Effect of smear layer and root-end cavity thickness on apical sealing ability of MTA as a root-end filling material: A bacterial leakage study

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Objective. The aim of this study was to investigate the effect of the cavity thickness and smear layer on apical sealing ability of mineral trioxide aggregate (MTA) as a root-end filling material.
Study design. Seventy single-rooted maxillary central teeth were used in this study. All teeth were instrumented to size 50 using a step-back technique. The selected teeth were randomly divided into 4 groups, each containing 15 experimental samples and 5 positive and 5 negative control samples. In the first and second groups (smear+), the teeth were irrigated with only 5.25% NaOCl. In the third and fourth groups (smear−), the teeth were irrigated with 17% EDTA and 5.25% NaOCl to remove the smear layer. Also, in the first and third groups, cavities were prepared as 3 mm. In the second and fourth groups, cavities were prepared as 5 mm. All the root-end cavities were then filled with MTA. Nail varnish was applied to all external root surfaces to the level of the resected root-ends to prevent lateral microleakage. Samples were sterilized in an ethylene oxide sterilizer for 12 hours. The apical 3-4 mm of the roots were immersed in brain heart infusion culture medium with phenol red indicator within culture chambers. The coronal access of each specimen was inoculated every 48 hours with a suspension of *Enterococcus faecalis*. Bacterial leakage was monitored every 24 hours for 4 weeks. The data obtained were analyzed using a chi-squared test, with $\alpha = .05$ as the level for statistical significance.
Results. There were no statistically significant differences in rate of bacterial leakage among the experimental groups at 1-4 weeks ($P > .05$). Also, there was no difference between the groups when the 2 thickness groups were combined ($P > .05$). However, there was statistically significant differences when the 2 smear groups were combined for 4-week observation periods ($P < .05$). Removal of the smear layer caused significantly more apical microleakage than when the smear layer was left intact for 4 weeks.
Conclusion. The thickness of root-end cavity (3 or 5 mm) had no influence in the bacterial leakage of the root end filled with MTA. Removing the smear layer may not be necessary in root-end cavities filled with MTA. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e67-e72)**

Studies have demonstrated that bacteria play an important role in the development of apical pathosis.[1,2] Microorganisms and their byproducts or toxins have the ability to penetrate along the root canal system and adversely affect the periradicular tissues.[3] The aim of nonsurgical endodontic treatment is to remove these irritants from the root canal system and 3-dimensionally fill the canal space.[4] In those instances where nonsurgical endodontic treatment is unsuccessful, periradicular surgery may be necessary to facilitate healing.[4] The surgical procedures involve exposure of the tooth apex, periradicular curettage, resection and preparation of root end, and placement of a root-end filling. The quality of the apical seal is an important determinant for successful periradicular surgery.[4,5]

To date, several different materials have been proposed to seal the root-end cavity, including amalgam, gutta-percha, zinc oxide–eugenol cements, composite resin with and without dentin-bonding adhesives, polycarboxylate cement, glass ionomer cement, compomers, resin-modified glass ionomers, resin cements, and more recently mineral trioxide aggregate (MTA).[6-8] MTA was

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developed at Loma Linda University and is recommended for use as repair material. It has been shown to be very effective in sealing pathways of communication between the root canal system and the external surface of the tooth.[9] It has several potential clinical applications as a result of its superior sealing property,[10] ability to set in the presence of blood,[11] bactericidal effects,[12] and biocompatibility.[13] However, there are disadvantages when using MTA, such as the extended setting time and the difficult handling.[14]

Microleakage of the root canal has been defined as the passage of bacteria, fluids, and chemical substances between the tooth and the filling material of the root canal.[15] It is considered to be a common reason for the clinical failure of endodontic therapy. Leakage through an obturated root canal is expected to take place at the interfaces between sealer and dentin or sealer and gutta-percha or through voids within the sealer.[16] But leakage after surgical endodontic treatment is a complex subject, because many variables might influence leakage, such as root-canal filling techniques, the physical and chemical properties of root-end filling materials used, apical root resection, root-end cavity thickness, and the smear layer. For these reasons the aim of the present study was to evaluate the effect of the cavity thickness and smear layer in root-end cavities filled with MTA material by using a bacterial leakage method.

MATERIALS AND METHODS

Preparation of specimens

Seventy freshly extracted single-rooted human mature maxillary central teeth without caries or cracks were used in this study. External surfaces of the teeth were cleaned with curettes. The teeth were stored in a physiologic saline solution until use. The crowns of all of the teeth were removed using a carbide bur in a high-speed handpiece with a cooling system at 14 mm length. Patency of the apical foramen was determined with a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland). Working lengths were established 1 mm short of the apical foramen, and the roots were instrumented to size 50 with K-files using a step-back technique. A size 15 K-file was used during root canal preparation to maintain patency of the canal. The coronal part of the root canals was completed with Gates-Glidden drills (Technical and General, London, U.K.). Between each file use, the canals were irrigated with 2 mL 5.25% NaOCl solution.

Root-end resections were made by removing 3 mm of the apex at a 90° angle to the long axis of the root with a cylindric carbide bur using a high-speed handpiece with water spray coolant. The roots were randomly divided into 2 groups according to the created root-end cavity sizes, 3 and 5 mm in thickness. Root-end cavity thickness were created using a #4 no-rond steel bur. These 2 groups were then randomly divided into 2 subgroups, smear+ and smear− groups. In the smear+ groups (smear+/3 mm and smear+/5 mm; each n = 15), the root canals were irrigated with 2 mL 5.25% NaOCl only. In the smear− groups (smear−/3 mm and smear−/5 mm; each n = 15), 2 mL 17% EDTA followed by 2 mL 5.25% NaOCl was used to remove the smear layer.

To provide an intracanal matrix to pack the root-end filling material against, a gutta-percha cone was firmly inserted into the canal leaving a root-end void of 3 or 5 mm that was confirmed by radiographs. The cavity thickness was also measured and confirmed with periodontal sond. The root canals were dried with sterile paper points before the root-end fillings were placed.

Root-end filling technique

A Messing gun was prepared to apply the MTA (ProRoot MTA; Dentsply/Tulsa, Tulsa, OK) within the root-end cavity. The material was mixed according to the manufacturer's recommended proportions and placed into the root end with the Messing gun and applied within the canal with the endodontic plugger, and this application was maintained until the root-end cavity was totally filled.

Ten roots were used as positive and negative control samples. For the 5 negative control samples, the entire root surface was coated with 2 layers of nail varnish after filling the root-end preparation with red rope wax. Positive control samples consisted of 5 teeth with unfilled root-end preparation.

After placement of the root-end fillings, all samples were examined under ×20 magnification to ensure proper adaptation and that there were no voids or marginal discrepancies of the material. The intracanal barrier was removed and radiographs were taken in 2 buccolingual and mesiodistal directions to verify the density and depth of the filling material. The restored teeth were stored in an incubator at 37°C in 100% humidity for 48 h.

Bacterial leakage test

The model used in this study was modified from a technique developed by Maltezos et al.[17] The tapered ends of 1.5 mL polypropylene centrifuge tube (Greiner, Mannheim, Germany) were cut, and the obturated roots were inserted into the tubes until the roots protruded through the end. The junction between each tube and root was sealed with cyanoac-

Table I. Samples that leaked in each group

Group	1 wk	2 wks	3 wks	4 wks	Total
Smear+/3 mm	1/15	—	—	1/15	2/15
Smear+/5 mm	—	—	—	1/15	1/15
Smear−/3 mm	—	1/15	2/15	—	3/15
Smear−/5 mm	3/15	3/15	—	—	6/15
Positive control	5/5	—	—	—	5/5
Negative control	0/5	0/5	0/5	0/5	0/5

rylate to prevent leakage of the connection. Two coats of nail varnish were applied to all external root surfaces to the level of the resected root-ends. The entire apparatus was then gas sterilized using ethylene oxide for a 12-hour cycle using the Anprolene AN 74C Gas Sterilizer (Andersen Products, Haw River, NC). To ensure sterilization, the whole system was incubated at 37°C for 3 days.

A volume of 7-8 mL brain heart infusion (BHI) broth (Difco, Detroit, MI) with 0.01% phenol red indicator was introduced into sterile 13 × 100 mm disposable culture tubes. The previously assembled portions of tooth and its container were then inserted in the culture tubes so that a minimum 3-4 mm of the apical part of each root was immersed in BHI broth. Each assembled apparatus was then placed in a 16 × 125 mm sterile test tube and covered with a polypropylene cap. The bacterial suspension of the *Enterococcus faecalis* organism (American Type Culture Collection 29212; RSHSE, Ankara, Turkey) was used to contaminate root canals. Each access cavity was aseptically inoculated with 15 μL freshly prepared *E. faecalis* culture (10^6 cells/mL) twice per week. All samples were incubated in a humid environment at 37°C for 4 weeks and examined daily for color change from red to orange-yellow each day. When the color of the broth had changed to orange-yellow, 10 μL of the culture broth was subcultured onto sheep blood agar to confirm purity. To assure sterility, all testing was performed in a class II laminar airflow cabinet.

To prevent bacterial leakage through the root surfaces, the roots of all teeth were coated with 2 coats of nail varnish, except over the apex. The apices of the negative control teeth were also sealed with nail varnish.

Statistical analysis

Statistical analysis of the data was accomplished using a chi squared test, with $\alpha = .05$ as the level for statistical significance.

RESULTS

The numbers of samples with bacterial leakage at the end of each week are listed in Table I. No growth was observed when checking the sterilization of the whole apparatus. None of the negative control samples showed any color change, indicating no leakage, and all of the positive control samples demonstrated a color change, indicating leakage within the first 24 hours. The broth cultures producing growth were then subcultured onto sheep blood agar to confirm that there was no contamination of the samples by bacteria other than *E. faecalis*.

There were no statistically significant differences in rate of bacterial leakage among the experimental test materials at 1-4 weeks ($P > .05$). In 4-week observation periods:

Smear+/3 mm versus smear−/3 mm. There was no statistically significant difference between the smear+/3 mm and smear−/3 mm groups ($P > .05$).

Smear+/5 mm versus smear−/5 mm. The smear+/5 mm group showed less microleakage than the smear−/5 mm group, but there was no statistically significant difference between them ($P > .05$).

Smear−/3 mm versus smear−/5 mm. There was no statistically significant difference between the smear−/3 mm and smear−/5 mm groups ($P > .05$).

Smear+/3 mm versus smear+/5 mm. There was no statistically significant difference between the smear+/3 mm and smear+/5 mm groups ($P > .05$).

Cavity thickness: MTA/3 mm (n = 30) versus MTA/5 mm (n = 30). There was no statistically significant difference between the 2 cavity thicknesses ($P > .05$).

Smear layer: MTA/smear+ (n = 30) versus MTA/smear− (n = 30). The MTA/smear+ groups showed significantly less microleakage than the MTA/smear− groups ($P < .05$).

DISCUSSION

Leakage through a filled root canal may take place along the sealer-dentin and sealer–filling material interfaces or through voids within the sealer, and it is a possible cause of root treatment failure.[16] Pashley[18] stated that microleakage is a serious clinical problem because most dental materials exhibit varying degrees of microleakage. To date many in vitro methods have been used to evaluate the sealing ability of root-end filling materials by using dyes,[11] scanning electron microscopy,[19] fluid filtration techniques,[20] electrochemical methods,[21] radioisotopes,[22] and bacteria.[23] Among these techniques, the bacterial leakage model is considered to be the most clinically relevant. *Enterococcus faecalis* has been currently identified as

the bacterial species most commonly found in root canals of teeth presenting failure after endodontic treatment,[24] and it has been used in several studies on leakage.[23,25] Therefore, in the present bacterial leakage study, *E. faecalis* was used.

The purpose of placing a root-end filling after root-end resection and preparation is to establish an effective barrier between the root canal and the periapical tissues.[26] Researchers have suggested that insufficient apical seal is a major cause of endodontic surgical failure.[8,27] Apical leakage studies show that the ideal root-end preparation depth is ≥3 mm or more.[8] This increases the contact length of the material in the cavity and decreases the probability of leakage. Furthermore, the depth of <3 mm most likely does not remove all of the lateral canals and apical ramifications, thereby posing a risk of reinfection and eventual failure. Mineral trioxide aggregate as a root-end filling material provides as good or better apical seal than materials such as amalgam,[6,11] zinc oxide–eugenol (ZOE) preparations,[6,11,13] and a conventional glass ionomer material[28] after apical resection. Apart from selecting the sealing material, another important factor is the thickness of the root-end filling. Some researchers have suggested different cavity thicknesses (2-5 mm) for MTA to effectively seal the apical area.[6,26,29] In the present study, the effects of the cavity thickness and smear layer in teeth filled with MTA as a root-end filling were investigated. Results from the present experiment demonstrated that there was no statistically significant differences between the two cavity thicknesses (3 and 5 mm) regarding bacterial microleakage ($P > .05$).

In clinical practice, cavity preparation by hand or by means of rotary instruments gives rise to formation of a thin layer covering dentin and/or enamel, known as a smear layer. In endodontic therapy, smear layer formation results from root canal preparation and is mainly composed of inorganic components (dentin debris) and organic materials, such as pulp tissue remnants, bacteria, and blood cells.[30] To date, many studies have focused on the influence of the smear layer on root canal filling and especially on root canal sealers. But the advantages and disadvantages of the presence of the smear layer in instrumented root canals are still controversial issues,[31] because leakage studies in which the smear layer has been removed have yet to prove that removal is consistently beneficial. Earlier studies[32,33] reported that the presence or absence of the smear layer had no significant effect on the apical seal. In addition, although some investigations[34] indicated that removal of the smear layer might improve the sealing ability, Timpawat et al.[15] showed that removal of the smear layer caused significantly more apical microleakage than when the smear layer was left intact. These conflicting findings might result from a variety of factors: using different forms of chelating agents to remove the smear layer, the type of sealer used, film thickness, flow rate, or type of filling technique.[15] Thus, no clear consensus has been reached in the endodontic community on whether the smear layer should be removed or left intact before obturation of the root canal space.

The microleakage of MTA used for root canal filling has been reported by several studies.[35,36] Vizgirda et al.[35] reported that MTA displayed more microleakage than laterally condensed as well as thermoplasticized gutta-percha. But Al-Hezaimi et al.[36] found that both gray and white MTA allowed less apical microleakage than warm vertically condensed gutta-percha. They also showed that no significant difference in leakage between gray MTA and white MTA, but importantly noted that root canal filling with MTA materials would severely limit retreatment options and should be considered in only select cases. But those studies didn't consider the effect of the smear layer when the MTA material was used as filling material. In a recent study, Yildirim et al.[20] investigated the effect of the smear layer on the sealing ability of MTA in the long term. They demonstrated that removal of the smear layer increased the amount of microleakage along the root canal filling. The microleakage of MTA used as a root-end filling material has also been evaluated by many studies using different leakage methods.[4,6,10,11,17,19,21,26,28,29] According to these studies, MTA appears to be a better sealing agent. Continue from the other studies, the present study evaluated the sealing ability of MTA under specific conditions. We chose to investigate the effect of the apical sealing ability with or without the smear layer or in different cavity thicknesses, with the potential of using MTA as a root-end filling material. Results of the present study showed that the MTA/smear+ groups showed significantly less microleakage than the MTA/smear− groups at the end of the 4-week period ($P < .05$). This result confirmed the earlier study.[20]

Decreased leakage in smeared versus smear-free MTA-obturated root-end cavities might have been due to several factors, such as hydrophilic properties of MTA and particle size of MTA. The smear layer might act as a "coupling agent" enhancing the bond between the MTA and root-canal dentin. MTA is a type of hydraulic cement that can set in the presence of water. Hydraulic cements are finely ground materials that, when mixed with water, gradually or instantly set and harden either in air or in water.[6] The reaction results in the formation of hydrated compounds whose strength

increases with time. The smear layer is a more or less moist layer. The moist environment caused by the smear layer might have a positive effect on the adaptation of MTA to the root canal wall. Komabayashi and Spångberg[37] reported that the cumulative percentage of particles 1.5-3 μm was 70% for Gray ProRoot MTA. They also reported that the diameter of these particles was 2.44-3.05 μm. Garberoglio and Brännström[38] showed that the tubule diameter was 0.9-2.5 μm at different levels of the root canal in a scanning electron microscopy study. Consequently, the particle size of MTA might not be suitable for full penetration into the tubules.

CONCLUSION

Based on the results, the thickness of root-end cavity (3 or 5 mm) had no influence in the bacterial leakage of the root-end filled with MTA. Removing the smear layer may not be necessary in root-end cavities filled with MTA.

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