

Original Article

Root-end filling with cement-based materials: An *in vitro* analysis of bacterial and dye microleakage

Majid Kazem¹, Faranak Mahjour², Omid Dianat³, Saman Fallahi⁴, Mohammad Jahankhah⁵

¹Iranian Center for Endodontic Research, Dental Research Center, ²General Dentist, Dental Research Center, ³Department of Endodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, ⁴Department of Prosthodontics, Dental School, Hamadan University of Medical Sciences, Hamadan, ⁵General Dentist, Private Practice, Assalouyeh, Iran

ABSTRACT

Background: One ideal property of a root-end filling material is its apical sealing ability. The aim of this *in vitro* study was to assess bacterial and dye microleakage of white and gray mineral trioxide aggregate (WMTA and GMTA), Portland cement and calcium-enriched mixture (CEM) cement used as root-end filling material, and to assess the agreement between these two test methods.

Materials and Methods: Fifty-four single-rooted teeth were used. The roots were randomly divided into four study and two control groups. After decoronation, root canals were instrumented and filled with gutta-percha and AH26 sealer. Root-ends were resected 3 mm above the root-end and 3 mm deep cavities were prepared. Root-end cavities were filled with each material. *Enterococcus faecalis* and methylene blue dye were used for determination of bacterial and dye leakage respectively. Data were analyzed using Fisher's Exact Test, one-way ANOVA, Kaplan-Meier analysis, and Cohen's Kappa.

Results: There was 100% bacterial leakage in Portland cement and CEM cement, 58.3% in GMTA, and 91.7% in WMTA. GMTA showed significantly less bacterial leakage than Portland cement and CEM cement ($P < 0.05$). In those samples with leakage occurrence, times of observation of leakage were not significantly different; however, by survival analysis, the results of the GMTA group were significantly different from those of the CEM cement and Portland groups. The difference in complete dye leakage was not significant. There was poor agreement between dye and bacterial leakage methods.

Conclusion: CEM cement provides leakage results comparable to other commonly used root-end filling materials such as WMTA.

Key Words: *Enterococcus faecalis*, microleakage, mineral trioxide aggregate

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Address for correspondence:

Dr. Omid Dianat,
Department of Endodontic,
Dental School, Shahid
Beheshti University of
Medical Sciences, Evin,
Tehran, Iran.
E-mail: omidianat@gmail.com

INTRODUCTION

Penetration of microorganisms and their byproducts into filled root canal systems causes failure in root canal treatments.^[1,2] If the orthograde retreatment has been either unsuccessful or impossible, the only alternative treatment would be endodontic surgery.^[3]

The procedure of placing a root-end filling material during periapical surgery is suggested to guarantee the complete sealing of the root canal.^[4] A perfect apical sealing avoids recontamination and leads to reduction of microorganisms, and results in successful treatment. Therefore, one of the ideal properties of a root-end filling material is its apical sealing ability.

Several materials have been suggested as root-end filling materials; however, each of these materials has its own limitations.^[5] In recent decades, mineral trioxide aggregate (MTA), suggested as a root-end filling material by Torabinejad^[6,7] has been marketed as gray-colored (GMTA) and white-colored (WMTA). Both these materials are composed of Portland

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cement, bismuth oxide, and gypsum.^[8] The most notable difference between gray and white ProRoot MTA is the disparity in concentrations of Al_2O_3 , MgO, and, especially, FeO.^[9] MTA has been broadly examined, and the experiments have resulted in interesting outcomes. This material is reported to have less dye and bacterial leakage, and better adaptation than amalgam, Super-EBA and IRM.^[10] Although associated with some drawbacks such as extended setting time, difficult handling and high price, MTAs have become the gold standard for root-end filling, primarily because of their ideal characteristics.^[11,12] As a result of virtually the same chemical composition, MTA and Portland cement demonstrate similar physical properties, antimicrobial activity, and biocompatibility (the only difference in the composition of these materials is that Portland cement does not contain bismuth, and this results in a lack of radiopacity in this material).^[13,14] Based on this similarity, we have viewed the reported properties of Portland cement as a reference, since the manufacturers of MTA have confirmed that Portland cement is one of the components. If Portland cement proves to be an appropriate root-end filling material, it can be an alternative for MTA since it has some advantages such as low cost.

Recently, calcium-enriched mixture (CEM) cement consisting of different calcium compounds (e.g., calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, calcium hydroxide and calcium chloride) has been suggested as a proper root-end filling medicament^[3] with physical properties conforming to ISO 6876:2001. CEM cement shows favorable results such as good performance in apexogenesis, treatment of inflammatory external root resorption, and obturation of immature necrotic teeth.^[15,16] As a root-end filling material, it takes part in regeneration of periapical tissue.^[17] It also has better biocompatibility and antibacterial effects than those of MTA.^[18-20] When used as a root-end medicament, CEM stimulates hard-tissue healing and forms a seal as effective as MTA.^[3,21,22] In comparison to MTA, CEM has an increased flow, decreased working time, appropriate setting time and suitable handling characteristics, and sets in aqueous environments more easily.^[23,24] However, the sealing ability of CEM cement when used as root-end filling material has not been examined yet.

The main challenge in laboratory-based leakage

testing is designing a reliable experimental procedure that can be easily repeated and will clearly result in conclusion. In an attempt to solve this problem, different methods such as dye and bacterial leakage methods have been suggested to assess the sealing of different root-end filling materials.^[10,25] The aim of this *in vitro* study was to assess bacterial and dye microleakage of four different root-end filling materials and to evaluate the agreement degree between these two methods.

MATERIALS AND METHODS

Selection and standardization of the samples

Fifty-four single-rooted teeth, extracted for different periodontal problems were selected for this *in vitro* experimental study. Teeth were stored in distilled water and thymol (0.2%) until use. Preoperative radiographs of each tooth were taken to confirm the presence of a single canal and a fully formed apex, and the lack of internal or external resorption, calcification, or root caries. Standard infection control protocols were followed during all phases of the study. Their surfaces were scaled to remove calculus and were immersed in NaOCl 5.25% for 1 h in order to remove organic tissue. Afterwards, the teeth were rinsed with and stored in normal saline. Using a #012 long-fissure bur (Mani Inc., Tochigi-ken, Japan) in a high-speed handpiece with water spray, the crowns were removed above Cementoenamel junction (CEJ) level, perpendicular to the long axis of the tooth. Root length was standardized to 13 mm (from root apex to coronal border).

After initial radiographs, root canals were instrumented to the master apical file #40 (Dentsply/Maillefer, Ballaigues, Switzerland) using step back technique under thorough irrigation of 2.5% sodium hypochlorite. The canals got completely prepared by cleaning and shaping up to the file #80. Each root canal was instrumented at 1 mm short of the radiographic apex. Root canals were dried with paper points (Aryadent, Tehran, Iran), and obturated with gutta-percha (Diadent, Seoul, Korea) and AH26 sealer (Dentsply, DeTrey, Konstanz, Germany) by the lateral condensation method. Afterwards, the sealer set completely for 24 h in an incubator under a 99% humidity atmosphere at 37°C. A high-speed handpiece with a #008 diamond-fissure bur (Mani Inc., Tochigi-ken, Japan) under a continuous water spray was used to cut off the apical 3 mm of each

root-end perpendicular to the long axis of the tooth; 3-mm-deep root-end cavities were prepared using a #008 diamond-fissure bur. Then, the apical preparation was irrigated with 1 ml of normal saline and dried with paper points. The roots were then randomly divided into four study groups of 12 each and two control groups of three each. Root-end cavities were filled with either one of the studied materials: WMTA (ProRoot MTA, Densply Tulsa Dental, Tulsa, OK), GMTA (ProRoot MTA, Densply Tulsa Dental, Tulsa, OK), Portland Cement Type IV (Isfahan cement, Isfahan, Iran) or CEM cement. All materials were prepared according to their instructions, were placed by using an MTA carrier (Sybro Endo, Orange, CA, USA), and were packed with a cotton pellet. A cotton pellet moistened with sterile distilled water was placed and incrementally placed up to preparation surface level. All teeth were placed in 99% humidity at 37°C for 24 h to allow complete setting of retrofill materials. The setting of materials was checked by an explorer. Except the end-prepared portion of the root, external surfaces of roots were entirely coated with two layers of nail polish (Max Factor, Cosmetics and Fragrances, Los Angeles, CA, USA) to prevent penetration of dye and bacteria through dentinal tubules and accessory canals. Gutta-percha was then removed using Hedstrom file and reamer (Dentsply, Maillefer, Ballaigues, Switzerland).

Positive controls

The three teeth in this group were not filled after cavity preparation.

Negative controls

The canals and root-end cavities were filled with sticky wax. All external surfaces were coated with two layers of nail polish including the sectional apical portion.

Apparatus used to evaluate microleakage

Dual-chamber leakage model, which is based on the straight fitting of two tubes, was employed in this study. The 1.5 mm end of three-millimeter micropipettes (Supa Co., Tehran, Iran) was cut, and the tooth was placed in the cap end part. Sticky wax was then used to fill in the space between tooth and micropipette.

The system was sterilized using ethylene oxide gas and placed in sterile test tube containing 10 ml sterile TSB (Trypticase Soy Broth) (Merck, Darmstadt, Germany), whose diameter was equal to that of micropipettes in order to make a firm fit.

The aim of this process was to limit the pathway between the microbial reservoir and TSB to the root canal [Figure 1]. This technique would permit evaluating microleakage that might occur through the retrofill materials.

Bacterial leakage test

The coronal chamber was inoculated with 0.5 ml TSB containing *Enterococcus faecalis* ATCC 29212 (Pasteur Institute, Tehran, Iran) (with a concentration of about 1.5×10^8 cells/mL, adjusted to 0.5 McFarland turbidity standard). All the samples were prepared with such concentration and induction. After that, the samples were incubated at 37°C. At five-day intervals, bacteria suspension was added to each tube; the micropipettes and test tubes were sealed with parafilm (Supa Co., Tehran, Iran), and the samples were again incubated.

As a reference, the turbidity of the culture medium, microbial leakage was assessed daily for 70 days. The turbidity was considered an indicator of microbial contamination. Turbid tubes were selected, and inocula were spread on blood agar, simple agar, and bile esculin under identical incubation conditions and also Gram staining was carried out to verify the existence of *E. faecalis*. The day of turbidity presence was recorded.

Dye leakage test

Chlorhexidine and distilled water were used to rinse the samples. After the samples dried, methylene blue 1% dye was placed on the upper area of the retrofill material. Dye penetration between root filling material and apical tooth walls was monitored with stereomicroscope $\times 40$ (Olympus, Tokyo, Japan) after

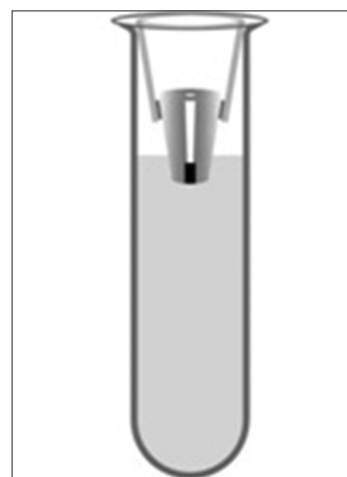


Figure 1: Schematic presentation of the dual-chamber apparatus

72 h. If dye leakage was observed at the interface of the root-filling material and the tooth root, it would be considered as a complete leakage.

Bacterial and complete dye leakage existence of the different materials was statistically analyzed by Fisher's Exact Test to investigate significant differences among the materials. One-way ANOVA was employed to analyze the time of bacterial microleakage. In order to evaluate the trend of leakage after 70 days of the experiment, Kaplan-Meier survival analysis was used. Cohen's kappa was applied to evaluate the agreement between the two methods. The statistical significance level was established at $P < 0.05$.

RESULTS

The results of bacterial leakage showed complete leakage within three days in the positive control samples. Negative samples showed no leakage after 70 days. The results of bacterial leakage in each material are shown in Table 1. GMTA showed significantly less bacterial leakage than Portland cement and CEM cement ($P = 0.01$).

In those samples in which the leakage had occurred, time of observation of the leakages was recorded. No significant differences were observed among the groups ($P = 0.49$). Based on the observed time of leakages, the trend of leakage after 70 days of the experiment was calculated. Significant differences were observed between GMTA and CEM cement, and between GMTA and Portland cement ($P = 0.14$) [Table 2, Figure 2].

All positive controls exhibited complete dye leakage, while the negative control group did not demonstrate any leakage. The results of dye leakage in each material are shown in Table 1. The differences between groups were not significant ($P = 0.095$).

There was no significant agreement between the results of the two methods ($P = 0.88$). Kappa = 0.007 was considered as poor agreement. The overall agreement was 36.1% between bacterial leakage and dye leakage.

DISCUSSION

In the present study, the apical sealing of four different root-end filling materials was compared by dye and bacterial leakage methods. The study was performed for a period of 70 days, a duration that seems to

be suitable according to the Torabinejad, *et al.*, study.^[26] Using two methods of leakage measurement helped us to gather more information, and to have a comparison between two methods. Dye leakage method determines material adaptation along the canal walls, and, because of the small size of dyes, it may demonstrate bacterial byproduct penetration. On the

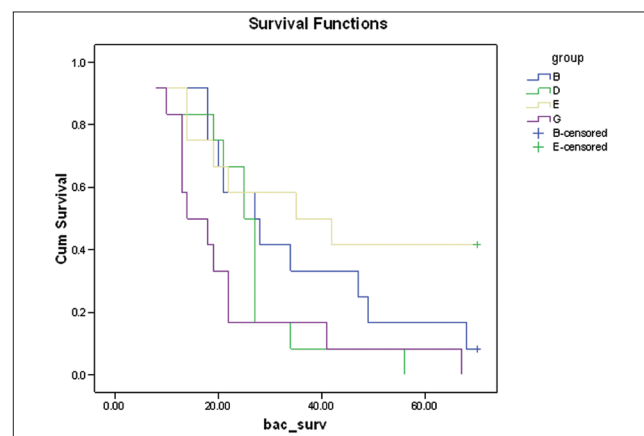


Figure 2: Plot of Kaplan-Meier estimates the trend of leakage in GMTA, WMTA, CEM cement, and Portland cement groups. Superscript letters represent a statistically significant difference ($P < 0.05$)

Table 1: Percentage of bacterial leakage and complete dye leakage in GMTA, WMTA, calcium-enriched mixture cement, and Portland cement groups

Group	Bacterial leakage (%)	Complete dye leakage (%)
GMTA	58.3 ^{ab}	0
WMTA	91.7	16.7
CEM cement	100 ^a	16.7
Portland cement	100 ^b	41.7

Superscript letters represent a statistically significant difference ($P < 0.05$), GMTA: Gray mineral trioxide aggregate; WMTA: White mineral trioxide aggregate; CEM: Calcium-enriched mixture

Table 2: Mean and standard error of days of bacterial leakage by observation and survival analysis in GMTA, WMTA, CEM cement and Portland cement groups

Group	Mean	Standard error	Confidence interval 95%	
			Inferior limit	Superior limit
GMTA	22 (42)	4.6 (7.3)	10.7 (27.7)	33.3 (56.3)
WMTA	30.7 (34)	5.3 (5.6)	19 (23)	42.5 (44.9)
CEM cement	21.7 (21.7)	4.8 (4.8)	11 (12.2)	32.2 (32.3)
Portland cement	25.5 (25.5)	3.5 (3.5)	17.8 (18.6)	32.2 (32.4)

GMTA: Gray mineral trioxide aggregate; WMTA: White mineral trioxide aggregate; CEM: Calcium-enriched mixture; (Numbers in parentheses indicate the results of survival analysis)

other hand, bacterial leakage measurements evaluate the sealing ability of all portions against bacteria.

Different types of bacteria have been used in different studies.^[8,10,27] In this study *E. faecalis* was used, mainly because it is the prime bacterium in chronic apical periodontitis and failed root canal treatment.^[28,29] While some other studies have used other bacteria instead of *E. faecalis*, their results are in agreement with ours in demonstrating a not significant difference between GMTA and WMTA in terms of bacterial leakage.^[27,30] Studies on leakage in Portland cement cases have reported incompatible results. In one study, Portland cement and MTA had same leakages, while some other studies have reported less leakage for MTA than for Portland cement, which is in agreement with our study.^[13,27]

In the present study, the mean times for microbial leakage in different material groups were close in the 70 days of observation. The trends after the 70th day were not followed; however, by using Kaplan-Meier calculations we could complete our data for the days after the 70th day. The results of survival analysis showed that the behavior of these materials diverges after 70 days such that there would be a significant difference between GMTA and CEM cement and also Portland cement. In regard to the time of bacterial leakage, Estrela *et al.*,^[13] have reported different results on MTA and Portland cement. These differences can be explained by the use of different types of Portland cement, or different bacteria in the experiments.

Our results are consistent with those of Islami, *et al.*,^[25] in that no significant difference was observed among dye leakages of GMTA, WMTA, and Portland cement. Some studies have reported that there was no complete dye leakage in GMTA, WMTA, and Portland cements.^[25,31] These differences in results may be attributed to the upper position of the dye to the root-end filling material, or to the role of gravitational force in reinforcing the penetration process.

The microleakage of CEM cement, which is comparable with MTA and Portland cement, indicates its good apical sealing. Due to the other good properties of this material such as biocompatibility,^[19] antibacterial^[18,32,33] and low cytotoxic effect,^[20] flow ability, good clinical handling,^[23] CEM cement is suggested as a proper root-end filling material.

It is not yet determined whether we can simulate the penetration of bacteria and their byproducts in apical periodontitis with dye leakage method. Kersten,

et al.,^[34] showed that the smaller the dye molecules, the more they can penetrate. As a result, one may conclude that dye molecules that are significantly smaller than bacteria, are capable of a deeper penetration.

Surprisingly, in dye microleakage studies, dye did not completely penetrate between the sealing material and tooth, even after two weeks. One reason for such a phenomenon might be the presence of air bubbles that can inhibit the penetration process.^[35] In our study, dye was placed over root-end filling material so that gravity force could enhance the act of penetration. Even with such an arrangement, dye penetration was less than that of bacteria. Barthel *et al.*,^[36] have listed some possible causes for this phenomenon. For example, in a clinical condition, several factors such as pH, temperature, or ionic charge, are altered with bacteria; however, these situations cannot be simulated with the dye method. Moreover, the bacteria's ability for active movement and growth, and their ability to change their shape and size, will consequently result in more penetration.

CONCLUSION

There was a poor agreement between the dye and bacterial leakage methods; therefore, bacterial leakage cannot be predicted by dye leakage results. It can be concluded that CEM cement provides leakage results comparable to other commonly used root-end filling materials such as WMTA. However, further *ex vivo* and *in vivo* studies are needed to assess other properties of this novel material.

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