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# Dye Leakage of Four Root End Filling Materials: Effects of Blood Contamination

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**The purpose of this study was to compare the amount of dye leakage (in the presence versus absence of blood) in root end cavities filled with amalgam, Super EBA, IRM, and a mineral trioxide aggregate.**

**After removing the anatomical crowns of 90 extracted human teeth, their roots were instrumented and obturated. Except for their apical 2 mm, the root surfaces were sealed with nail polish. After removal of the apical 2 to 3 mm of each root, a standardized root end cavity was prepared. Five root ends were filled with gutta-percha and no sealer, and another five root ends were filled with sticky wax. These served as positive and negative controls, respectively. The remaining 80 roots were divided into four equal groups and filled with the test materials. For each material, half of the root end cavities were dried prior to placing the filling material. The remaining half were filled after they were contaminated with blood. All 90 roots were then immediately placed in 1% methylene blue dye for 72 h. Finally, the roots were split and linear dye penetration was measured and statistically analyzed (analysis of variance).**

**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 the root end filling materials ($p < 0.0001$). Mineral trioxide aggregate leaked significantly less than other materials tested with or without blood contamination of the root end cavities.**

Apical surgery is performed in the presence of persistent periradicular pathosis when orthograde endodontic treatment is unfeasible. Because some endodontic failures are due to inadequate cleaning of the root canals and egress of antigens into the periradicular tissues, a number of investigators have recommended placement of root end fillings in teeth that require root end resection (1–3).

The primary function of the root end filling is to improve the seal of the root canal system following apical surgery. This should prevent leakage of irritants from the root canal into the periradicular tissues. The procedure usually consists of exposure of the involved root apex, resection of the apical end of the root, preparation of a class I cavity, and insertion of a root end filling material (4).

The ideal characteristics required for root end filling materials are the same as those for root canal filling materials. An ideal root end filling material should adhere to the preparation walls and seal the root canal system; it should be nontoxic, be well tolerated by periradicular tissues, and promote healing. It should not corrode, be electrochemically active, or stain the periradicular tissues; it should be easy to manipulate and be radiopaque. In addition, it should be dimensionally stable, nonabsorbable, and not be affected by the presence of moisture (5).

Amalgam has been used as a root end filling material for many years. Its potential disadvantages, however, include (a) initial leakage, (b) secondary corrosion, (c) mercury and tin contamination, (d) moisture sensitivity, (e) need for an undercut in the cavity preparation, (f) staining of hard and soft tissues, and (g) scatter of amalgam particles (5). Because of these disadvantages, zinc oxide-eugenol-based cements such as IRM and Super EBA have been advocated as root end filling materials. However, zinc oxide-eugenol-based cements have potential disadvantages: (a) moisture sensitivity, (b) irritation of vital tissue, (c) solubility, and (d) difficulty in clinical handling of the material (6).

An experimental material, mineral trioxide (MT) aggregate, has recently been developed to seal off all pathways of communication between the root canal system and the external surface of the tooth. In an in vitro study, Torabinejad et al. (6) used rhodamine B fluorescent dye and a confocal microscope to evaluate the sealing ability of amalgam, Super EBA, and a MT aggregate when used as root end filling materials. Thirty single-canal teeth were cleaned, shaped, and obturated with gutta-percha and root canal sealer. After application of nail polish to the external surface, the apical 3 mm of each root was resected and 3-mm-deep root end preparations were made. The roots were randomly divided into three groups and the dry root end preparations filled with the experimental materials. All roots were then exposed to an aqueous solution of rhodamine B fluorescent dye for 24 h, longitudinally sec-

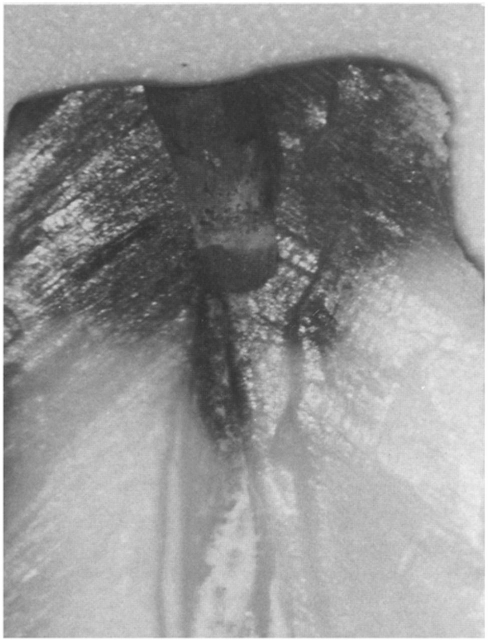

FIG 1. Complete leakage between gutta-percha and the root end cavity preparation in a positive control sample.

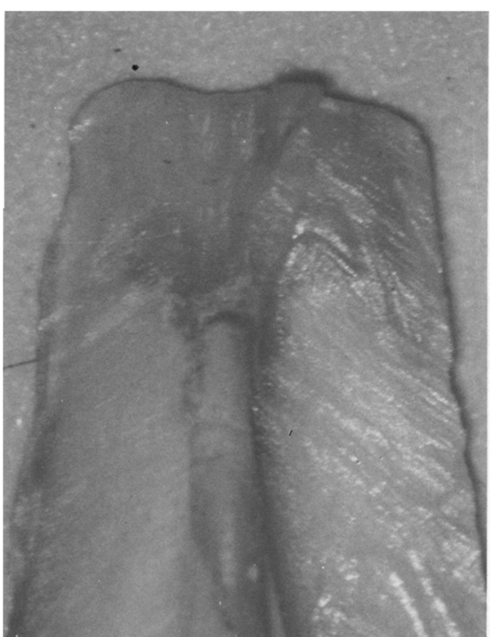

FIG 2. No dye leakage between the dentinal walls of a root end cavity filled with sticky wax in a negative control sample.

tioned, and the extent of dye penetration was measured using a confocal microscope. Statistical analysis showed that the MT aggregate leaked significantly less than amalgam and Super EBA.

Clinically, it is inevitable that moisture or blood will contaminate the root end cavity preparations and filling materials as they are placed. Based on available knowledge and information, presence of moisture or blood should affect the amount of leakage of the root end filling materials (6, 7). However, most studies to date, which have evaluated the sealability of root end filling materials, are based on methods by which the root end filling materials are placed in extracted teeth under dry conditions (6). To assess the possible effect of moisture on the sealing ability of root end filling materials, we compared the apical seal achieved with amalgam, IRM, super EBA, and MT aggregate placed in dry or blood contaminated root end cavities.

## MATERIALS AND METHODS

Ninety freshly extracted, single-rooted, human anterior teeth with mature apices were used in this study. All teeth were stored in sterile water before use.

The anatomical crown of each tooth was resected, leaving a caries-free root surface. Working length was determined by placing a #15 file into the canal until it reached the apical foramen. The apical foramen of each root was enlarged to a size #40 file. After opening the foramen to a #40 file, stepback filing was performed to a #70 file at 0.5-mm increments. We prepared root canals with a reaming motion and with copious irrigation with 5.25% sodium hypochlorite. Middle and coronal canal flaring was performed using Gates Glidden burs. After instrumentation was completed, a #40 file was again passed through the apex to ensure patency. The root canals were obturated with laterally condensed gutta-percha and Roth's Root Canal Sealer (Roth International, Chicago IL). After sealing the coronal access openings with Cavit (Premier Dental Products Co., Philadelphia, PA), we applied two coats of fingernail polish to the surface of each root.

The apical 2 to 3 mm of each root was resected using a high-speed tapered fissure bur at approximately 90 degrees to the long axis of the root. Root end preparations were made using a high-speed #331 bur with constant water spray. We standardized cavity preparations with the cutting blades of #331 bur measuring 1 mm in diameter and 2 mm in depth.

The prepared roots were placed randomly into the following groups. Five root ends were filled with gutta-percha without sealer and used as positive controls. Another five root ends were filled with sticky wax and used as negative controls. The remaining 80 roots were divided into four equal groups of 20 roots each. Four materials were tested, both with (10 samples) and without (10 samples) blood present in the root end preparations at the time of filling placement. The materials tested were Valiant PhD amalgam (L. D. Caulk, Milford, DE), IRM (L. D. Caulk), Super EBA Cement (Bosworth Co., Skokie, IL), and the MT aggregate.

A constant supply of fresh blood was obtained by venipuncture using a 23-gauge butterfly catheter. We performed sterile saline flush between draws to prevent clotting in the system. Root end cavity preparations were filled with blood before placement of root end filling materials in half of the roots assigned to each filling material. Immediately after placement of the root end filling materials, the roots were immersed in 1% methylene blue dye. We removed the roots from the methylene blue dye after 72 h. The roots were grooved on the buccal and lingual surfaces down to the gutta-percha. They were then split into two sections, and the retrograde filling materials were removed. Dye penetration was measured linearly to its furthest extent within the root end cavity using a Wolfe Dissecting Stereo Microscope (Carolina Biological Supply Co., Burlington, NC) with a 0.5-mm ocular

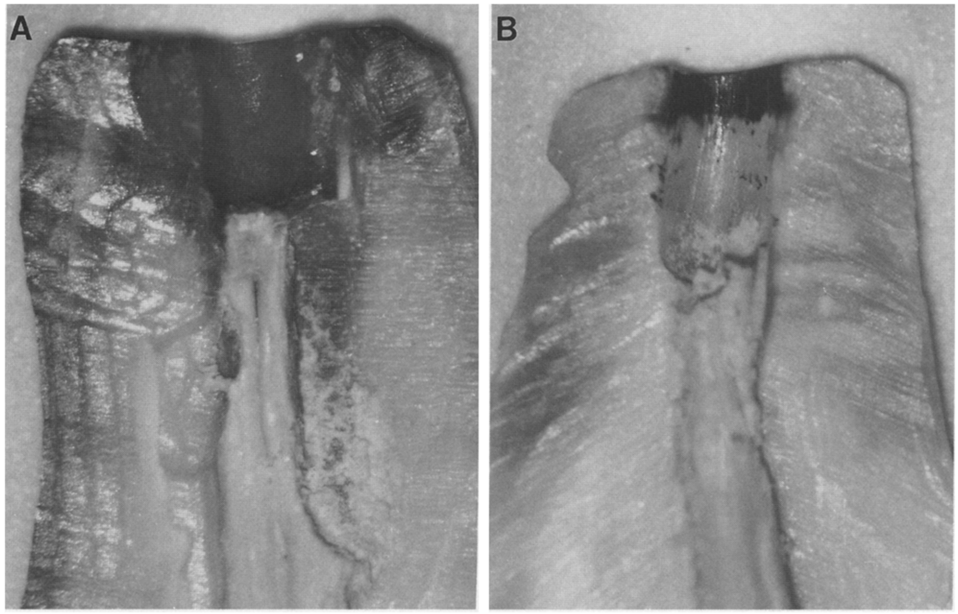


FIG 3. Significant dye leakage occurred between dry root end cavity walls and amalgam (*A*); less leakage occurred in blood-contaminated root end cavities (*B*).

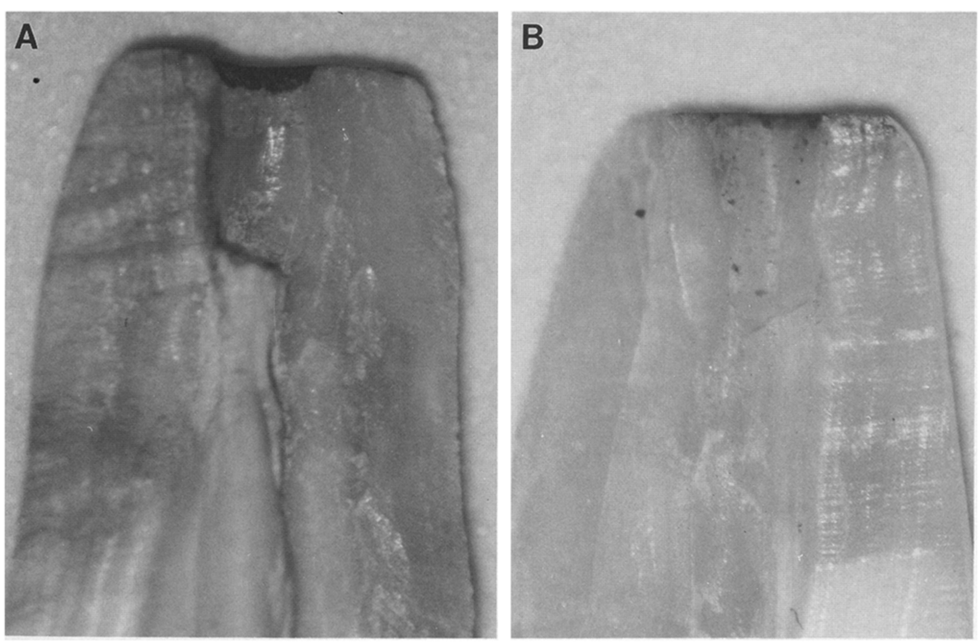


FIG 4. Minimal leakage occurred when the MT aggregate was used in dry (*A*) or blood-contaminated (*B*) root end cavities.

**TABLE 1. Extent of linear dye penetration in dry root end cavities with various materials**

| Group | No. | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|---|
| Amalgam | 10 | 0.30 | 1.00 | 0.81 | 0.24 |
| Super EBA | 10 | 0.10 | 1.00 | 0.68 | 0.38 |
| IRM | 10 | 1.00 | 1.00 | 1.00 | 0.00 |
| MT Aggregate | 10 | 0.02 | 0.73 | 0.31 | 0.26 |

**TABLE 2. Extent of linear dye penetration in blod-contaminated root end cavities with various materials**

| Group | No. | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|---|
| Amalgam | 10 | 0.25 | 1.00 | 0.50 | 0.27 |
| Super EBA | 10 | 1.00 | 1.00 | 1.00 | 0.00 |
| IRM | 10 | 1.00 | 1.00 | 1.00 | 0.00 |
| MT Aggregate | 10 | 0.06 | 0.52 | 0.28 | 0.14 |

grid at ×10. In addition, the length of each root end cavity preparation also was measured. We then converted dye leakage measurements into percentages of the cavity preparation depth to accommodate for minor deviations in the depth of the individual root end preparations. Statistical analysis was performed using a one-way analysis of variance with the

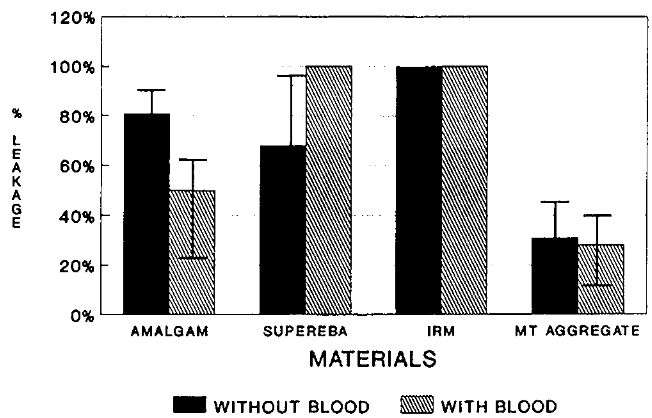


FIG 5. Percentage of dye leakage of various materials placed in dry and blood-contaminated root end cavities.

Duncan multiple comparison test at a significance level of $p < 0.05$.

## RESULTS

We observed complete leakage between gutta-percha and root end cavity walls in the positive control group (Fig. 1). In contrast, no leakage was observed in the negative control group (Fig. 2). Varying degrees of dye leakage occurred between the dentinal walls and amalgam (Fig. 3) and IRM and Super EBA, when used as root end filling materials. Very little leakage occurred in root end cavities filled with MT aggregate (Fig. 4). The extent of linear dye penetration for each material placed in dry root end cavities is shown in Table 1. The extent of linear dye penetration of various materials placed in root end cavity preparations in the presence of blood is shown in Table 2. Percentages of dye leakage for various groups are shown in Fig. 5.

Statistical analysis of the data showed no significant difference between groups exposed to blood and those not exposed to blood during root end filling. However, the analysis of variance test revealed a highly significant difference ($p < 0.0001$) between the various materials used. The Duncan multiple comparison test specifically showed that the MT aggregate leaked significantly less than other materials, either in the presence or absence of blood. Linear dye leakage differences observed between other materials in either treatment were not statistically significant.

## DISCUSSION

The aim of this study was to evaluate amalgam, Super EBA, IRM, and MT aggregate in their ability to prevent apical dye leakage in vitro with or without blood present in the root end preparation. The reason for comparing the materials, both with and without blood contamination, was to simulate the clinical conditions. During endodontic surgery many means are utilized to control hemorrhage in the surgical site. Local anesthetic with increased concentration of vasoconstrictor, chemical cauterization, electric cautery, and mechanical barriers such as bone wax are usually employed. Yet, with all of these means, the goal of a dry apical field is not always attainable. Root end preparations may be dried with paper points, but their effectiveness is unknown. Directing compressed air into the surgical site as a means to remove blood or moisture from the root end preparation is not recommended.

Many in vitro dye leakage studies have been performed. However, no study has intentionally left the root end preparations contaminated with blood in order to examine the apical seal achieved under these more relevant clinical conditions. Moisture may be an important factor due to its potential effects on the physical properties and sealing ability of the restorative materials.

According to Phillips (7), moisture contamination of amalgam can occur during handling from the operators hand, trituration, or because the field of operation was not kept dry during condensation. Zinc-containing amalgam is known to be significantly affected by moisture contamination during condensation. Zinc-containing amalgams have been shown to exhibit increased setting expansion when contaminated with moisture. In addition to increased expansion, there is an increase in corrosion with zinc-containing amalgam. This type of amalgam would be subject to surface pitting due to hydrogen release, which would diminish the amalgam's strength and could affect its biocompatibility (7).

Setting expansion is not reported to occur with nonzinc-containing amalgam. Therefore, zinc-free amalgam should be considered the amalgam of choice when moisture contamination is possible. Zinc-free amalgam, on the other hand, tends to have more internal porosity and diminished physical properties relative to zinc-containing amalgam (7).

Zinc oxide-eugenol-based cements such as IRM and Super EBA are also affected by moisture contamination (7). Moisture acts as an accelerator and dramatically decreases the setting time of these materials. This may be troublesome because the material may set before it contacts or becomes closely adapted to the dentinal walls. If additional material needs to be added, it may not be cohesive or able to produce an adequate seal in a moisture-contaminated environment.

In this study, IRM and Super EBA did not seal well in the presence of blood. IRM leaked more than amalgam, which is in contrast to the findings reported by Bondra et al. (8) and Smee et al. (9) who found IRM to seal better than amalgam. This discrepancy may in part be due to the type of dye used. These studies (8, 9) used India ink to observe leakage, while in our investigation we used 1% methylene blue dye as the tracer.

In this study, the presence or absence of blood made no difference in the extent of leakage observed with IRM. Super EBA showed a trend toward increased apical dye leakage when tested in the presence of blood. However, this difference was not statistically significant. Like IRM, the Super EBA also failed to seal as effectively as amalgam. This finding conflicts with reports by Bondra et al. (8) and Beltes et al. (10) but concurs with the study reported by King et al. (11).

The MT aggregate, which is a new potential root end filling material, showed promise in this dye leakage study. The amount of dye leakage with the MT aggregate was significantly less than that of amalgam, IRM, and Super EBA. The presence or absence of blood seemed not to affect the sealing ability of the MT aggregate. Although the results of the present studies show that the presence of blood has no significant effect on the amount of dye leakage of the MT aggregate and IRM, decreases leakage of amalgam, and increases the dye leakage in Super EBA, direct extrapolation and relevance of dye leakage studies to clinical settings are debatable (12, 13). However, when a filling material does not allow penetration of small molecules such as dye particles, it has the potential to prevent leakage of bacteria which have larger molecular sizes.

Removal of root end filling materials from their respective cavities provided the examiners with a tridimensional view of the extent of the dye leakage. Dye penetration into the interface between the root end filling materials and the dentinal walls was uneven, with deeper penetration in some areas than others. The furthest extent of linear dye penetration was used to represent the deepest gap between the root end filling materials and the dentinal walls. Although the dye molecules are much smaller than bacteria, existence of dye leakage indicates presence of a potential gap for bacterial penetration.

Goldman et al. (14) as well as Spangberg et al. (15) have questioned the validity of dye leakage studies because of the possible effect of entrapped air on the ingress of the dye solutions. Because of dissimilarity between the tested surfaces (glass and resin tubes) in these studies and prepared root canal walls, Masters et al. (16) compared the degree of dye leakage of filled and unfilled glass tubes and root canals with and without vacuuming. They found no significant difference in the degree of dye leakage between obturated root canals with or without vacuuming. These results question the value of vacuuming in dye studies in obturated root canals.

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## You Might Be Interested

This brief report emphasizes the wisdom of the hoary dictum; "never give a diagnostic opinion until you have all the facts." An endodontist was shown the X-ray in Fig. 1 and was told that the bicuspid was sensitive and mobile, there had been surgery in the area for the endodontic failure, and "wasn't there a radiolucency around the most apical of the misplaced retrogrades or whatever they are?" See the next "You Might Be Interested" in this issue for the startling conclusion.

*M. Kureey*