

Original Article

In vitro Comparison of Bacterial Leakage and Dye Penetration in Perforated Primary Molars Restored with MTA Angelus and Cold Ceramic

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KEY WORDS

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ABSTRACT

Background: Various surgical and non-surgical techniques have been proposed to restore the furcal defects, with non-surgical techniques being preferred due to their non-invasiveness, convenience, and fewer side effects. Several materials with different physical properties and treatment success rates have been used for this purpose. However, the efficacy of cold ceramic (CC) for furcal and root canal wall perforation repair in primary teeth has not been previously evaluated.

Purpose: This study aimed to compare bacterial leakage and dye penetration in perforated primary molars restored with mineral trioxide aggregate (MTA) Angelus and CC.

Materials and Method: This *in vitro* study was conducted on 50 extracted primary molars. A perforation was created in their pulpal floor by using a round-end diamond bur with 1mm diameter. The teeth were then randomly divided into 4 groups: furcal perforation repair with CC (group 1; n=20), furcal perforation repair with MTA Angelus (group 2; n=20), unrestored furcal perforation (positive control; n=5), and no furcal perforation (negative control; n=5). Bacterial leakage was evaluated using a two-chamber bacterial leakage model with *Enterococcus faecalis* (*E. faecalis*) while dye penetration was assessed stereomicroscopically by the dye penetration technique using basic fuchsin. Data were analyzed by the Mann-Whitney U, Kaplan-Meier, log-rank, Chi-square, and Fisher's exact tests ($\alpha=0.05$).

Results: MTA and CC had no significant difference in dye penetration depth (p Value= 0.896) or bacterial leakage ($p=0.434$).

Conclusion: Despite the study limitations, it can be concluded that both CC and MTA have acceptable sealing properties for furcal perforation repair in primary molars under *in vitro* conditions since they had no significant difference in dye penetration or bacterial leakage tests. Considering the characteristics of MTA, such as its long setting time, high cost, and difficult handling, CC may be used as a suitable alternative material for treatment of furcal perforations in primary teeth.

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Introduction

Selection of a suitable material with ideal physical and biological properties, and minimal microleakage is highly important for clinical success of pulp therapy in primary teeth [1-2]. Root canal wall or furcal perfora-

tions are among the common procedural errors during endodontic treatment [3], which allow for the microorganisms to penetrate from the root canal into the surrounding bone tissue, and cause infection and inflammation, damaging the periodontal tissue. Furcal or root

canal wall perforation is often caused by extensive caries, internal/external root resorption, or iatrogenic causes such as inadequate access cavity preparation, excessive filing, or errors in creating or cleaning the canal path [2-4].

Following the occurrence of root canal perforation, the most important point to consider is the time to restore the perforation and prevent the entry of microorganisms. If the perforation is not restored immediately, penetration of microorganisms into the surrounding tissues causes inflammatory reactions and subsequent bone loss, and can even damage the underlying permanent tooth bud [4].

Various surgical and non-surgical techniques have been proposed to restore the furcal defects, with non-surgical techniques being preferred due to their non-invasiveness, convenience, and fewer side effects. Non-surgical techniques involve rapid placement of a restorative material in the perforation area from within the canal and prevention of bacterial leakage [5]. Various materials with different physical properties and treatment success rates are used for this purpose. However, no material has yet been found that offers all the desired properties at once [6-7]. These properties include non-toxicity, high biocompatibility, induction of osteogenesis and cementogenesis, no shrinkage after placement in the canal, impermeability to fluids such as saliva and blood, no discoloration, ease of use, and reasonable cost [6-7]. Currently, various materials such as Biodentine, calcium enriched mixture cement, and mineral trioxide aggregate (MTA) with high sealability are commonly used to repair the root canal wall and furcal perforations [8-9].

The optimal sealability and biocompatibility of MTA, and its favorable adaptation to the cavity walls have been well confirmed [8]. Despite all the desirable properties of MTA evaluated *in vitro*, the possibility of leakage following the use of MTA increases in the clinical setting due to its poor handling and long setting time. These two properties prevent rapid healing of the lesion and increase marginal leakage [9].

Cold ceramic (CC) is a newly introduced ceramic material with applications similar to those of other calcium silicate-based cements [10]. This material was first introduced by Modaresi and Talakoob [11] in Iran in 2000. CC is a material similar to MTA that can be used

as a root-end filling material, root perforation repair material, and apical barrier in open-apex teeth, and can potentially be considered as a paste for root canal sealing as well as a capping agent for pulp capping and pulpotomy [12].

Perforations should be sealed with appropriate restorative materials. Materials used to manage perforations in teeth should have properties such as adequate sealability, non-resorbability, non-toxicity, biocompatibility, radiopacity, and moisture tolerance [7, 13]. In addition, these materials should be easy to handle, and have the ability to induce osteogenesis and cementogenesis [14]. Despite having desirable properties, MTA has a long setting time, and is also expensive [15].

To the best of the authors' knowledge, the efficacy of CC for furcal and root canal wall perforation repair in primary teeth has not been previously evaluated. Thus, this study was designed aiming to compare bacterial leakage and dye penetration in perforated primary molars restored with MTA and CC. The null hypothesis of the study was that no significant difference would be found in bacterial leakage or dye penetration between MTA and CC used for perforation repair in primary teeth.

Materials and Method

This *in vitro*, experimental study was conducted on 50 primary molars that had been extracted since they were non-restorable or non-retainable, after obtaining written informed consent from the ethics committee of Islamic Azad University (IR.IAU.DENTAL.REC.1403.103).

Sample size

The minimum sample size was calculated to be 20 in each group for the bacterial leakage test according to a study by Nazari Moghadam *et al.* [10] using the log-rank test power analysis of SPSS 11, and assuming $\alpha=0.05$, $\beta=0.2$, and hazard ratio of 0.94 in the control group and 0.35 in the test group. Since the control group specimens were only used to confirm the accuracy of the methodology, only 3 to 5 specimens in each group were considered as the control group for each test.

The minimum sample size was calculated to be 19 in each group for the dye penetration test according to a study by Reddy *et al.* [16] using the two-sample t-test power analysis of SPSS 11 assuming $\alpha=0.05$, $\beta=0.2$, mean standard deviation of microleakage=0.142, and significant difference of 0.1. Since both the dye penetra-

tion and bacterial leakage tests were performed on the same specimens, 20 specimens were considered for each group.

Eligibility criteria

The inclusion criteria were primary molars with no root fracture or crack with standardized roots in terms of presence of at least two-thirds of the root length. The exclusion criteria were physiological or pathological internal/external root resorption, history of pulpotomy or pulpectomy, and perforation of the pulp chamber floor.

Specimen preparation

The debris present on the tooth surface was removed by a #15 surgical scalpel (Scalpel blade, ATP, Trion Co, Germany) and the teeth were cleaned by a disposable prophylaxis brush (Melorin Co., China) connected to a low-speed hand-piece under water irrigation. The teeth were rinsed with saline (Shiraz Serum Co., Iran) after extraction, and stored in 0.5% thymol solution for 1 week. They were then stored in distilled water (3Sib Co, Iran) at 4°C. All roots were cut by a diamond disc (Crown Cutter; DFS Diamon Co., Germany) at high speed under water coolant slightly above their cemento-enamel junction. The residual pulp tissue at the canal orifice was completely removed, and the canal orifice and apical part of the root were etched with 35% phosphoric acid, sealed by using a bonding agent and composite resin (Charisma Diamond, Heraeus Kulzer Co., Germany), and cured for 40 seconds with a LED curing unit (Woodpecker LED D, China). Next, the external surface of the specimens was coated with nail varnish. A perforation was then created in the pulpal floor by using a round-end diamond bur (Jota Co., Switzerland) with 1 mm diameter. Next, the specimens were randomly assigned to 4 groups: furcal perforation repair with CC as instructed by the manufacturer (group 1; n=20), furcal perforation repair with MTA Angelus as instructed by the manufacturer (group 2; n=20), unrestored furcal perforation (positive control; n=5), and no furcal perforation (negative control; n=5).

MTA Angelus (Angelus, Londrina, PR, Brazil,) and CC (SJM Company, Iran) were delivered into the furcal defects by a MTA carrier (PD - Produits Dentaires SA, Swiss) and packed with a plugger (Medesy, Italy) [17]. The specimens were placed in plastic vials, and incubated at 37°C for 24 hours. The specimens were then

placed in microtubes with a perforated end. Their inter-radicular region was extruded through the perforated end, and the microtube was sealed with sticky wax. Next, this part was placed in a penicillin vial to separate the upper and lower chambers. The specimens were then individually packed and gamma-sterilized with 25 kGy dose by a GC-220 gamma cell calibrated according to ISO/ASTM 51026:2015 standard [18].

Microbial culture

Enterococcus faecalis (*E. faecalis*; ATCC29212) was obtained from the Iranian Research Organization for Science and Technology and its inoculation suspension was prepared in 0.5 McFarland standard concentration containing 1.5×10^8 colony forming units/milliliter. *E. faecalis* was cultured in brain heart infusion broth and incubated at 37°C and 10% CO₂ for 48 hours.

Bacterial leakage test

Each specimen pack was opened under a Class II hood (Labcaire, UK), and 500 µL of *E. faecalis* inoculation suspension was poured into the upper chamber. Sterile brain heart infusion broth was poured into the lower chamber. To ensure bacterial viability, the culture medium in the upper chamber was refreshed daily. Presence/absence of turbidity in the culture medium in the lower chamber was evaluated on a daily basis for 30 days spectrophotometrically (BioTek, Cytation, USA) [19]. In case of turbidity, bacterial culture was performed to ensure that the turbidity was caused by *E. faecalis*.

Dye penetration test

After the bacterial leakage test, the specimens were stored in saline for 2 weeks [20]. Next, basic fuchsin dye (Neutron, Iran) was poured into the access cavities for 72 hours. The specimens were then rinsed under running water for 30 minutes to remove excess dye. They were then mesiodistally sectioned parallel to their longitudinal axis. The dye penetration depth was measured under a stereomicroscope (X20 ZSM1001, Zist Rah Danesh, Iran) [21]. Each specimen was scored according to ISO 11405 as score 0: no microleakage, score 1: dye penetration to half of the cavity wall depth (moderate microleakage), and score 2: dye penetration exceeding half of the cavity wall depth (severe microleakage) (Figures 1-2).

Statistical analysis

Quantitative data were analyzed using the Mann-Whitn-



Figure 1: Stereomicroscopic images of a perforation repaired with MTA: (right) no dye penetration; (left) dye penetration

ey U test (for dye penetration), Kaplan-Meier analysis and log-rank tests (for bacterial leakage), and the Chi-square and Fisher’s exact tests (for the frequency and severity of leakage). $p < 0.05$ was considered statistically significant. To validate the statistical power of the study, a power analysis was performed.

Results

For the bacterial leakage test (log-rank test), the study power was calculated to be 80.16% with 40 specimens (n=20 in each experimental group), which was above the minimum standard power of 80%, indicating that the study had enough power to detect a hazard ratio=0.35 (clinically significant difference). For the dye penetration test (t-test), the study power was calculated to be 80.32% with 38 specimens (n=19 in each experimental group), indicating that the study had enough power to detect a mean difference of 0.1 (with a standard deviation of 0.1). Thus, sufficient statistical power confirmed the validity of negative results (absence of a difference between the groups).

Dye penetration

Table 1 presents the dye penetration score in the two experimental groups. The Mann-Whitney U test (applied

due to non-normal data distribution) revealed no significant difference between the two groups in the mean dye penetration score ($p = 0.896$).

Bacterial leakage

To assess the resistance of materials to leakage over time (time to leakage), the Kaplan-Meier analysis and log-rank test were used (Figure 3). The Kaplan-Meier analysis showed that after 30 days, 75% of MTA and 65% of CC specimens had no leakage, yielding a mean resistance time of 26.95 days for MTA and 24.65 days for CC. The CC and MTA groups had no significant difference in bacterial leakage ($p = 0.434$). The Chi-square test and Fisher’s exact test showed no significant difference in overall leakage scores either ($p = 0.490$ and $p = 0.731$, respectively).

Discussion

This study compared bacterial leakage and dye penetra-

Table 1: Dye penetration score in the two experimental groups

Group	No leakage	Moderate leakage	Severe leakage	Total
MTA	8 40.0%	7 35.0%	5 25.0%	20 100.0%
Cold Ceramic	9 45.0%	4 20.0%	7 35.0%	20 100.0%
Total	17 42.5%	11 27.5%	12 30.0%	40 100.0%



Figure 2: Stereomicroscopic images of a perforation repaired with CC: (right) no dye penetration; (left) dye penetration

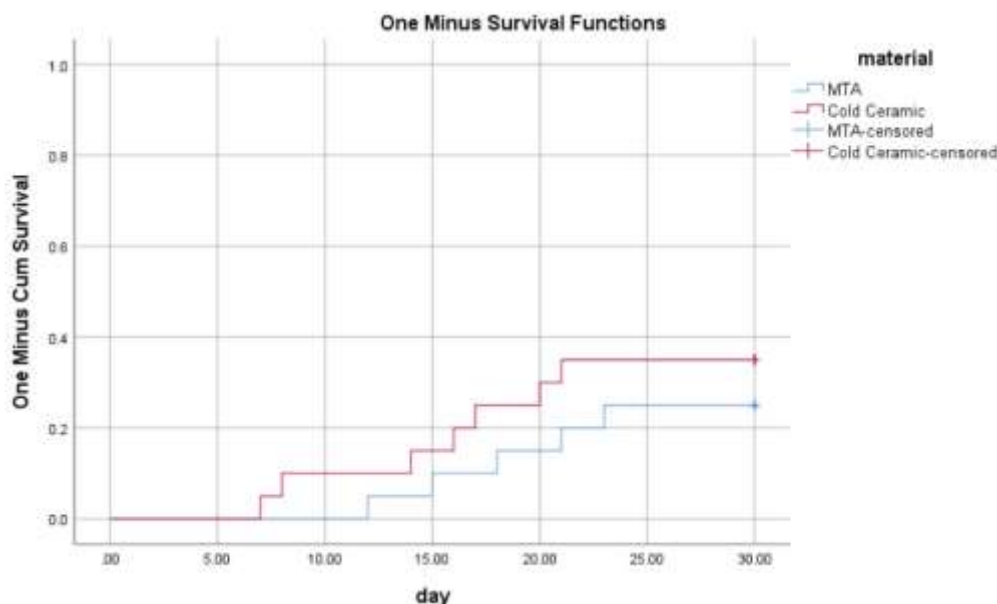


Figure 3: Kaplan-Meier analysis for bacterial leakage

tion in perforated primary molars restored with MTA and CC. The results showed no significant difference between MTA and CC in dye penetration or bacterial leakage. Thus, the null hypothesis of the study was accepted.

The aim of perforation repair is to create a mechanical and biological seal between the peri-radicular tissues and the access cavity, which is possible by using a biocompatible material that induces favorable tissue healing [22]. Among the various materials, MTA, which was first introduced in dentistry in 1993 [23], is considered the most suitable material for this purpose in terms of sealability and tissue compatibility [24]. It has special properties such as optimal sealability in presence of moisture, resistance to microorganisms, low cytotoxicity, antibacterial activity, biocompatibility, and induction of osteogenesis and dentinogenesis [9]. MTA contains Portland cement (75%), bismuth oxide (20%), and calcium sulfate (5%) [25]. MTA Angelus is one of the new products, which consists of tricalcium silicate and iron oxide powder and a liquid. The powder is mixed manually with the liquid, resulting in formation of calcium hydroxide and calcium silicate. The setting time of this material is about 3 hours [26].

On the other hand, CC is another new product containing MTA, the main ingredient of which is calcium hydroxide. The alkaline nature of this material improves its antibacterial activity. The initial setting time of CC is 15 minutes and it sets completely within 24 hours in

presence of moisture [12, 27]. Therefore, the initial setting time of 15 minutes in CC may improve the prognosis in the first 24 hours. On the other hand, working with MTA can be challenging and requires time and practice, while CC is easy to handle in such situations [17]. CC has a shorter setting time than MTA and in addition to fine particles, it also has coarser particles in its structure, which ultimately makes the material easier to handle.

The current findings showed that MTA and CC did not differ significantly in terms of bacterial leakage. Also, the mean duration of resistance time to leakage was 26.95 days for MTA and 24.65 days for CC, which was not significantly different. In line with the current findings, Roda *et al.* [5] used CC to repair a strip perforation in a mandibular first molar and showed that this material was effective for perforation repair and improved the clinical symptoms. In line with the present study, Asgari and Hajihassani [28] used CC to repair a perforation caused by internal root resorption in a maxillary lateral incisor and reported that after one year, the tooth was functional and asymptomatic, and the treatment with CC was successful. These findings indicate successful management and healing of endodontic lesions with CC and its appropriate setting time compared to MTA, which can make this material a suitable alternative to MTA.

Khedmat *et al.* [29] compared MTA Angelus and CC in their *in vitro* study in terms of viability, odonto-

genic differentiation, and calcification potential of human dental pulp stem cells and periodontal ligament fibroblasts. They reported that both materials were highly biocompatible and increased the expression of alkaline phosphatase enzyme, which plays a key role in the initial formation of mineralized tissues and induction of hydroxyapatite deposition in collagen matrices. Their findings were similar to the current results, suggesting that CC may be a suitable material for vital pulp therapy and root perforation repair. It is worth noting that clinical studies are also required to confirm these findings.

In contrast to the current results, Jahromi *et al.* [30] compared CC and ProRoot MTA for treatment of furcal perforations and healing of the surrounding periodontal tissue in dogs and reported that ProRoot MTA had better results in terms of healing of the surrounding tissues under the same conditions, but after 1 and 2 months, there was no significant difference between the two materials. Variations in the study results can be due to comparison of different materials under different conditions. The current study compared the amount of microleakage, while Jahromi *et al.* [30] focused on histological improvement of the surrounding periodontal tissues.

Modaresi and Hemati [12] reported that the sealing properties of CC were comparable to those of MTA in dry and saliva-contaminated conditions, and superior to MTA in blood-contaminated conditions. This finding was similar to the current results regarding no significant difference between MTA and CC in microleakage. Therefore, it appears that CC may be used for perforation repair due to its almost similar or superior properties compared to MTA.

However, in contrast to the present results, Mokhtari *et al.* [31] showed that the mean marginal gap of CC was significantly greater than that of MTA Angelus. To date, different methods have been used to assess the sealability of materials used for furcal perforation repair, including bacterial leakage models, dye penetration test, air pressure, and electrochemical methods [31]. In the current study, a bacterial leakage model and dye penetration test were used to compare the sealability of MTA and CC. The difference between the current study result and that of Mokhtari *et al.* [31] may be attributed to different methods used to assess the sealability of the materials. However, more clinical studies are needed to address this discrepancy.

The dye penetration test is a common and easy method for measuring microleakage. Factors such as the pH of the dye, chemical reactions, and the size of the dye particles affect the amount of dye microleakage [32]. Wu *et al.* [33] stated that the optical density of methylene blue decreases due to the acidity of MTA, which can lead to an underestimation of the actual amount of microleakage. Moreover, the increase in pH during mixing of MTA powder with water causes the surfaces to be stained by methylene blue [33-34]. Therefore, in the current study, fuchsin dye, which has alkaline properties, was used.

De-Deus *et al.* [35] reported that the bacterial leakage and dye extraction methods have low sensitivity in showing the difference between different materials. Contrary to their findings, Mehdipour *et al.* [36] stated that the dye extraction method provides more reliable findings compared to the dye penetration test because it quantitatively evaluates all the dye absorbed in the root. On the other hand, Dastorani *et al.* [19] stated that although the bacterial leakage method can better simulate the clinical conditions than the dye penetration test, it cannot be reliable in measuring the sealability due to the specific antibacterial properties of materials such as MTA. However, many studies in this field have used bacterial leakage models to measure the sealability of restorative materials such as MTA [37-38]. Therefore, considering the differences in the methods of measuring the sealability among different studies, the current study used two different methods of bacterial leakage and dye penetration test, which was a strength point of this study.

The main limitations of this study included *in vitro* design, lack of occlusal forces, saliva, and different types of oral microorganisms, and lack of investigation and comparison of MTA and CC regarding cementum formation and periodontal tissue healing. To achieve clinical success, perforation repair materials should ideally induce the formation of new bone, PDL, and cementum. Since cementum is a biological barrier against the spread of microorganisms within the root canal system, it is desirable for cementogenesis to occur around perforation repair biomaterials [21]. Thus, this topic should be investigated in future studies. Also, *in vivo* studies are required to obtain more generalizable results.

Conclusion

Despite the study limitations, it can be concluded that both CC and MTA have acceptable sealing properties for furcal perforation repair in primary molars under *in vitro* conditions since they had no significant difference in dye penetration or bacterial leakage tests. Considering the characteristics of MTA, such as its long setting time, high cost, and difficult handling, CC may be used as a suitable alternative material for treatment of furcal perforations in primary teeth.

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Conflict of Interest

None to declare

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