots with root-end fillings. These findings suggest RRM and MTA both have good sealing ability.

RRM Induced More Cementum-like Tissue Formation than That of MTA—In the majority of sections, mineralized tissue was observed on the surfaces of the resected dentin (marked as C in Fig. 2B and E–G and Fig. 4B, E, H, and K) and the root-end filling material (also marked as C in Fig. 2A, B, G, and H, Fig. 3A–H, and Fig. 4C, F, I, and L). Mineralized tissue on the resected dentin surface was often seen at the most peripheral area (junction of resected surface and lateral root surface) in continuum with the cementum on the lateral root surface (Fig. 2E) and extending to varied distances. Mineralized tissue observed directly on the material also covered the surface to varying degree (compare Figs. 3B and F and 4C and I). This new tissue either had similar characteristics to cementum on the lateral root surface (Fig. 2I) or that of bone (Fig. 2G). In many sections, this mineralized tissue extended onto the adjacent resected dentin surface (Figs. 2B and G, 3D and H, and 4C), and the thickness of the tissue decreased toward the peripheral (Fig. 2F, blue arrow; Fig. 4B, yellow arrow). To avoid confusion, “cementum-like tissue” will be used when referring to the mineralized tissue on root-end surface. On the basis of histometric analysis, a significantly greater area was covered on the resected dentin surface and material surface when RRM was used (Fig. 3J and K).

More Periodontal Ligament-like Tissue and Bone Formation Were Associated with Roots Filled with RRM than Those Filled with MTA—Dense fibrous tissue and bone were often observed underneath the cementum-like tissue (Figs. 2F and G, 3A–H, and 4). Fibrous tissue was in continuum with the periodontal ligament (PDL) on the lateral root surface (Fig. 2H). Some fibers were seen inserted into cementum-like tissue on the root end (Figs. 2B and 4B and E), suggesting a likelihood of PDL regeneration. Therefore, “PDL-like tissue” will be used when referring to the dense fibrous tissue in proximity to root-end surface. In a few cases, a complete bony bridge covered the root-end surface in continuum with the buccal and lingual lamina dura, indicating a completed regeneration and reconstitution of the lamina dura (Figs. 2H and 3C). However, in most samples, gaps were seen in this layer of newly formed bone, which was occupied by loose connective tissue similar to bone marrow tissue (Fig. 3A and F). When the data were further analyzed, a significantly greater area was covered by PDL-like tissue and bone adjacent to the root-end surface filled with RRM (Fig. 3L and M).

Cortical Plate Healing Is Material Independent—Cortical plate reestablishment was observed in the majority of roots (Fig. 3A, C, E, and G), and the difference in cortical bone

healing was not significant between the 2 materials (average score was 2.67 ± 0.13 in MTA group and 2.52 ± 0.09 in RRM group) (Fig. 3N).

Radiographic Assessment

Superior Healing Associated with RRM Could Not Be Detected on Periapical Radiographs—None of the treated roots showed unsatisfactory healing. Overall, 84.3% roots showed complete healing, 9.8% incomplete healing, and 5.9% uncertain healing. If complete and incomplete healing were combined as success, the overall success rate of root-end surgeries in this study was 94.1% (Table 4, Fig. 5).

In the RRM group, the number of roots diagnosed as complete healing was higher than that in the MTA group (92.6% vs 75%), but the difference was not statistically significant ($P = .073$).

CBCT and Micro CT Images Were Able to Detect the Healing Difference between RRM and MTA—In terms of healing associated with different materials (Fig. 6), results were similar between two 3-dimensional (3D) imaging modalities. More samples showed score 2 healing (better healing) in the RRM group than in the MTA group, and significant differences were found in some parameters, including healing on the resected root-end surface and healing in the periapical area on both buccolingual and mesiodistal sections (Fig. 6A and B, $P = .000$ to $.027$). However, no significant difference was observed in cortical plate healing between the 2 materials (Fig. 6C, $P = .401$ and $.365$ in CBCT and micro CT).

The alignment and length of root-end filling were evaluated on micro CT images. There were 84.3% fall samples aligned adequately, and the average length of root-end filling was 2.23 ± 0.090 mm. No significant difference was found in RRM and MTA groups with regard to these 2 parameters ($85.2\%/2.21 \pm 0.112$ mm in RRM group, $83.3\%/2.25 \pm 0.089$ mm in MTA group). However, the average filling length in the roots with adequate alignment was significantly longer than those with inadequately aligned filling (2.37 ± 0.091 mm and 1.50 ± 0.121 mm). The effect of alignment and length of root-end filling on healing tendency was further evaluated, and no statistical difference was found (detailed data were not shown).

Discussion

In the present study, roots filled with RRM demonstrated outstanding healing of periapical tissue, which was evaluated with histologic methods and different radiographic modalities. It is the first *in vivo* experiment testing this new bioceramic material so far.

To simulate clinical conditions, apical periodontitis was induced, and lesions were confirmed on periapical radiographs before root-end surgery. The presence of bacteria in the root canals was further confirmed on histologic slides by using Brown and Brenn stain (Fig. 2C and D). In a clinical situation, the primary aim of root-end surgery is to seal any remaining bacteria in the canal and prevent future leakage into the periapical area. If a root-end filling material has good sealing ability, bacteria and contaminants left behind in the

root canal will not cause periapical irritation, and healing will take place. Therefore, our experiment design investigates not only the biocompatibility of the materials but also their sealing ability.

The biocompatibility and sealing ability of MTA have been investigated thoroughly and proved to be outstanding (1). However, the cytotoxicity of RRM has only been investigated in several *in vitro* studies, and the results are inconsistent (9–12, 16–20). Our study showed minimal or no inflammatory reaction adjacent to both materials, and healing took place in all the surgical sites. These results suggest that RRM is comparable to MTA in biocompatibility and sealing ability. The sealing ability of RRM was indirectly inferred from the data; no significant difference between healing parameters exists when comparing infected root canals and disinfected canals.

Another important feature of MTA as a root-end filling material is cementum-like tissue formation directly on its surface, which has been observed in many previous animal experiments (3–8). In the present study, mineralized tissue was also seen on the surfaces of RRM and MTA and the resected dentin. In some sections, mineralized tissue covered the entire root-end surface and was in continuum with cementum on the lateral root surface, suggesting the newly formed mineralized tissue is likely to be regenerated cementum. However, cementum and bone have very similar characteristics, and there is no specific staining technique to differentiate between the two. Hence, the term *cementum-like tissue* was used, mainly on the basis of the location of this mineralized tissue and some features observed on hematoxylin-eosin stained slides. Craig and Harrison (21) suggested that newly formed cementum originates from PDL-derived tissue. However, isolated cementum-like tissue was also found on the root-end filling surface without conjunction of the cementum on the lateral surface, and the thickness decreased toward peripheral in many sections (Fig. 2F). These observations are in line with the findings of Baek et al (7). It is possible that cementum-like tissue on peripheral root-end surface and isolated mineralized tissue on the material have different cell origins. The former may originate from PDL-derived tissue, and the latter was from connective tissue in alveolar bone (4, 7). Progenitor cells in bone are stimulated by root-end filling materials and become mineralized tissue producing cells (osteoblasts/cementoblasts). Another explanation is that cementum-like tissue on the material was connected to the cementum from the mesial or distal surface, and it cannot be seen easily in buccolingual sections. The nature of this mineralized tissue and mechanism of its formation needs further investigation.

The ultimate success of root-end surgery is regeneration and reconstitution of a functional PDL. Dense fibrous tissue adjacent to the resected root-end surface was observed in many roots, and this was in continuum with the PDL on the lateral root surface (Figs. 2H and 3A, C, E, and G). Furthermore, fibrous insertion mimicking Sharpey fibers were observed in the cementum-like tissue (Figs. 2B and 4B). It is likely that this tissue is in the process of becoming functional PDL, suggesting restoration of the original tissue.

While comparing histologic healing in roots filled with RRM and MTA, several differences were noticed. A significantly greater area of the root-end surface was covered by cementum-like tissue, PDL-like tissue, and bone in the RRM group than in the MTA group (Fig. 3J–

M). However, the degree of inflammatory infiltration and cortical plate healing were not associated with a specific root-end filling material (Fig. 3I and N). These histologic findings are in agreement with the radiographic findings by using CBCT and micro CT in which RRM presented significantly better healing on the resected root-end surface and in the periapical area but not cortical plate reestablishment (Fig. 6). However, the most commonly used radiographic method, periapical radiography, could not detect the difference (Fig. 5A). It can be inferred that minute differences such as reformation of PDL, cementum, and quality of bone cannot always be observed on periapical x-rays, even though the success criteria were based on the correlation between histologic findings and radiographic features (14, 15).

To achieve a thorough assessment of 3D images, a scoring system was developed in the present study in which healing in different areas in both sagittal and coronal views was evaluated separately (Fig. 1). The results obtained from CBCT and micro CT corresponded to the histologic results. It suggests that CBCT may be a better evaluation method for clinical studies comparing 2 root-end filling materials, and it also suggests that micro CT is a valid tool to obtain preliminary results before a long duration for histology processes. However, the correlation between CBCT, micro CT images, and histologic data needs to be further analyzed.

In addition to healing parameters, the length and alignment of the root-end filling were also evaluated on micro CT images because they correlate with a proper seal (22). By using 3D images, the shortest distance between canal space and resected root-end surface can be measured accurately. Furthermore, buccally and lingually misaligned fillings can be detected, which is very difficult to do by using periapical radiographs. Our results showed that these 2 parameters in both groups were similar, which indicates that the technical quality of the surgeries in both groups was comparable and that healing was not influenced by iatrogenic variables. To our knowledge, there is no study investigating the effects of length and alignment of root filling on apical healing. It is reasonable to hypothesize that a shorter or inadequately aligned filling is associated with less favorable healing. However, no statistical difference was found in this study. This outcome may be explained because of the small number of samples with inadequately aligned and short fills (8 roots with misaligned filling and 7 fillings shorter than 1.5 mm).

Although several studies have shown superior healing of MTA used in root-end surgery in comparison to amalgam, intermediate restorative material, and SuperEBA (3, 4, 6–8), our study was the first animal experiment comparing MTA with a bioceramic material. RRM performed significantly better in regard to higher healing scores. It is possible that RRM has better mineralized tissue inductive/conductive properties, thus accelerating the deposition of cementum-like tissue on root-end surface accompanied by PDL-like tissue and bone. This hypothesis is partly supported by the data from our pilot cell culture studies in which RRM showed a mitogenic effect on osteogenic/odontogenic progenitor cells and induced osteogenic/cementogenic differentiation in those cells. Furthermore, it is reasonable to expect similar cortical plate healing in the 2 groups, because the plate is not in close proximity to the root-end filling material and hence not directly affected by its properties. However, the mechanism of accelerated healing needs to be further investigated.

Some clinical implications can be drawn from the present study. Using RRM as root-end filling material may achieve better or faster healing in the area adjacent to the resected root-end surface as compared with MTA. In addition, root-end surgery can lead to successful regeneration of apical tissues, even though the remaining root canal is still infected/contaminated. Therefore, nonsurgical retreatment may not always be necessary before root-end surgery as long as the root-end filling material has a good sealing ability. Furthermore, using CBCT as the evaluation tool may be beneficial in clinical research because of its ability to detect a minute difference.

However, there are some limitations in this study. Because of the limited number of animals, healing was assessed only at 1 point (6 months after surgery). Hence, the healing dynamic associated with the materials is unknown. The differences between RRM and MTA might be more dramatic or nonsignificant at an earlier or extended follow-up period. Furthermore, bacteria profiles present in the canal may not be as complex as most clinical situations. In the present study, few well-established biofilm structures were found in Brown and Brenn stained slides. It may be attributed to the fact that intentional induction of apical periodontitis is different from the natural process of the disease development. Therefore, caution must be taken in directly applying these results in clinical conditions. In addition, the superior performance of RRM in the present study should not be extrapolated for other potential clinical applications.

In conclusion, RRM is a biocompatible material with good sealing ability. When used for root-end cavity filling, RRM is associated with a significantly greater area covered by cementum-like tissue, PDL-like tissue, and bone adjacent to the resected root-end surfaces as compared with those filled with MTA in an animal model. The superior healing tendency associated with RRM could be detected by CBCT and micro CT but not periapical radiographs.

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Figure 1.

Healing parameters on micro CT and CBCT images. On *left* are buccolingual sections, and on *right* are mesiodistal sections. Only micro CT images are shown. (A–C) Healing on resected root-end surface. (A) Hard tissue completely covered resected root-end surface. (B) Hard tissue partially covered resected root-end surface. (C) Absence of hard tissue covering resected root-end surface. (D–F) Healing of periapical area. (D) Normal trabecular pattern in periapical area. (E) Thinner and less dense trabecular bone in periapical area. (F) Absence of trabecular bone in periapical area. (G–I) Healing of cortical plate. (G) Intact cortical plate reestablishment. (H) Cortical plate is porous or not in full integrity. (I) Absence of cortical plate. (J and K) Alignment of root-end filling. (J) Adequately aligned root-end filling. (K) Inadequately aligned root-end filling.

Figure 2.

(A) RRM remnants in periapical area did not elicit inflammatory reaction (*yellow arrow*). Mineralized tissue (marked as C) formed adjacent to RRM. (B) No inflammatory infiltration around MTA remnants in periapical area (*yellow arrow*). Mineralized tissue formed on surface of MTA, with fibrous insertion from adjacent dense fibrous tissue mimicking Sharpey fiber (*blue arrow*). (C and D) Presence of bacteria (*green arrow*) in root canal (Fig. 1C) and in dentinal tubules (Fig. 1D). Bacteria were stained as blue or red in Brown and Brenn stain. (E) Mineralized tissue (*yellow arrow*) was usually observed on resected dentin surface at peripheral, and it is in continuum with cementum on lateral root surface (*blue arrow*). (F) Mineralized tissue (*yellow arrow*) was found on root-end filling material surface (MTA). It extended on to resected dentin surface, and thickness decreased (*blue arrow*). MTA remnants did not induce inflammatory reaction in periapical area. (G) Mineralized tissue (*yellow arrow*) on filling material surface (RRM) showed similar characteristics to bone. (H) Fibrous tissue formed adjacent to root-end surface, and it was in continuum with PDL on lateral root surfaces (*yellow arrow*). Mineralized tissue completely covered root-end cavity (*blue arrow*). (I) Cementum on lateral root surface, characterized with basophilic line (*yellow arrow*). B, bone; C, mineralized tissue on root-end surface/cementum-like tissue; D, dentin; MTA, mineral trioxide aggregate; RC, root canal; RRM, EndoSequence Root Repair Material.

Figure 3.

Representative of RRM group (A–F) and MTA group (G–L). (A–C and G–I) Hematoxylin-eosin stain. (D–F and J–L) Masson trichrome stain (collagen stained as blue color, nuclei stained as brown color). (A and D) Overview of periapical area. Cementum-like tissue, PDL-like tissue, and bone covered most of resected root-end surface, which is similar to original structure. RRM remnants were surrounded by loose connective tissue with very few inflammatory cells (blue arrow). (B and E) At higher magnification, cementum-like tissue covered entire resected dentin surface, although the thickness varies (yellow arrow). Fibrous insertion into cementum-like tissue was also observed (green arrow). (C and F) Mineralized tissue formed on surface of RRM (yellow arrow). (G and J) Overview of periapical area. Mild inflammatory infiltration was noted (blue arrow). However, cementum-like tissue, PDL-like tissue, and bone were also found adjacent to root-end surface. (H and K) Cementum-like tissue was observed on entire resected dentin surface (yellow arrow). (I and L) Mineralized tissue was found on surface of MTA (yellow arrow). B, bone; C, mineralized tissue on root-end surface/cementum on lateral root surface; D, dentin; MTA, mineral trioxide aggregate; RRM, EndoSequence Root Repair Material.

Figure 4.

Periapical response of roots filled with RRM (A–D) and MTA (E–H). (A and C) Overview of cross section, both showed complete cortical plate reestablishment. (B) Magnified view of box in (A). Cementum-like tissue covered surface of RRM completely (yellow arrow). Few inflammatory cells were found in periapical area around RRM remnants (blue arrow). (D) Magnified view of box in (C). Mineralized tissue (yellow arrow) on material surface and resected dentin surface formed a continuum, which may seal a potential gap between root-end filling material and dentinal wall. (E and G) Overview of cross section. Incomplete cortical plate formation in (G). (F) Magnified view of box in (E). Cementum-like tissue formed on MTA surface (yellow arrow). However, it did not completely cover entire root-end cavity. (H) Magnified view of box in (G). Mineralized tissue (yellow arrow) on surface of MTA and resected dentin formed a bridge. (I–N) Distribution of healing scores associated with RRM and MTA in each healing parameter. Average scores and *P* values were also listed in the charts. Significant differences were found in cementum-like tissue on material surface (J), cementum-like tissue on resected dentin surface (K), PDL-like tissue formation (L), and bone formation adjacent to root end (M). In all of those parameters, RRM achieved better healing. No significant difference between the 2 materials was found in the parameters of inflammatory infiltration (I) and cortical plate formation (N). B, bone; C,

mineralized tissue on root-end surface/cementum on lateral root surface; D, dentin; MTA, mineral trioxide aggregate; RRM, EndoSequence Root Repair Material.

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Figure 5.

Distribution and averages of healing scores diagnosed by using CBCT and micro CT. (A and B) In general, significantly more score 2 was observed in RRM group than in MTA group, and none of the roots in positive control group were diagnosed as score 2, regardless of healing parameters, imaging modalities, and different sections. Results indicate RRM is associated with significantly more hard tissue formation on the root-end surfaces and in the periapical areas. (C) Cortical plate healing in RRM and MTA groups is not significantly different, and a few roots in positive control group also showed intact cortical plate surface. (D) Average scores of both materials in different healing parameters also demonstrated the superiority of RRM. B-L, buccolingual; M-D, mesiodistal.

Figure 6.

Examples of healing on different imaging modalities. In all panels, 1 is post-surgery periapical radiograph, 2 is 6-month follow-up periapical radiograph; 3 is mesiodistal (MD) section and 4 is buccolingual (BL) section of CBCT images at 6-month follow-up; 5 is MD section and 6 is BL section of micro CT image at 6-month follow-up. (A) Periapical radiographs were not able to detect periapical bony defect effectively. (A1 and A2) Comparing post-surgery and follow-up radiographs, it was diagnosed as complete healing. (A3 and A4) Periapical radiolucency was clearly shown on CBCT images. The root was diagnosed as absence of hard tissue covering the root-end surface (score 0) and absence of trabecular bone in the periapical area (score 0). (A5 and A6) Micro CT image demonstrated same results. (B) Root filled with RRM. (B1 and B2) Periapical radiographs showed complete healing. (B3 and B4) Diagnosed as hard tissue completely covered the root-end surface (score 2) and normal trabecular pattern in the periapical area (score 2) on both sections. (B5 and B6) Micro CT images showed the same results. (C) Root filled with MTA. (C1 and C2) Diagnosed as complete healing by using periapical radiographs. (C3 and C4) CBCT showed hard tissue completely covered the root-end surface (score 2) and normal trabecular pattern in the periapical area (score 2) on both sections. (C5 and C6) Micro CT images showed the same results except the finding of less dense trabecular bone in the periapical area (score 1) on BL section. (D) Root filled with MTA showed uncertain healing on periapical radiographs. (D1 and D2) Size of radiolucency has decreased but was larger

than twice the width of PDL space. (*D3* and *D4*) CBCT showed no hard tissue forming on the resected root-end surface and in the periapical area (score 0) on both MD and BL sections. However, intact cortical plate was observed (score 2). (*D5* and *D6*) Micro CT images showed absence of hard tissue on the resected root-end surface (score 0), but some trabecular bone was observed in the periapical area (score 1). F/U, follow-up.

Table 1
Positive Control Group in Which Canals Remain Infected without Root-end Fillings

	Material control group		Experimental group					
	First premolar		Second premolar		Third premolar		Fourth premolar	
	Single		Mesial	Distal	Mesial	Distal	Mesial	Distal
Intentionally infect the root canal (day 0)	+		+	+	+	+	+	+
Disinfect the root canal before the surgery (6 weeks)	+		-	-	-	-	-	-
Root-end filling (6 weeks)	+ (MTA or RRM) + (3 roots filled with MTA, and 3 filled with RRM in one side of the jaw)							
Radiographic evaluation (7.5 months)	Radiographs acquired (periapical radiography, CBCT, micro CT)							
Histologic evaluation (10 months)	Serial sections stained with hematoxylin-eosin, Masson trichrome, and Brown and Brenn stain							

Distal, distal root; Mesial, mesial root; Single, single root.

Table 2
Radiographic Healing Parameters

Parameters	Score	Criteria
Healing on resected root-end surface in M-D and B-L sections	2	Hard tissue covering resected root-end surface completely (Fig. 1A)
	1	Hard tissue covering resected root-end surface partially (Fig. 1B)
	0	Absence of hard tissue covering resected root-end surface (Fig. 1C)
Healing of periapical area in M-D and B-L sections	2	Reformation of normal trabecular pattern (Fig. 1D)
	1	Trabecular bone is thinner and less dense compared with noninvolved area (Fig. 1E)
	0	Absence of trabecular bone in previous osteotomy site (Fig. 1F)
Healing of cortical plate in B-L sections	2	Presence of cortical plate with intact surface (Fig. 1G)
	1	Presence of immature cortical plate. The surface is porous or not in full integrity (Fig. 1H)
	0	Absence of cortical plate (Fig. 1I)
Alignment of root-end filling	1	Along and within the original root canal (Fig. 1J)
	0	Inadequately aligned (Fig. 1K)
Length of root-end filling		The shortest distance from most coronal point of filling to most apical point and parallel to root canal. These measurements were only obtained on micro CT images.

B-L, buccolingual; M-D, mesiodistal.

Table 3
Scoring Criteria for Histologic Assessments

Parameters	Score	Criteria
Degree of inflammatory infiltration	1	Number of inflammatory cells adjacent to resected root end is <25% of that in the positive control
	2	Number of inflammatory cells is >25% but <50% of that in the positive control
	3	Number of inflammatory cells is >50% but <75% of that in the positive control
	4	Number of inflammatory cells is >75% of that in the positive control
Cementum-like tissue formation on root-end filling material surface	1	Cementum-like tissue on <25% of the material surface
	2	Cementum-like tissue on >25% but <50% of the material surface
	3	Cementum-like tissue on >50% but <75% of the material surface
	4	Cementum-like tissue on >75% of the material surface
PDL-like tissue formation	1	PDL adjacent to <25% of the resected root-end surface
	2	PDL adjacent to >25% but <50% of the resected root-end surface
	3	PDL adjacent to >50% but <75% of the resected root-end surface
	4	PDL adjacent to >75% of the resected root-end surface
New bone formation adjacent to root end	1	New bone covered <25% of the resected root-end surface
	2	New bone covered >25% but <50% of the resected root-end surface
	3	New bone covered >50% but <75% of the resected root-end surface
	4	New bone covered >75% of the resected root-end surface
Cortical plate formation	1	Absence of cortical plate formed at the osteotomy surface
	2	Incomplete cortical plate formation with defect communicating between gingival tissue and the osteotomy site
	3	Complete cortical plate formation
Presence of bacteria in root canal [*]	0	Absence of bacteria in the root canal
	1	Presence of bacteria in the root canal

^{*} Determined by the Brown and Brenn stained slides.

Table 4

Healing on Periapical Radiographs

	Complete	Incomplete	Uncertain	Unsatisfactory	Success/failure
MTA	18 (75%)	3 (12.5%)	3 (12.5%)	0 (0%)	21 (87.5%)/3 (12.5%)
RRM	25 (92.6%)	2 (7.4%)	0 (0%)	0 (0%)	27 (100%)/0 (0%)
Overall	42 (84.3%)	5 (9.8%)	3 (5.9%)	0 (0%)	47 (94.1%)/3 (5.9%)