

SCIENTIFIC ARTICLES

Bacterial Leakage of Mineral Trioxide Aggregate as a Root-End Filling Material

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Previous dye leakage studies have shown that mineral trioxide aggregate leaks significantly less than other commonly used root-end filling materials. This study determined the time needed for *Staphylococcus epidermidis* to penetrate a 3-mm thickness of amalgam, Super-EBA, Intermediate Restorative Material (IRM), or mineral trioxide aggregate (MTA) as root-end filling materials. Fifty-six single-rooted extracted human teeth were cleaned and shaped using a step-back technique. Following root-end resection, 48 root-end cavities were filled with amalgam, Super-EBA, IRM, or MTA. Four root-end cavities were filled with thermoplasticized gutta-percha without a root canal sealer (+ control), and another four were filled with sticky wax covered with two layers of nail polish (- control). After attaching the teeth to plastic caps of 12-ml plastic vials and placing the root ends into phenol red broth, the set-ups were sterilized overnight with ethylene dioxide gas. A tenth of a microliter of broth containing *S. epidermidis* was placed into the root canal of 46 teeth (40 experimental, 3 positive, and 3 negative control groups). In addition, the root canals of two teeth with test root-end filling materials and one tooth from the positive and negative control groups were filled with sterile saline. The number of days required for the test bacteria to penetrate various root-end filling materials was determined. Most samples whose apical 3 mm were filled with amalgam, Super-EBA, or IRM began leaking at 6 to 57 days. In contrast, the majority of samples whose root ends were filled with MTA did not show any leakage throughout the experimental period (90 days) in this study. Statistical analysis of the data showed no significant difference between the leakage of amalgam, Super-EBA, and IRM. However, MTA leaked significantly less than other root-end filling materials

maintained under test conditions for 90 days in this experiment ($p < 0.05$).

Substances that have been used as root-end fillings include gutta-percha, amalgam, zinc oxide-eugenol cements, cavite, composite resin, gold foil, glass ionomers, mineral trioxide aggregate (MTA), and a host of other restorative filling materials. Because most endodontic failures occur as a result of the presence of antigens in uncleaned and unobturated root canals, a root-end filling material should provide an apical seal that inhibits the egress of antigens from the root canal system into the periradicular tissues. The quality of the apical seal obtained by root-end filling materials has been assessed by various techniques (1-6).

Radioisotope and dye penetration techniques have been the most frequently used methods to evaluate the sealing ability of various root-end filling materials. Autoradiography is a subjective, quantitative technique for measuring apical leakage. Factors such as type of isotope, distance between radiation source and emulsion, and the length of exposure of the film can affect the results obtained by this technique (7). Matloff et al. (8) showed that methylene blue dye penetrated further up the canal than ^{45}Ca or ^{14}C -labeled urea or ^{125}I -labeled albumin. They questioned the validity of the results obtained in radioisotope studies, because radioisotope tracers are much smaller than bacteria and have dissimilar leakage patterns.

Despite their popularity and ease of use, dye leakage studies also have several disadvantages: (a) like radioisotope tracers, the molecular size of most dye particles is smaller than bacteria; (b) most dye leakage studies have measured the degree of leakage in one plane, making it impossible to evaluate the total leakage; and (c) compared with clinical conditions, in vitro dye studies are static and do not reflect the dynamic interaction between the root canals and periradicular tissues.

Magura et al. (9) examined salivary penetration through obturated root canals using histological examination and dye penetration, and reported that saliva penetration assessed in

histological sections was significantly less than that visualized with dye analysis (9). Switzer et al. (10) compared coronal bacterial leakage to dye leakage on the same teeth and found no correlation between the two leakage methods. Kersten and Moorer (11) compared the ability of four obturation methods to prevent leakage of bacteria-sized particles or large protein molecules and found leakage of the commonly used dye, methylene blue, was comparable with that of a small bacterial metabolic product of similar molecular size. Their findings showed that microleakage of the small molecules could not be prevented, whereas leakage of bacteria-sized particles and large size protein molecules could be prevented with some of the obturation techniques.

Because of inherent inadequacies in dye and radioisotope leakage studies and a lack of correlation between bacterial leakage and that of dye and isotope molecules, bacterial leakage studies have been recommended to test the suitability of potential root-end filling materials.

Kos et al. (12) evaluated the ability of poly-HEMA as root-end filling material and found that this material prevented leakage of *Proteus vulgaris*, an actively motile Gram-negative rod, and *Streptococcus salivarius*, a Gram-positive coccus and a normal human oral resides. Luomanen and Tuompo (13) compared the tightness of titanium screws versus amalgam as root-end filling materials using *Serratia marcescens* bacteria in an in vitro model. They found that the bacteria penetrated the apical titanium screw seals in 2 to 7 days and the retrograde amalgam fillings on the first day of the experiment. Staining of these teeth with India ink showed that penetration of bacteria had occurred at the tooth-filling margin.

Torabinejad et al. (6) used rhodamine B fluorescent dye and a confocal microscope to evaluate the sealing ability of amalgam, Super-EBA, and MTA as root-end filling materials. Statistical analysis of their data showed that the MTA leaked significantly less than amalgam or Super-EBA. In another in vitro study, they determined the amount of dye leakage (in the presence versus absence of blood) in root-end cavities filled with amalgam, Super-EBA, Intermediate Restorative Material (IRM), or MTA (14). They found that the presence or absence of blood had no significant effect on the amount of dye leakage. However, the results showed that there was a significant leakage difference between these root-end filling materials ($p < 0.0001$); MTA leaked significantly less than other materials tested with or without blood contamination of the root-end cavities.

The purpose of this study was to evaluate the ability of MTA as a root-end filling material to prevent bacterial leakage compared with amalgam, IRM, or Super-EBA.

MATERIALS AND METHODS

To examine bacterial leakage of various test materials, a refined in vitro model described by Kos et al. (12) and Torabinejad et al. (15) was used in this experiment.

Preparation of Teeth

Fifty-six single-rooted extracted, human teeth with straight canals were used in this experiment. The teeth had been stored previously in 10% formalin and were kept moist throughout the experiment. After initial radiographs, standard

access cavities were prepared, and the coronal portions of the canals were enlarged with #2 to #4 Gates Glidden drills.

To obtain a standardized diameter, the apical foramina of the teeth were enlarged and kept patent to a #40 file, using a step-back filing technique (15). Approximately 2 ml of 2.25% NaOCl were used between each file size to remove debris. The apical 3 mm of each root was removed with a fissure bur in a high-speed handpiece, under water spray, at 90 degrees to the long axis of the tooth (15).

A root-end cavity preparation with a 3-mm depth was made with a 330 bur on the resected root end, including the apical opening of each root. A #70 or #80 K-file with snug fit was placed 2.5 mm from the apical opening of each prepared canal. After removal, the apical 1/2 mm of each file was flattened using a large diamond bur. Forty-eight teeth were divided into four equal groups of 12 teeth each. Zinc-free amalgam (Sybraloy, Kerr Manufacturing Co., Romulus, MI), IRM (L. D. Caulk Co., Milford, DE), Super-EBA (Harry J. Bosworth Co., Skokie, IL), and MTA (Loma Linda University, Loma Linda, CA) were prepared according to the manufacturers' directions for use as root-end filling materials. After placing the flattened file in 40 experimental prepared root canals, 10 root-end preparations were filled with each of the four root-end filling materials. As controls, eight teeth were divided into two equal control groups of four each. To prevent bacterial leakage through the root surfaces, two layers of nail polish were applied to the external surfaces of all roots, except to the resected root ends and the root-end filling materials. Following placement of flattened file in the root canals of control teeth, four root-end cavities were filled with thermoplasticized gutta-percha without a root canal sealer (+ control), and the other four were filled with sticky wax covered with two layers of nail polish (- control). To test the sterility of the apparatus set-up, the root canals of two teeth with test root-end filling materials and one tooth from the positive and negative control groups were filled with sterile saline. The rest of root canals (46 samples) were filled with trypticase soy broth contaminated with *Staphylococcus epidermidis*. The distribution of the samples is shown in Fig. 1.

Apparatus Set-Up

Twelve-ml plastic vials with snap-on plastic caps (Nalgene, Rochester, NY) were used to suspend the prepared teeth in phenol red lactose broth. By using a high-speed handpiece

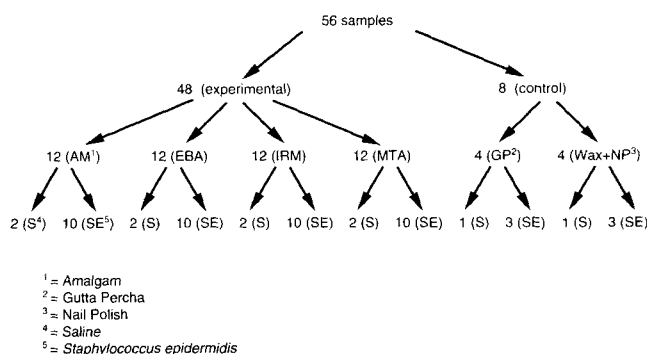


Fig 1. Distribution of samples between experimental and control groups.

and a #6 round bur, a hole was made through the center of every cap. Each tooth was placed into the fabricated hole in the cap, up to its cemento-enamel junction, and secured to the cap using sticky wax covered with two layers of nail polish. Securing each tooth to the vial cap in this manner allowed the root of the tooth to be within the vial and its crown outside of the vial when the lid was secured (snapped on) to the vial (Fig. 2). The prepared teeth along with their caps and the vials were sterilized overnight with ethylene oxide gas. Sterile phenol red broth with 3% lactose was placed in each vial to a level of 2 to 3 mm above the resected root end.

Bacterial Preparation

Using a sterile micropipette, an overnight culture containing $\sim 7.5 \times 10^6$ *S. epidermidis* in 0.1 ml of trypticase soy broth was carefully placed into the root canal of each tooth via the coronal access cavity preparation. Penetration of *S. epidermidis* from the root canals into the phenol red broth resulted in formation of an acid and a change of color in the indicator solution (red to yellow).

Monitoring of the Samples

To ensure viability of *S. epidermidis*, after removal of old culture, fresh overnight culture of the organism was added to the root canals every other day. When the bacterial culture was replenished, the old culture was plated to confirm continued viability of the microorganisms.

The samples were monitored daily until the phenol red broth at the bottom of the flask turned yellow. After color change, a sample of the yellow medium was plated on blood agar to ensure that it contained the same type of bacteria as that placed in the tubing. A Kruskal-Wallis one-way analysis of variance and multiple comparison tests were used to determine the statistical differences between various groups.

RESULTS

The bacteria present in the root canals of teeth in the positive control group caused a color change in the phenol red medium broth within 3 to 8 days with a median of 5.5 days. In contrast, the samples in the negative control group did not cause any color change in the phenol red medium. The phenol red medium broth did not change color in samples in which the coronal segment of the root canal was in contact with sterile saline throughout the experiment.

Table 1 shows the median time it took *S. epidermidis* to penetrate through 3 mm of various root-end filling materials and cause visible color change in the growth medium. In all cases when color change occurred, the bacteriological test showed that the bacteria present in the growth medium was *S. epidermidis*. Except for one sample, the samples whose apical 3 mm were filled with amalgam leaked within a 6- to 50-day interval. Eight of 10 samples with Super-EBA as root-end filling material leaked between 11 to 57 days. All samples with IRM as root-end filling material leaked between 8 to 52 days. Eight of 10 samples whose apical 3 mm were filled with MTA did not show any leakage throughout the experimental period (90 days). Two MTA samples caused color change in

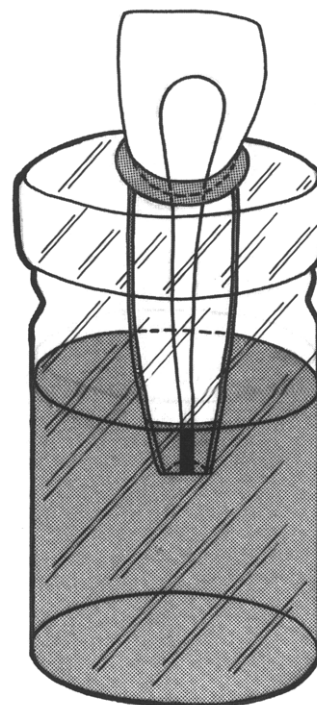


Fig 2. Diagram of a tooth suspended in a plastic vial.

TABLE 1. Median time of bacterial leakage of various test root-end filling materials

Material	No. of Days
Amalgam	28.5
Super-EBA	34.5
IRM	15.0
MTA	90.0

the phenol red medium: one on day 25 and one on day 41. Kruskal-Wallis one-way analysis of variance showed a significant statistical difference between experimental groups and positive control group ($p < 0.00001$). Multiple comparison test showed no significant difference between the leakage of amalgam, Super-EBA, and IRM. However, MTA leaked significantly less than other root-end filling materials ($p < 0.05$).

DISCUSSION

Except Kos et al. (12) and Luomanen and Tuompo (13), other investigators have determined the sealing ability of various root-end filling materials by placing the root ends in the tracers substances (6-8, 14, 16, 17). This study also determined the ability of commonly used root-end filling materials and MTA to prevent coronal bacterial leakage. In addition, the present study investigated the ability of 3-mm-thick root-end filling materials to prevent coronal leakage under the most severe contamination.

The color change in all samples in the positive control group indicates that root canal sealer is needed to improve gutta-percha as root canal filling material, as shown by Becker and Von Fraunhofer (16) with thermoplasticized gutta-percha with and without root canal sealer. It also confirms the findings by Torabinejad et al. (15), who used filled root canals

with single gutta-percha cone without root canal sealer as positive controls in an in vitro bacterial penetration of coronally, unsealed endodontically treated teeth.

In a pilot study, when we used 3-mm-thick sticky wax as root-end filling material and as negative control, the phenol red broth medium changed color in 6 to 19 days. Gas sterilization of the samples might have affected the sealing ability of wax that was used as a negative control group in a dye leakage study without gas sterilization (17). Application of two layers of nail polish on the wax surface prevented bacterial leakage and provided a negative control group that did not show signs of bacterial leakage throughout the experiment. An absence of color change in the phenol red broth medium in this group and the samples in contact with sterile saline indicates that our set-up did provide a contamination-free chamber. Like the set-ups used by Kos et al. (12) our apparatus determined the bacterial leakage of four root-end filling materials in coronal-apical direction. The main advantage of the present apparatus over set-ups used by Kos et al. (12) and Torabinejad et al. (15) is the fact that the tooth crown was outside of the vial. This reduces the chances for accidental overflowing of the root canal with bacterial solution via the latex tubing.

The results of the present bacterial leakage study confirm those of our previous dye leakage studies that collectively indicate that MTA provides a better apical seal as a root-end filling material than that obtained by amalgam, Super-EBA, or IRM (6, 14). MTA sealing ability is probably due to its hydrophilic nature and slight expansion when it is cured in a moist environment. Its superior sealing ability in this study might also be due to its antibacterial effect on *S. epidermidis*. In a preliminary in vitro study, we have compared the antibacterial effects of MTA with those of amalgam and Super-EBA cement on some selected oral bacteria (18). Our results show that fresh and set (24-h samples) MTA has some antibacterial effect against these organisms. Because dye molecules did not penetrate the tooth-filling (MTA) interface in our previous dye studies (6, 14) and the antibacterial effect of set (24-h) MTA was less than its fresh counterpart (18), it is reasonable to assume that its bacterial impermeability, under severe contamination condition, is due to its sealing ability, and not due to its antibacterial effects. Comparing the length of time needed for bacteria to penetrate a standardized thickness of Super-EBA, and IRM as root-end filling materials from this study with those needed for dye molecules to penetrate in our previous studies (6, 14), it appears that dye molecules leak faster than bacteria. Similar results have been reported by Kersten and Moorer (11), as well as by Magura et al. (9), in coronal dye leakage investigations. Comparing the length of time needed for bacteria to penetrate amalgam in this study with those of Kos et al. (12) and Luomanen and Tuompo (13), it appears that their test bacteria penetrated amalgam faster than *S. epidermidis* in the present study. The difference could be due to types of dyes and amalgam, and the thickness of root-end filling materials. Bacteria used in previous studies were *P. vulgaris* and *S. salivarius* (12), as well as *S. marcescens* (13), whose motility rates are different from *S. epidermidis* used in this study. Mattison et al. (7) compared the apical leakage of different thicknesses and compositions of dental amalgam electrochemically and showed that 3 mm of amalgam significantly reduced leakage

as compared with the 1-mm filling. Gilheany et al. (19) evaluated the apical leakage of various depths of root-end filling materials placed in root apices that had been resected at 0, 30, and 45 degrees to the long axis of the root. They also showed that increasing the depth of the root-end filling significantly decreased apical leakage. There was also a significant increase in leakage as the amount of bevel increased.

For ease of management of experimental conditions and interpretations of the data, the bacterial content of contaminated canals in this study was purposely limited to only one species. In a follow-up study (unpublished data), we used a similar set-up and placed the coronal portions of root-end filled root canals in contact with human saliva, and their root ends in trypticase soy broth and determined the number of days required for bacteria in saliva to penetrate various root-end filling materials (amalgam, Super-EBA, IRM, or MTA). Our results showed similar bacterial leakage patterns to those observed in the present study. A study is in progress to investigate the marginal adaptation of MTA, amalgam Super EBA and IRM under scanning electron microscope.

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References

- Chong BS, Pitt Ford TR, Watson TF. The adaptation and sealing ability of light-cured glass ionomer retrograde fillings. *Int Endod J* 1991;24:223-32.
- Shani J, Friedman S, Stabholz A, Abed J. A radionuclidic model for evaluating sealability of retrograde filling materials. *Int J Nucl Med Biol* 1984;11:46-52.
- King KT, Anderson RW, Pashly DH, Pantera EA. Longitudinal evaluation of the seal of endodontic retrofillings. *J Endodon* 1990;16:307-10.
- Tanzilli JP, Raphael D, Moodnik RM. A comparison of the marginal adaptation of retrograde techniques; a scanning electron microscopic study. *Oral Surg* 1980;50:74-80.
- Stabholz A, Shani J, Friedman S, Abed J. Marginal adaptation of retrograde fillings and its correlation with sealability. *J Endodon* 1985;11:218-23.
- Torabinejad M, Watson TF, Pittford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J Endodon* 1993;19:591-5.
- Mattison GD, Von Fraunhofer A, Delivanis PD, Anderson AN. Microleakage of retrograde amalgams. *J Endodon* 1985;11:340-5.
- Matloff IR, Jensen JR, Singer L, Tabibi A. A comparison of methods used in root canal sealability studies. *Oral Surg* 1982;53:203-8.
- Magura ME, Kafrawy AH, Brown CE, Newton CW. Human saliva coronal microleakage in obturated root canals: an in vitro study. *J Endodon* 1991;17:324-31.
- Switzer S, Moshonov J, Trope M. In vitro comparison of bacterial and dye leakage of obturated root canals. *J Endodon* 1992;18:194 [Abstract].
- Kersten HW, Moorer WR. Particles and molecules in endodontic leakage. *Int Endod J* 1989;22:118-24.
- Kos WL, Aulozzi DP, Gerstein H. A comparative bacterial microleakage study of retrofilling materials. *J Endodon* 1982;8:355-8.
- Luomanen M, Tuompo H. Study of titanium screws as retrograde fillings using bacteria and dye. *Scand J Dent Res* 1985;93:555-9.
- Torabinejad M, Higa RK, McKendry DJ, Pittford TR. Dye leakage of four root end filling materials: effects of blood contamination. *J Endodon* 1993;20:159-63.
- Torabinejad M, Ung B, Kettering JD. In vitro bacterial penetration of coronally unsealed endodontically treated teeth. *J Endodon* 1990;16:566-9.
- Becker SA, Von Fraunhofer JA. The comparative leakage behavior of reverse filling materials. *J Endodon* 1989;15:246-8.
- Torabinejad M, Kahn H, Bankes D. Isopropyl cynoacrylate as a root canal sealer. *J Endodon* 1984;10:304-7.
- Hong CU, Torabinejad M, Kettering JD. The effects of three retrofilling materials on selected oral bacteria. *J Endodon* 1993;19:200 AB 67.
- Gilheany PA, Figdor D, Tyas M. Apical dentin permeability and microleakage associated with root end resection and retrograde filling. *J Endodon* 1994;20:22-6.