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Comparison Of The Sealing Ability Of Three Biocompatible Materials Used For Furcation Repair Material: An In Vitro Study

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ABSTRACT

Introduction: Furcation perforation is a critical endodontic complication requiring effective sealing with biocompatible materials. Mineral Trioxide Aggregate (MTA), derived from cement, and has been successfully used to repair lateral root and furcal perforations in apexogenesis and as a vital pulp capping agent. This study aimed to compare the microleakage of BioMTA, Angelus MTA, and Root MTA in repairing furcation perforations in mandibular molars using a bacterial leakage model.

Materials & Methods: In this in vitro study, 55 extracted intact human mandibular molars were divided into three experimental groups (n=15) and positive and negative control groups (n=5 each). Perforation sites in Groups 1, 2, and 3 were repaired with Angelus MTA, BioMTA, and Root MTA, respectively. Sealing ability was assessed using an Enterococcus faecalis dual-chamber bacterial leakage model over 30 days. Data were analyzed using the chi-square test and Kaplan-Meier survival analysis ($p<0.05$was considered significant).

Results: All positive control samples showed turbidity, whereas no negative control samples exhibited bacterial leakage. No significant differences in microleakage or mean survival time were found among the three MTA materials. The microleakage rates were 26.7%, 33.3%, and 40.0% for BioMTA, Angelus MTA, and Root MTA, respectively.

Conclusion: Given the lack of significant differences among the three materials, selection may be based on ease of use, economy, and availability.

Keywords: Dental Leakage; Furcation Defects; Mineral Trioxide Aggregate; Root Canal Filling Materials; Enterococcus faecalis.

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Introduction

Perforations can be defined as a pathologic or mechanical connection between the root canal system and the external tooth surface, causing inflammation, bacterial infection, bone resorption, and proliferation of epithelial tissue [1, 2]. Perforations can result from tooth decay, resorption, or iatrogenic factors [3]. Accidental furcal perforation may arise during restorative or endodontic procedures [4]. Up to 29% of all endodontic mishaps were reported as an accidental perforation, and 87% were in the pulp chamber of molars. Furcal perforations have a worse prognosis than other locations, due to the nature of the anatomic and topographic complexity of the area [2]. Perforations adjacent to the crestal bone generally have lower treatment success rates [5]. Early diagnosis, timely repair, and selection of an appropriate material are considered key factors for successful perforation management [6]. The main goal of perforation repair is to inhibit the passage of bacterial contamination from the tooth to the surrounding periradicular tissues [5].

Three-dimensional sealing is one of the critical requirements in the repair of furcation perforations. An adequate seal is the result of proper marginal adaptation, adhesion, low solubility, and minimal dimensional changes of the cement used [7]. An ideal repair material should be capable of creating an effective seal, exhibit good biocompatibility, and promote both bone and cementum formation [6].

The perforation defect can be repaired surgically or non-surgically [2]. In recent decades, it has been suggested to use tricalcium silicate-based materials, such as mineral trioxide aggregate (MTA), portland cement, and Endosequence in managing perforations [8].

Mineral Trioxide Aggregate (MTA) is a bioactive calcium silicate–based material widely used in endodontics for procedures such as apexification, regenerative treatments, and repair of root perforations [9]. MTA exhibits adequate radiopacity, high pH, successful sealing ability, low solubility, and biocompatibility [2].

Angelus MTA(Angelus Co., Londrina, Brazil), has a reduced setting time and low solubility [10]. BioMTA (Cerkamed, Stalowa Wola, Poland) is considered a modified calcium silicate cement containing hydroxyapatite and nanoparticles [10], to reduce the long hardening time of MTA, increase its hardness, plasticity, and biocompatibility [11]. Root MTA (Lotfi, Tabriz, Iran) is a locally manufactured calcium silicate–based cement [10]. Various techniques have been employed to evaluate microleakage, including dye penetration, fluid filtration, bacterial and protein leakage tests, dye extraction methods, scanning electron microscopy, and radioactive isotope analysis [12].

The dye penetration technique is one of the commonly used methods; however, due to the chemical properties, pH, low molecular weight of the dye, and its ability to dissolve in restorative materials, it may either overestimate or underestimate the depth of penetration. In this study, the *Enterococcus faecalis* bacterial leakage model was used in a dual-chamber system, which is considered an improved version of the dye penetration technique. *Enterococcus faecalis* was selected because it is one of the most common microorganisms associated with endodontic treatment failures [12]. Given the critical implications of furcation perforation as well as sparse data comparing the sealing ability of Angelus MTA, BioMTA, and Root MTA for repairing the perforated teeth, the purpose of this *in vitro* study was to

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compare the bacterial leakage of Angelus MTA, BioMTA, and Root MTA in repairing the furcation perforations in human mandibular molars using a dual-chamber bacterial leakage model.

Materials & Methods

This in vitro experimental study was approved by the Research Ethics Committee of Babol University of Medical Sciences (Ethics code: IR.MUBABOL.HRI.REC.1398.024).

The minimum sample size was calculated using the following formula:

$$n=\frac{((Z(1-\alpha/2)+Z(1-\beta))^2\times[P1(1-P1)+P2(1-P2)])}{(P1-P2)^2}$$

Based on a previous study, a minimum of 14 specimens per group was required to achieve a 99% confidence level and 80% statistical power [13]. To compensate for potential specimen loss during the experimental procedures, 15 specimens were allocated to each experimental group. In addition, five positive-control and five negative-control specimens were included. Consequently, a total of 55 freshly extracted human mandibular molars were enrolled. Eligible teeth had normal furcation anatomy with completely separated roots, minimal caries (defined as at least 4 mm of sound tooth structure coronal to the cementoenamel junction (CEJ), and no history of endodontic treatment. Teeth with cracks identified by microscopic examination were excluded.

The selected teeth were cleaned thoroughly using a periodontal curette (1853; Dena Puya, Iran) and immersed in 0.5% sodium hypochlorite (Golrang, Tehran, Iran) for 24 h. They were subsequently stored in normal saline (0.9% NaCl; Darupakhsh, Tehran, Iran) until use. Initial endodontic access was prepared with a high-speed #12 diamond round bur (DentsplySirona, Switzerland). The roof of the pulp chamber was then completely removed, and the cavity walls were refined using an Endo-Z bur (DentsplySirona, Charlotte, NC, USA).

Each tooth was sectioned horizontally at the apical third of the root with a high-speed diamond disc (HLF 86; Dorsa, Tehran, Iran) under continuous water cooling. The canal orifices and resected root ends were subsequently sealed with a light-cured glass-ionomer cement (Fuji II LC; GC Corporation, Tokyo, Japan). Except for the negative-control group (n = 5), a standardized furcation perforation was created in all specimens using a #012 round bur (D&Z Co., Wiesbaden, Germany) mounted on a high-speed handpiece under continuous water irrigation.

All perforations measured 1.2 mm in diameter, corresponding to the bur size. Specimens with irregular perforations were excluded, and the bur was replaced after every five perforations to ensure consistent cutting efficiency. All specimens, except those assigned to the negative-control group (n = 5), in which no perforation was created, were randomly allocated to four groups using a computer-generated random number sequence: one positive-control group and three experimental groups. In Groups I, II, and III (n = 15 per group), furcation perforations were repaired with Angelus MTA, BioMTA (Cerkamed, StalowaWola, Poland), and Root MTA (Dr Lotfi, Tabriz, Iran), respectively. In the positive-control group (n = 5), perforations were created but left unrepaired.

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In the negative-control group (n = 5), no perforation was created, and the furcation area remained intact Following irrigation with normal saline, each repair material was prepared according to the manufacturer's instructions and delivered to the perforation site using an MTA carrier (Dental Devices, Sialkot, Pakistan). The material was gently condensed with a moist cotton pellet. The specimens were placed on a soft sponge moistened with normal saline and incubated at 37°C for 24 h to allow complete setting of the biomaterials. After the MTA placement had been confirmed, the external surface of each specimen was coated with two layers of nail polish, leaving the perforation site and an approximately 1-mm surrounding margin exposed.

In the negative-control group, the entire external surface was coated with two layers of nail polish. Microleakage was evaluated using a dual-chamber anaerobic bacterial leakage model. Each specimen was mounted in a plastic Eppendorf cylinder (Sigma-Aldrich Co., Hamburg, Germany) after removal of the apical portion of the root, allowing the external aspect of the perforation site to remain accessible. The interface between the specimen and the inner wall of the cylinder was completely sealed with cyanoacrylate adhesive (Super Bonder, Loctite, Itapeva, SP, Brazil). Each cylinder was then secured to the cap of a Falcon tube (Sigma-Aldrich Co., Hamburg, Germany) using the same adhesive (Figure 1A).

The assembled apparatus was sterilized with ethylene oxide gas for 24 h. The upper chamber was inserted into a 15-mL Falcon tube tube, which served as the lower chamber and contained 10 mL of sterile Tryptic Soy Broth (TSB; Sigma-Aldrich Co., Hamburg, Germany). The entire assembly was incubated at 37°C under 100% humidity for 48 h. The upper chamber subsequently served as the reservoir for *Enterococcus faecalis*.

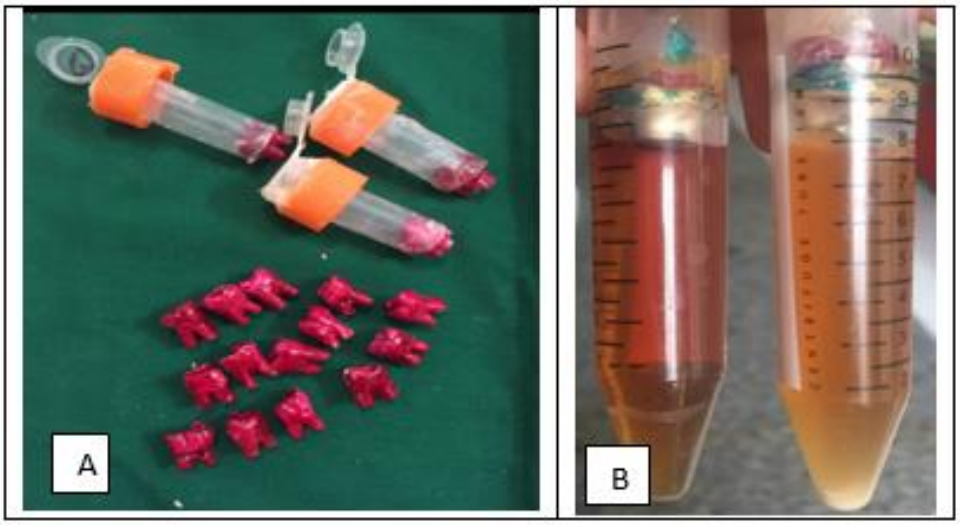

Figure 1. (A) Specimens mounted in the upper-chamber vials together with the corresponding components of the lower-chamber assembly; (B) Representative sample showing turbidity in the lower chamber, indicating bacterial microleakage

A standard strain of Enterococcus faecalis (ATCC 29212), a Gram-positive and catalase-negative coccus, was obtained from the microbial culture bank of the Department of Microbiology, Babol University of Medical Sciences. To obtain sufficient bacterial growth, the strain was cultured on 5% sheep blood agar and incubated at 37°C for 24 h. The identity

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of *E. faecalis* was confirmed by Gram staining and catalase testing. A bacterial suspension containing 9×10^8 colony-forming units (CFU)/mL was prepared by transferring a small number of colonies into Tryptic Soy Broth (TSB), followed by adjustment of the turbidity to the 3.0 McFarland standard. The 3.0 McFarland standard consisted of a mixture of 0.3 mL of 1% $BaCl_2$ and 9.7 mL of 1% H_2SO_4 [14].

Each specimen was inoculated with 100 µL of freshly prepared *E. faecalis* suspension (9×10^8 CFU/mL) every 3 days for 30 days. All specimens were incubated at 37°C and examined daily for turbidity, which was considered indicative of bacterial leakage throughout the experimental period (Figure 1B).

Results

Among the specimens without turbidity, Root MTA, Angelus MTA, and BioMTA accounted for 9 (30.0%), 10 (33.3%), and 11 (36.7%) cases, respectively. Among the specimens with turbidity, Root MTA, Angelus MTA, and BioMTA accounted for 6 (40.0%), 5 (33.3%), and 4 (26.7%) cases, respectively. The distribution of turbidity among the three MTA formulations was not statistically significant, as determined by the chi-square test ($P = 0.74$).

Kaplan-Meier survival analysis was performed to evaluate the time to microleakage occurrence among the three MTA groups. BioMTA exhibited the longest mean survival time (26.67 ± 6.27 days), followed by Angelus MTA (26.00 ± 6.72 days). The Root MTA group showed the shortest mean survival time (24.20 ± 8.40 days). However, no statistically significant differences were observed in mean survival times among the three groups.

Table 1 presents the number of turbidity events, indicating bacterial microleakage, detected in the culture medium of the experimental groups during the observation period. BioMTA demonstrated the lowest frequency of microleakage events among the three materials, with leakage detected on days 10, 17, 20, and 23.

Table1. Turbidity in different experimental groups, time of detection and number of samples

MTA Formulations	Turbidity	Day (Number)
Root MTA	6	9 (2) 13 (1) 17 (1) 18 (1) 27 (1)
Angelus MTA	5	10 (1) 16 (1) 17 (1) 20 (1) 27 (1)
BioMTA	4	10 (1) 17 (1) 20 (1) 23 (1)

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Discussion

The results of the present study showed no statistically significant differences in bacterial microleakage among the three types of MTA (BioMTA, Angelus MTA, and Root MTA).

According to our study, the findings of Francis et al. were consistent with our results, as no statistically significant difference was observed in the sealing ability of the tested calcium silicate-based materials [15]. The slight differences between the two studies may be attributed to variations in leakage assessment protocols, particularly the monitoring intervals and methodological conditions used for leakage detection.

Labaf et al. reported lower dye penetration for Pro Root MTA compared with Root MTA [13]. In contrast, no statistically significant difference was observed among the three tested MTA materials in the present study. This discrepancy may be related to differences in the physicochemical properties of the materials, including particle size and homogeneity, as well as variations in the methods used for microleakage assessment, which may influence the reported sealing performance.

The results of the present study differed from those of the aforementioned study, which reported significant differences among the tested materials and superior performance of Bio-C Repair [16]. In contrast, no statistically significant difference was observed among the materials in the current study. This discrepancy may be related to differences in the tested materials, leakage assessment methods, and experimental conditions.

The findings of Moradi et al. were in agreement with the present study, as no statistically significant difference was observed among the tested root canal repair materials [17]. However, the difference in evaluation methods should be considered when comparing the results, since Moradi et al. assessed marginal adaptation microscopically, whereas the present study evaluated bacterial leakage using a dual-chamber bacterial leakage model. Variations in assessment techniques and experimental conditions may explain minor differences in the reported performance of the materials.

In contrast to our findings, Makhlouf et al. reported that Biodentine demonstrated significantly better sealing ability compared with MTA Angelus [18]. However, no statistically significant difference was observed among the three tested materials in the present study. This discrepancy may be related to differences in the evaluation methods, as Makhlouf et al. used a dye penetration model, whereas the present study employed a bacterial leakage model with a dual-chamber system. In addition, variations in the observation period and experimental conditions may have influenced the outcomes.

Nagmode et al. reported inferior marginal adaptation of Angelus MTA compared with light-cure MTA [19]. In contrast, no statistically significant difference was observed among the tested materials in the present study. This discrepancy may be attributed to differences in the methods used to evaluate sealing ability, as Nagmode et al. used scanning electron microscopy, whereas a bacterial leakage model was employed in the present study. These differences highlight the influence of evaluation methods and observation periods on outcomes.

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One limitation of the present study was the relatively short duration of microleakage evaluation. It is therefore recommended that future investigations assess and compare the microleakage behavior of these materials over extended periods to better simulate long-term clinical conditions.

Conclusion

Despite the limitations of this study, the three tested MTA materials demonstrated comparable sealing abilities. Selection may be based on ease of use, economy, and availability. Further studies with longer follow-up periods and clinical evaluations are warranted to confirm these findings.

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Conflict of interest

The authors declare that they have no competing interests.

Author's Contribution

Sina Mirzaee Rad and Akam Saeidi proposed the study title, supervised, and critically reviewed the manuscript. Fatemeh Bour performed the experimental procedures and collected the data. Marzieh Alimohammadi conducted the literature search, search and wrote and edited the manuscript. Abolfazl Davudabadi supervised the microbiology section and bacterial culture. Hemmat Gholinia carried out the statistical analysis. All authors read and approved the final version of the manuscript.

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