Healing of experimental apical periodontitis after apicoectomy using different sealing materials on the resected root end

Kaori OTANI1, Tsutomu SUGAYA1, Mahito TOMITA2, Yukiko HASEGAWA3, Hirofumi MIYAJI1, Taichi TENKUMO1, Saori TANAKA1, Youji MOTOKI1, Yasuhiro TAKANAWA1 and Masamitsu KAWANAMI1

1Department of Periodontology and Endodontology, Division of Oral Health Science, Hokkaido University Graduate School of Dental Medicine, N13 W7 Kita-ku, Sapporo 060-8586, Japan
2Dental Office Mahito, 2-2 Kawanacho, Showa-ku, Nagoya 466-0856, Japan
3Kinikyo Sapporo Dental Clinic, 7-25 Kikusui 4-1, Shiroishi-ku, Sapporo 003-0804, Japan
Corresponding author, Kaori OTANI; E-mail: kaohtani@den.hokudai.ac.jp

This study evaluated apical periodontal healing after root-end sealing using 4-META/MMA-TBB resin (SB), and root-end filling using reinforced zinc oxide eugenol cement (EBA) or mineral trioxide aggregate (MTA) when root canal infection persisted. Apical periodontitis was induced in mandibular premolars of beagles by contaminating the root canals with dental plaque. After 1 month, in the SB group, SB was applied to the resected surface following apicoectomy. In the EBA and MTA groups, a root-end cavity was prepared and filled with EBA or MTA. In the control group, the root-end was not filled. Fourteen weeks after surgery, histological and radiographic analyses in a beagle model were performed. The bone defect area in the SB, EBA and MTA groups was significantly smaller than that in the control group. The result indicated that root-end sealing using SB and root-end filling using EBA or MTA are significantly better than control.

Keywords: Root-end sealing, 4-META/MMA-TBB resin, Apicoectomy, Retrograde, Root-end filling

INTRODUCTION

The spread of bacteria from the root canal to surrounding tissues should be prevented when treating apical lesions. When conventional root canal treatment has failed, or when orthograde retreatment is not feasible because the root canal is obliterated, severely curved, or contains a fractured instrument or a large post/core, endodontic surgical procedures like apicoectomy and intentional reimplantation can be considered. Root-end filling should be performed properly for the success of endodontic surgical procedures, such as apicoectomy. Regrettably, however, conventional retrograde root filling is not always successful.

A variety of materials have been used as root-end filling materials. When infected substances are present in the root canal, it is important that the retrograde filling prevents leakage of these substances from the root canal to the periapical tissue. Some reports have suggested that microleakage is not completely inhibited in vitro with amalgam, zinc oxide eugenol cement or glass-ionomer cement1-7). Microleakage with mineral trioxide aggregate was significantly less than that with amalgam, gutta-percha and zinc oxide eugenol in a dye leakage test7-10), and mineral trioxide aggregate showed significantly less leakage than reinforced zinc oxide eugenol cement when evaluated using a bacterial leakage system11). However, microleakage of mineral trioxide aggregate has been reported to increase with time12).

Applying a bonding agent to a resected root end without cavity preparation has been shown to reduce microleakage by sealing both the root canal and dentinal tubules13). Rud et al. reported good results using Gluma® (Gluma desensitizer, Heraeus Kulzer G.m.b.H., Werheim, Germany) and Retroplast™ (Retroplast Trading, Ronne, Denmark)14-17). However, resin is known to be sensitive to moisture during application. Contamination of the resected surface with blood and humidity in expiration during the application procedure causes inferior bonding and resin detachment.

Since 4-META/MMA-TBB resin adheres to dentin both in vitro and in vivo18), it has been widely used in orthopedics and prosthetic dentistry. In our previous studies, 4-META/MMA-TBB resin showed a number of advantages, such as high adhesiveness to dentin and cementum19). According to Tanaka et al.20), dye leakage of root-ends sealed without cavity preparation using 4-META/MMA-TBB resin was significantly less than that of root-end fillings using amalgam and reinforced zinc oxide eugenol cement.

Some reports have suggested that 4-META/MMA-TBB resin offers good biocompatibility after complete curing21-24). Furthermore, 4-META/MMA-TBB resin has a high adhesiveness and biocompatibility to periodontal tissue when used for bonding fractured teeth25,26). Thus, 4-META/MMA-TBB resin might be suitable for retrograde root sealing following apicoectomy or intentional replantation27,28).

In this study, we evaluated apical periodontal healing after root-end sealing using 4-META/MMA-TBB resin and root-end filling using reinforced zinc oxide eugenol cement or mineral trioxide aggregate when root canal infection persisted.
MATERIALS AND METHODS

Animals
Five healthy female beagle dogs, 27–28 months old, were used in this experiment. Seventy roots from forty mandibular premolars were used. The experimental protocol (No. 06013) followed the guidelines for the care and use of laboratory animals of the Graduate School of Medicine, Hokkaido University.

Surgical procedure
Surgical procedures were performed under general anesthesia with medetomidine hydrochloride (0.1 mL/kg, Domitor®, Meiji Seika, Tokyo, Japan) and ketamine hydrochloride (0.1 mL/kg, Ketaral 50®, Sankyo, Tokyo, Japan), and local anesthesia with lidocaine hydrochloride (2% with 1:80,000 epinephrine, Xylocaine®, DENTSPLY SANKIN, Tokyo, Japan).

The pulp tissue was removed and dental plaque was placed into the canals and sealed with glass ionomer cement (Fuji IXG® GC, Tokyo, Japan). Apical periodontitis developed in 1 month and was verified radiographically (baseline). The area of periradicular radiolucency was evaluated using Scion Image® (National Institute of Health). The area of periradicular radiolucency was measured using Scion Image® (National Institute of Health). The area of periradicular radiolucency at 14 weeks was compared with that at baseline. Periapical healing of the roots was evaluated according to the criteria by Rud et al.[16,29] as follows. Complete healing: Complete bone regeneration around the apex with or without a recognizable periodontal space. Incomplete healing: A periapical rarefaction with an irregular, often asymmetrical outline and an angular connection to the periodontal ligament. Uncertain healing: Uncertain healing or cases undergoing phases of healing. A rarefaction located symmetrically around the apex with funnel-shaped connection to the periodontal ligament space and which gradually decreased compared to postoperative radiograph. Unsatisfactory healing: The same radiographic signs as for uncertain healing, except that the rarefaction is either enlarged or unchanged compared to the postoperative or a previously taken follow-up radiograph. It is impossible to distinguish between bone resorption and sealed 4-META/MMA-TBB resin because 4-META/MMA-TBB resin appears radiolucent. Although the thickness of 4-META/MMA-TBB resin is less than 0.1 mm. Then, in the SB group, the area of periodontal radiolucency is measured as the bone resorption. Statistical differences were analyzed using Chi-square test with SPSS 10.0 J (S.P.S.S. Co. Ltd., Tokyo, Japan).

Histological procedure
The animals were euthanized with an overdose of sodium pentobarbital (0.5 mL/kg, Nembutal® injection, Abbott Laboratories, Abbott Park, IL, USA) following general anesthesia 14 weeks after surgery. The tissue blocks were fixed in 10% buffered formalin, decalcified in 10% formic-citric acid, and embedded in paraffin wax. Six-micrometer-thick sections were serially prepared along the long axis of the tooth and the sections close to the center of the root were stained with hematoxylin and eosin (H–E).

Three HE-stained sections were taken; one approximately from the center of the root, and the other two 100 μm from either side of the center. The mean value of these three sections was established as a measured value. The following measurements were performed under light microscope:
1. The area of the bone defect, which was the area
surrounded by the resected dentinal surface and bone (Fig. 1).

2. The percentage of cementum-like tissue on the resected dentinal surface, which was the percentage of the length of newly deposited cementum-like tissue on the resected dentinal surface (a) / the length of the resected dentinal surface (b+c) (Fig. 2).

3. The percentage of cementum-like tissue on the root-end filling materials, which was the percentage of the length of newly formed cementum-like tissue on the surface of the root-end filling materials (a) / the length of the root-end material (b) (Fig. 3).

Histological measurements were taken using Scion Image® (National Institute of Health). Means and standard deviation of the histomorphometric parameters were calculated for each group. Two-by-two analyses were performed by Mann-Whitney U test with Bonferroni correction. P values were considered statistically significant at <0.01 and <0.05. All statistical procedures were performed using SPSS 10.0 J.

RESULTS

Two SB specimens were excluded from the study because of technical problems that occurred during histological preparation. Three EBA specimens were excluded because perforations were made when root-end filling cavities were prepared. One EBA and two MTA specimens were excluded because failures in the root-end filling material were detected on the radiographs after surgery. Consequently, a total of 17 SB, 14 EBA, 17 MTA and 14 control specimens were available for radiographic and histological examination.

Observations of periradicular radiolucency

The areas of preoperative periradicular radiolucency in the SB, EBA, MTA and control groups were 2.24±1.51 mm², 2.10±0.98 mm², 2.12±1.00 mm², and 2.15±1.67 mm², respectively. There were no significant differences in the area of preoperative periradicular radiolucency among the 4 groups. The area of postoperative periradicular radiolucency in the SB, EBA, MTA and control groups was 8.55±4.01 mm², 8.92±3.15 mm², 8.05±3.39 mm², and 8.41±3.86 mm², respectively. There were no significant differences in the area of postoperative periradicular radiolucency among the 4 groups. Healing was evaluated radiographically 14 weeks after the surgery. The areas of periradicular radiolucency in the SB, EBA, MTA and control groups were 8.55±4.01 mm², 8.92±3.15 mm², 8.05±3.39 mm², and 8.41±3.86 mm², respectively. There were no significant differences in the area of postoperative periradicular radiolucency among the 4 groups. Healing was evaluated radiographically 14 weeks after the surgery. The areas of periradicular radiolucency in the SB, EBA, MTA and control groups were 1.22±1.07 mm², 1.37±1.07 mm², 1.27±1.29 mm², and 6.17±2.18 mm², respectively (Fig. 4 & Table 1). The healing in the SB, EBA and MTA groups was significantly better than that in the control group (p<0.01). The periradicular lesion in the 14 weeks after the surgery of the SB, EBA and MTA groups had become significantly better than the preoperative lesion (p<0.05), whereas the healing in the control group was significantly worse (p<0.01).

The healing was also evaluated clinically and radiographically 14 weeks after the surgery. In the SB group, complete and incomplete healing was observed in
Fig. 4 Observations of periradicular radiolucency.
There were no significant differences in all groups at immediately after surgery. The SB, EBA and MTA groups had became significantly better than the control group \((p<0.01)\) and the preoperative lesion \((p<0.05)\).

<table>
<thead>
<tr>
<th></th>
<th>Area of preoperative radiolucency [mm²] mean (SD)</th>
<th>Area of postoperative radiolucency [mm²] mean (SD)</th>
<th>Area of periradicular radiolucency at 14 weeks [mm²] mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB</td>
<td>2.24 (1.51)</td>
<td>8.55 (4.01)</td>
<td>1.22 (1.07)*†</td>
</tr>
<tr>
<td>EBA</td>
<td>2.10 (0.98)</td>
<td>8.92 (3.15)</td>
<td>1.37 (1.07)*†</td>
</tr>
<tr>
<td>MTA</td>
<td>2.12 (1.00)</td>
<td>8.05 (3.39)</td>
<td>1.27 (1.29)*†</td>
</tr>
<tr>
<td>Control</td>
<td>2.15 (1.67)</td>
<td>8.41 (3.86)</td>
<td>6.17 (2.18)**</td>
</tr>
</tbody>
</table>

* Significantly different from the area of preoperative radiolucency \((p<0.05)\)
** Significantly different from the area of preoperative radiolucency \((p<0.01)\)
† Significantly different from control group \((p<0.01)\)

Table 2 Radiographic classification of healing

<table>
<thead>
<tr>
<th></th>
<th>Complete</th>
<th>Incomplete</th>
<th>Uncertain</th>
<th>Unsatisfactory</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>SB*</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>EBA*</td>
<td>9</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>MTA*</td>
<td>12</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>10</td>
<td>14</td>
</tr>
</tbody>
</table>

* Significantly different from control group \((p<0.01)\)
12 and 5 of the 17 roots, respectively (Table 2). In the EBA group, complete and incomplete healing was observed in 9 and 5 of the 14 roots, respectively. In the MTA group, complete and incomplete healing was observed in 12 and 5 of the 17 roots, respectively. In the control group, uncertain and unsatisfactory healing was observed in 4 and 10 of the 14 roots, respectively. In the SB, EBA and MTA groups, the healing 14 weeks after apicoectomy was classified as “almost good”.

The healing in the SB, EBA and MTA groups was significantly better than that in the control group \((p<0.01)\). There were no significant differences among the SB, EBA, and MTA groups.

**Histological observations**

In the control group, infiltrating inflammatory cells, composed of leukocytes, lymphocytes and some macrophages, were observed in the apical periodontal tissue (Fig. 5).

In the SB group, few inflammatory cells were observed in the apical periodontal tissue facing 4-META/MMA-TBB resin. It was observed that 4-META/MMA-TBB resin was encapsulated with thin fibrous tissue. Alveolar bone had formed close to 4-META/MMA-TBB resin (Fig. 6).

In the EBA group, the tissue facing reinforced zinc oxide eugenol cement contained a few inflammatory cells (Fig. 7).

In the MTA group, bone regeneration was observed (Fig. 8).

---

**Fig. 5** Control group at 14 weeks (H–E staining).
- a) Bone defect and marked inflammatory cells (arrow) are shown. Scale bar, 500 µm.
- b) Higher magnification of framed area (b) in a. Scale bar, 50 µm.

**Fig. 6** SB group at 14 weeks (H–E staining).
- a) Well-marked bone regeneration was observed. 4-META/MMA-TBB resin was dissolved during the preparation of specimens. Scale bar, 500 µm.
- b) Higher magnification of framed area (b) in a. Thin fibrous tissue (arrow) on the surface of 4-META/MMA-TBB resin and few inflammatory cells are shown. Scale bar, 50 µm.

**Fig. 7** EBA group at 14 weeks (H–E staining). EBA: reinforced zinc oxide eugenol cement.
- a) Bone regeneration was observed. Scale bar, 500 µm.
- b) Higher magnification of framed area (b) in a. Cementum-like tissue (arrow) on the dentin surface and a few inflammatory cells on reinforced zinc oxide eugenol cement are shown. Scale bar, 50 µm.

**Fig. 8** MTA group at 14 weeks (H–E staining). MTA: mineral trioxide aggregate.
- a) Bone regeneration was observed. Scale bar, 500 µm.
- b) Higher magnification of framed area (b) in a. Cementum-like tissue (arrow) on the dentin surface and mineral trioxide aggregate and few inflammatory cells are shown. Scale bar, 50 µm.
cells, such as lymphocytes and plasma cells. Cementum-like tissue formation was only observed on the resected dentin surface (Fig. 7).

In the MTA group, inflammatory cells were rarely seen in the apical periodontal tissue facing mineral trioxide aggregate. Cementum-like tissue formation was observed on both the resected dentin surface and mineral trioxide aggregate in 14 of the 17 sections. Mostly, cementum-like tissue was formed in succession to the original cementum at the root ends (Fig. 8).

Area of bone defect
The areas of bone resorption in the SB, EBA, MTA and control groups were 1.23±1.06 mm², 1.37±1.03 mm², 1.00±0.87 mm², and 6.45±1.04 mm², respectively (Table 3). Bone resorption in the SB, EBA and MTA groups was significantly less than that in the control group (p<0.01). There were no significant differences among the SB, EBA, and MTA groups.

Percentage of cementum-like tissue on resected dentinal surface
The percentages of cementum-like tissue deposition on the resected dentinal surface in the SB, EBA, MTA and control groups were 5.8±9.2%, 55.1±19.5%, 74.8±25.3%, and 4.0±10.0%, respectively (Table 3). The percentages of cementum-like tissue deposition on the resected dentinal surface in the EBA and MTA groups were significantly higher than those in the SB and control groups (p<0.01). The MTA group showed significantly more deposition than the EBA group (p<0.05) (Table 3).

Percentage of cementum-like tissue on root-end filling materials
The percentage of cementum-like tissue deposition on the root-end filling materials in the MTA group was 73.1±38.1% (Table 3). This was significantly greater than that in the SB, EBA and control groups (p<0.01). Cementum-like tissue deposition on the root-end filling materials was not observed with 4-META/MMA-TBB resin or reinforced zinc oxide eugenol cement.

DISCUSSION
In this study, pulp tissues were removed from the canals and apical periodontitis was induced by contaminating the root canals with dental plaque. Periradicular radiolucency was verified. No significant difference was observed in the area of preoperative periradicular radiolucency among the 4 groups. It was therefore assumed that the preoperative condition of the 4 groups was almost identical.

The radiographic and histological findings of the present study indicate that apical periodontal healing was stimulated by root-end treatment with reinforced zinc oxide eugenol cement, mineral trioxide aggregate and 4-META/MMA-TBB resin. These results suggest that reinforced zinc oxide eugenol cement, mineral trioxide aggregate and 4-META/MMA-TBB resin may prevent leakage of contamination from the root canals to the surrounding apical tissue. Upon histological observation, the morphological characteristics of the healing in each of the groups were different.

The tissue facing reinforced zinc oxide eugenol cement contained a few inflammatory cells and this result was in agreement with those of Baek et al.30). However, the degree of inflammation was extremely mild and the bone resorption was not significantly different among the SB, EBA, and MTA groups. As Rubinstein et al.31) reported long-term success when using reinforced zinc oxide eugenol cement as the root-end filling material, a few inflammatory cells facing reinforced zinc oxide eugenol cement might be clinically acceptable32). In contrast, reinforced zinc oxide eugenol cement has been reported to have inadequate long-term sealing ability6,10). In the EBA group, cementum-like tissue deposition was observed on the resected dentinal surface, but not on reinforced zinc oxide eugenol cement. Thus, newly formed cementum-like tissue may not prevent leakage, and inflammation of apical periodontal tissues might recur over the long term when contamination is present in the root canal.

Cementum-like tissue formation on the resected dentinal surface and mineral trioxide aggregate has been reported30,33-36). In the present study, cementum-like tissue deposition was observed on the resected dentinal...
surface in all sections and on the mineral trioxide aggregate in 14 of the 17 sections. Continuous cementum-like tissue formation on dentin and the filling material may prevent leakage of contaminated substances. Many in vitro studies have demonstrated that mineral trioxide aggregate has good long-term sealing ability, although a gradual increase in leakage over long periods has also been reported. Cementum-like tissue might be useful for sealing if the leakage of mineral trioxide aggregate increases over time. There are few studies regarding the sealing ability of cementum-like tissue on the apical dentinal surface and mineral trioxide aggregate. In the present study, the percentage of cementum-like tissue on the apical dentinal surface and on the mineral trioxide aggregate was 74%, and cementum-like tissue deposition was not observed on the mineral trioxide aggregate in 3 of the 17 sections. Further investigations might be necessary to determine whether cementum-like tissue induced by mineral trioxide aggregate is effective in sealing the root canal system.

After root-end sealing using 4-META/MMA-TBB resin, cementum-like tissue was not formed on the resin surface, but few inflammatory cells were observed in the tissue facing 4-META/MMA-TBB resin. This could be attributed to the high sealing ability and biocompatibility of the material. Resin is known to be sensitive to moisture during application, which decreases sealing ability and biocompatibility. As a polymerization initiator 4-META/MMA-TBB resin uses TBB. TBB is believed to promote radical formation in the presence of oxygen and water. Therefore, 4-META/MMA-TBB resin might show high-level polymerization under wet conditions, high sealing ability, little cytotoxicity and high biocompatibility after complete curing. Our previous studies using dye leakage tests suggested that the leakage values of dentin and cementum increased until the third month and then leakage tests suggested that the leakage values of dentin after complete curing. Our previous studies using dye sealing ability, little cytotoxicity and high biocompatibility significantly better than apicoectomy with no material in trioxide aggregate in root canal infection were reinforced zinc oxide eugenol cement and mineral resin following apicoectomy and root-end filling using 4-META/MMA-TBB resin, reinforced zinc oxide eugenol cement and mineral trioxide aggregate has good long-term sealing ability, although a gradual increase in leakage over long periods has also been reported. Cementum-like tissue might be useful for sealing if the leakage of mineral trioxide aggregate increases over time. There are few studies regarding the sealing ability of cementum-like tissue on the apical dentinal surface and mineral trioxide aggregate. In the present study, the percentage of cementum-like tissue on the apical dentinal surface and on the mineral trioxide aggregate was 74%, and cementum-like tissue deposition was not observed on the mineral trioxide aggregate in 3 of the 17 sections. Further investigations might be necessary to determine whether cementum-like tissue induced by mineral trioxide aggregate is effective in sealing the root canal system.

After root-end sealing using 4-META/MMA-TBB resin, cementum-like tissue was not formed on the resin surface, but few inflammatory cells were observed in the tissue facing 4-META/MMA-TBB resin. This could be attributed to the high sealing ability and biocompatibility of the material. Resin is known to be sensitive to moisture during application, which decreases sealing ability and biocompatibility. As a polymerization initiator 4-META/MMA-TBB resin uses TBB. TBB is believed to promote radical formation in the presence of oxygen and water. Therefore, 4-META/MMA-TBB resin might show high-level polymerization under wet conditions, high sealing ability, little cytotoxicity and high biocompatibility after complete curing. Our previous studies using dye leakage tests suggested that the leakage values of dentin and cementum increased until the third month and then remained fairly constant until the sixth month, and sealing durability that prevents coronal leakage seemed to be maintained for 1 year. This suggests that root-end sealing using 4-META/MMA-TBB resin might have a good prognosis clinically. From these results, it is possible that root-end sealing using 4-META/MMA-TBB resin is effective in sealing the root canal following apicoectomy. In particular, root-end sealing with 4-META/MMA-TBB resin is likely to be effective when a root-end cavity cannot be prepared, for example, in teeth with long post-core or in roots in which the morphology makes it difficult to prepare a root cavity.

CONCLUSION

The histological reaction of apicoectomy with 4-META/MMA-TBB resin, reinforced zinc oxide eugenol cement or mineral trioxide aggregate showed different healing morphology. Root-end sealing using 4-META/MMA-TBB resin following apicoectomy and root-end filling using reinforced zinc oxide eugenol cement and mineral trioxide aggregate in root canal infection were significantly better than apicoectomy with no material in histological analysis and radiography. Only root-end filling using mineral trioxide aggregate was observed cementum-like tissue formation on the root-end filling materials.

REFERENCES


