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Histologic Assessment of Mineral Trioxide Aggregate as a Root-End Filling in Monkeys

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Mineral Trioxide Aggregate (MTA) has been shown in a number of experiments to be a potential root-end filling material. The purpose of this study was to examine the periradicular tissue response of monkeys to MTA and amalgam as root-end fillings. The pulps were removed from all the maxillary incisors of three monkeys. The root canals were prepared and filled with laterally condensed gutta-percha and sealer, and the access cavities were restored with amalgam. Buccal mucoperiosteal flaps were raised, and root-end resections were performed before root-end cavity preparation with burs. The root-end cavities in half of the teeth were filled with MTA, while amalgam was placed in the other cavities. After 5 months the periradicular tissue response was evaluated histologically. The results showed no periradicular inflammation adjacent to five of six root ends filled with MTA; also five of six root ends filled with MTA had a complete layer of cementum over the filling. In contrast, all root ends filled with amalgam showed periradicular inflammation, and cementum had not formed over the root-end filling material, although it was present over the cut root end. Based on these results and previous investigations, MTA is recommended as a root-end filling material in man.

An experimental material, mineral trioxide aggregate (MTA), has recently been investigated as a potential alternative to the presently used root-end filling materials (1–9). The sealing ability of MTA has been shown in dye and bacterial leakage studies (1–3) to be superior to that of amalgam or SuperEBA. The cytotoxicity of MTA was investigated, using the agar overlay and radiochromium release methods (7) and found to be less than that of IRM or SuperEBA. With implantation of MTA in Guinea pig mandibles (8) no observable difference was found between MTA and SuperEBA. Furthermore, the use of MTA as a root-end filling material in dogs provided superior results to amalgam, and it has been shown to have an inductive effect on cementoblasts (9).

The purpose of this study was to investigate the response of periradicular tissues of monkeys to MTA and amalgam when used as root-end filling materials in teeth in which bacterial contamination of the root canals was avoided.

MATERIALS AND METHODS

The left and right maxillary central and lateral incisors of three healthy 4-yr-old Cynomolgus monkeys, each weighing 12 to 15 lb, were used in this experiment. The procedures were carried out according to the guidelines approved by the Research Committee of Loma Linda University. Anesthesia was provided by an initial intramuscular injection of 0.5 ml Ketamine (Fort Dodge Laboratories Inc, Fort Dodge, IA) and 0.05 ml Rompun (Miles Inc, Shawnee Mission, KA). This was supplemented by intraoral local anesthesia obtained by buccal mucosal infiltration of 2% lidocaine with 1:50,000 epinephrine.

The teeth were isolated with rubber dam and the pulps exposed through a standard occlusal access opening. The root canals were debrided and enlarged to a #40 size master apical file. The root canals were obturated with laterally condensed gutta-percha and Roth root canal sealer (Roth International Ltd, Chicago, IL) before restoration of their access cavities with amalgam.

Periradicular surgery was carried out 1 week after root canal obturation. A full thickness buccal mucoperiosteal flap with two vertical incisions (mesial of first premolars) was raised to gain access to the periradicular tissues of the four maxillary incisors. After reflection of the flap and removal of the cortical bone with a #6 round bur in a high-speed handpiece with the use of copious sterile water spray, the unerupted maxillary cuspid teeth were removed to allow the incisor roots to be resected at the juncture of their apical and middle thirds with a fissure bur under constant sterile water spray. Root-end cavities were prepared to a depth of 2 mm using a #2 round bur in a high speed handpiece and sterile water spray. The root-end cavities on one side were randomly selected to be filled with zinc-free amalgam (Kerr Manufacturing Co, Romulus, MI) and on the other side with MTA (Loma Linda University, Loma Linda, CA). The surgical flaps were sutured with absorbable gut sutures, and the animals were placed on a soft diet. Each animal received an intramuscular injection of penicillin fol-

Table 1. Histologic findings in periradicular tissues of root ends filled with amalgam or MTA.

	Amalgam	MTA
No. Roots	6	6
Concentration of inflammation	4 S	1 S
Severe: S	2 MO	5 H
Moderate: MO		
Mild: MI		
Healed: H		
Width (mm) of inflammation	1 1.0	1 1.0
	5 0.5	5 Healed
Predominant cell	6 L	1 L
Macrophage: M		5 Healed
Polymorph: P		
Lymphocyte: L		
Fibrous Capsule Yes/Total	4/6	0/6
Cementum over material Yes/Total	0/6	5/6
Cementum over root end Yes/Total	6/6	6/6
New bone formation Yes/Total	6/6	6/6

lowing surgery as well as analgesics for 3 days. The animals were monitored daily for 1 week for possible postsurgical complications; however, healing took place uneventfully in all animals.

The animals were reanesthetized 5 months after surgery and perfused with 10% buffered formalin. Block sections containing incisor teeth and their surrounding tissues were then removed and placed in formalin for 2 weeks. The specimens were demineralized in EDTA, embedded in paraffin, and sectioned buccolingually at a thickness of 6 μm. Step-serial sections at approximately 50 μm intervals were stained with hematoxylin and eosin, Masson's trichrome, or by the Brown and Brenn method.

Concentration, extent of inflammation, and predominant inflammatory cell type in the periradicular tissues adjacent to the root-end filling materials were recorded. The severity of the inflammation was recorded as: none, no inflammatory cells; mild, few inflammatory cells; moderate, inflammatory cells did not obscure the normal tissues; and severe, inflammatory cells replaced normal tissues. The extent of inflammation from the surface of root-end filling material was recorded as $\leq$ 0.2 mm, $\leq$ 0.5 mm, or $\leq$ 1 mm. Presence or absence of bacteria within the teeth, a fibrous capsule, cementum deposition on the root end and root-end filling materials, and new bone formation were also recorded. Histopathologic examination of the specimens was performed by two investigators jointly without knowledge of which material had been placed in the root end.

RESULTS

A total of six amalgam and six MTA samples were available for histologic examination. The histopathologic findings are summarized in Table 1. The periradicular tissues of all roots with amalgam as the root-end filling material had moderate to severe inflammation (Fig. 1), while only one root that had been filled with MTA displayed inflammation, which was severe. When amalgam was used as the root-end filling material, lymphocytes were the predominant inflammatory cell in the bulk of the lesions; however,

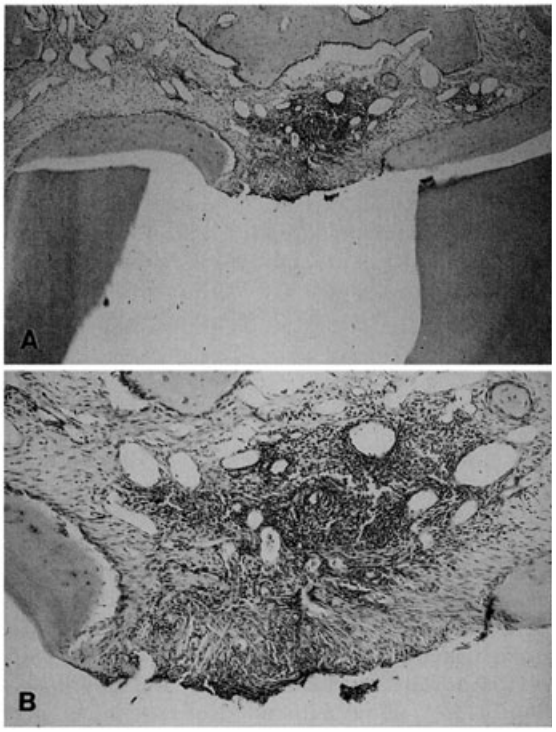

Fig 1. Tissue response to an amalgam root-end filling. *(A)* New cementum has grown over the cut root-end dentin but not over the root-end filling, where there is inflamed connective tissue (Original magnification ×20). *(B)* The connective tissue over the root-end filling has a moderate infiltrate of inflammatory cells (Original magnification ×50, Hematoxylin and eosin).

close to the amalgam polymorphonuclear leukocytes were frequently observed. A fibrous tissue capsule was found over most amalgam root-end fillings.

Cementum formation was observed over the dentinal surface of the resected root ends for both groups, but it was not seen over any of the amalgam root-end fillings. In contrast, a thick layer of cementum was present over five of six MTA root-end fillings (Fig. 2); an incomplete layer had partly formed over the other. The cementum showed incremental lines (Fig. 3); in some places periodontal fibers could be seen inserted into the new cementum (Fig. 4).

Bacteria were not observed at the root ends or in the root canals of any teeth. New bone formation occurred after periradicular surgery in the site of the former apex, regardless of the type of root-end filling material placed in the remaining root.

DISCUSSION

The number of roots in this study was small because of the limited number of suitable teeth for endodontic surgery in each animal, the need to minimize the number of monkeys, and the high cost of animal purchase and upkeep. Nevertheless, the differences between the tissue responses to the two root-end filling materials were marked and did not support an increase in sample size.

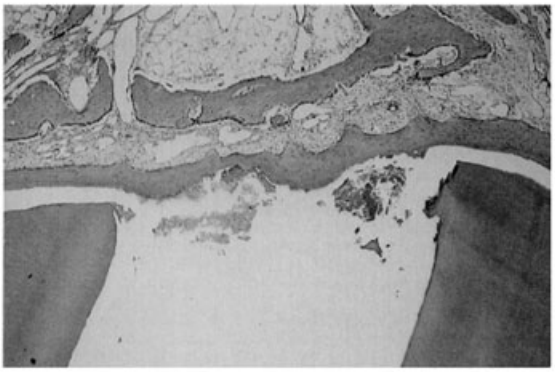

FIG. 2. Tissue response to an MTA root-end filling. New cementum has grown over the cut root-end dentin and over the root-end filling; there is no inflammation in the adjacent connective tissue (Original magnification ×20, Hematoxylin and eosin).

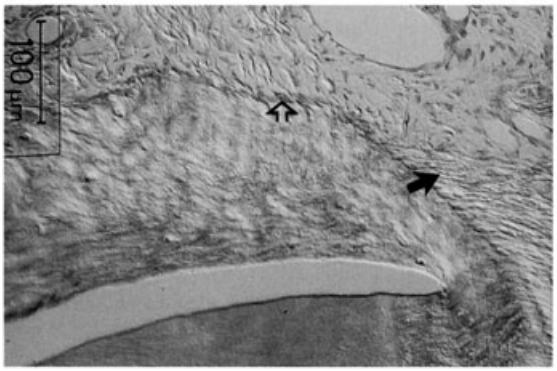

FIG. 3. Tissue response to an MTA root-end filling. New cementum has grown over the root-end filling; there are incremental lines in the cementum parallel to the surface (Original magnification ×50, Hematoxylin and eosin, polarized light).

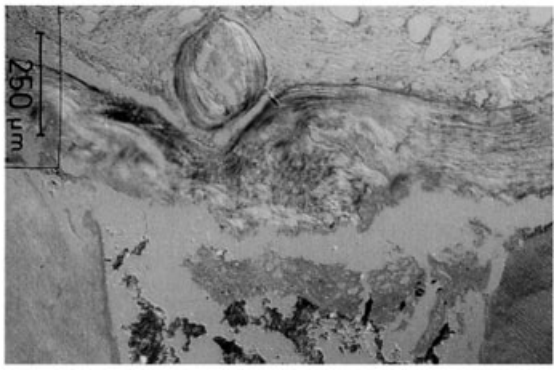

FIG. 4. Tissue response to an MTA root-end filling. New cementum has grown over the cut root-end dentin; there are new periodontal fibers inserted into the surface (arrow) and blast cells on the surface (open arrow) (Original magnification ×50, Hematoxylin and eosin, polarized light).

The conditions of the experimental design in endodontic surgery can have a profound influence on the outcome (10). If the entire root canal has been well filled, the tissue response is normally favorable, as determined by an absence of inflammation (10, 11). However, if the canal is contaminated with bacteria, the tissue response is characterized by severe inflammation (10, 12). Previous histologic studies of root-end fillings have usually placed root canal fillings before undertaking root-end resection (11, 13–15), and the present study was undertaken to allow more direct comparison with these.

Amalgam root-end fillings have recently been criticized because of associated periradicular inflammation in histologic studies (9, 12); however, in these studies the canal space was infected. It was therefore decided in the present study to reexamine the tissue response to amalgam with infection excluded as far as practicable. Root canal treatment was carried out aseptically, and sections of the teeth were stained to check for the absence of bacteria. The tissue response to amalgam root-end fillings in this ideal situation was unfavorable, characterized by inflammation at the root end in all specimens. It demonstrates the unsuitability of this material for root-end filling. The major inflammatory cell was the lymphocyte, but close to the root-end filling surface polymorphonuclear leukocytes were observed. The reason for the presence of polymorphs is unclear, but it may be related to corrosion product action.

The overall favorable tissue response to MTA root-end fillings as evidenced by a lack of inflammation is similar to, but an improvement on, previous studies in dogs, one of which examined root ends (9) and the other furcal perforations (16). Inflammation was observed around one root end filled with MTA but the reason is unclear. This particular animal had intermittent pyrexia near the end of the experiment, and on histologic examination the cancellous bone away from the teeth was heavily infiltrated with lymphocytes. The possibility exists that the inflammation seen at the root end of this tooth was unrelated to the root-end filling material. It is worth noting that cementum had formed over the cut root-end dentin even though it had not over the MTA and that new bone had formed in the position of the former apex. The other tooth in this animal with MTA root-end filling had a layer of cementum over the root-end filling, and there was an absence of periradicular inflammation.

A thick layer of cementum was observed over five of six MTA root-end fillings. The layer was continuous with that over the resected dentin, and incremental lines and cell inclusions were observed in it (Fig. 3). Some of the cementum surface was characterized by fiber insertion, mimicking Sharpey's fibers, whereas in other areas blast cells were observed on the surface (Fig. 4). The new cementum was attached to the original cementum at the sides of the root (Fig. 4). Two possibilities exist for the source of the new cementum: either it is derived from the remaining periodontal ligament and has grown in from the sides, or it is derived from the ingrowing connective tissue from bone. Previous work (9) indicates that the blood clot in the bony cavity organizes within 2 weeks. Therefore, the progenitor cells could well be derived from bone rather than from the periodontal ligament. Further the presence of blast cells on the surface of the cementum (Fig. 4) suggests a bone origin for this bone of attachment. If the cementum had grown from the lateral aspects, incremental lines would have been expected running diagonally in the cementum, rather than the parallel ones that were observed. The suggested mode of healing differs from that proposed by Craig and Harrison (17) who indi-

cated that the new cementum was derived from the existing periodontal ligament. In the present study a thick layer of cementum had grown over the dentin root end in contrast to the limited amount reported by Craig and Harrison (17). They recommended etching the tooth surface to remove the smear layer; however, it would appear that when MTA is used for root-end filling, etching of the cut dentin is unnecessary.

The layer of cementum over the MTA showed irregularities in some sections (Fig. 3), although no defects or soft tissue inclusions were noted. In some root ends the MTA was not flush with the dentin surface; however, cementum had grown directly against the MTA. The formation of cementum against MTA may be due to a number of factors such as sealing ability (1), biocompatibility (7), or alkaline pH on setting (5). Recent in vitro work has demonstrated the ability of MTA to stimulate cytokine release from bone cells (18) indicating that it actively promotes hard tissue formation rather than being inert (10, 11) or being irritant like existing root-end filling materials (12).

Based on the results of this study and previous experiments (9, 12), it appears that amalgam is an unsuitable root-end filling material and its use should be discontinued because it does not prevent microleakage (1–3) and does not allow regeneration of the dentoalveolar structure. The results of this study and previous investigations (1–9) support the use of MTA as a root-end filling in man.

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References

1. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. J Endodon 1993; 19:591–5.

2. Torabinejad M, Higa RK, McKendry DJ, Pitt Ford TR. Dye leakage of four root end filling materials: effects of blood contamination. J Endodon 1994;20:159–63.

3. Torabinejad M, Falah Rastegar A, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root-end filling material. J Endodon 1995;21:109–12.

4. Torabinejad M, Wilder Smith P, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. J Endodon 1995;21:295–9.

5. Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. J Endodon 1995;21: 349–53.

6. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. J Endodon 1995;21:403–6.

7. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Cytotoxicity of four root end filling Materials. J Endodon 1995;21:489–92.

8. Torabinejad M, Hong CU, Pitt Ford TR, Kariyawasam SP. Tissue reaction to implanted Super-EBA and Mineral Trioxide Aggregate in the mandible of guinea pigs: a preliminary report. J Endodon 1995;21:569–71.

9. Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. J Endodon 1995;21.

10. Pitt Ford TR, Roberts GJ. Tissue response to glass ionomer retrograde root fillings. Int Endod J 1990;23:233–38.

11. Zetterqvist L, Anneroth G, Nordenram A. Glass-ionomer cement as retrograde filling material. Int J Oral Maxillofac Surg 1987;16:459–64.

12. Pitt Ford TR, Andreasen JO, Dorn SO, Kariyawasam SP. Effect of IRM root end fillings on healing after replantation. J Endodon 1994;20:381–85.

13. Marcotte LR, Dowson J, Rowe NH. Apical healing with retrofilling materials amalgam and gutta-percha. J Endodon 1975;1:63–5.

14. Corcoran JF, Sieraski SM, Ellison RL. Osseous healing kinetics after apicoectomy in monkeys: II. A quantitative histological appraisal. J Endodon 1985;11:269–74.

15. Maher WP, Johnson RL, Hess J, Steiman HR. Biocompatibility of retrograde filling materials in the ferret canine. Oral Surg Oral Med Oral Pathol 1992;73:738–45.

16. Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. Oral Surg Oral Med Oral Pathol 1995;79:756–62.

17. Craig KR, Harrison JW. Wound healing following demineralization of resected root ends in periradicular surgery. J Endodon 1993;19:339–47.

18. Koh ET, Pitt Ford TR, Torabinejad M, McDonald F. Mineral trioxide aggregate stimulates cytokine production in human osteoblasts. J Bone Min Res 1995;10S:S406.