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ORIGINAL ARTICLE

Repair of furcal perforation using a new endodontic cement

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Abstract The aim of this study was to compare the histologic response elicited by repairing furcal perforations with mineral trioxide aggregate (MTA) and a new endodontic material in the name of "calcium enriched mixture (CEM) cement" in dogs' teeth. Thirty-four premolars were randomly divided into four groups: MTA (n=15), CEM (n=15), positive, and negative controls (n=4). Root canal therapy were carried out; perforations were made, and the furcation areas were then repaired with MTA or CEM cement. The animals were sacrificed after 3 months. The teeth and their adjacent structures were processed and stained with hematoxylin and eosin stain for histological evaluation. Chi-square test was used to evaluate hard tissue formation, and Mann–Whitney U test was used for the histological evaluation of inflammation. Specimens in positive controls showed severe inflammatory infiltration, prominent granulation tissue, and epithelial proliferation; negative controls demonstrated normal periodontal ligament without inflammatory reactions. Hard tissue formation was observed in all the specimens of the two experimental groups. In inflammatory evaluation, mild inflammation was detected in the experimental groups, and no statistically significant differences were observed between them. MTA and CEM cement showed similar favorable biological response in furcation perforation repair, especially in inducing the formation of cementum-like hard tissue.

Keywords Perforation · Furcation defects · Endodontics · CEM cement · NEC · New material · MTA

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Introduction

Perforation in the floor of pulp chamber of multirooted teeth causes an inflammatory response in the periodontium, which can lead to irreversible loss of periodontal attachment in the area [1]. Some etiologic factors involved in pulp chamber perforations include large carious lesion, internal or external root resorption, and iatrogenic damages [2].

The involved teeth will have a poor prognosis if furcal perforations are not properly repaired [1]. Therefore, appropriate and prompt treatment of the involved teeth is necessary to retain such teeth. Two main techniques have been proposed for the repair of such defects [2]. Since surgical procedures for the repair of such defects may lead to pocket formation, nonsurgical methods—especially in inaccessible areas—are advocated [2, 3]. Ideally, perforations should immediately be repaired with a biocompatible material to quit the communication between perforation site

and gingival sulcus so that favorable prognosis could be achieved [2, 4]. Numerous materials have been used for furcal and root perforation repair [1–9], but none met all requisite qualifications for an ideal biomaterial, i.e., the ability to induce osteogenesis and cementogenesis, biocompatibility, nontoxicity, noncarcinogenicity, availability, easy handling, and reasonable price [7–9].

Mineral trioxide aggregate (MTA) was introduced for repair of lateral perforations in 1993 by Loma Linda University [10]. Since then, more than 600 articles have been published on the properties and applications of this water-based cement. MTA is derived from Portland cement (type I) and composed of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite [11]. This cement has a long setting time (~4 h) and an alkaline pH (~12.5) [12]. The color changes between gray and white MTA are due to the lower amount of iron in white MTA [13]. Interestingly, there is no significant difference between the dominant compounds in both white and gray MTAs and associated Portland cements except for bismuth oxide which is present in MTAs [14, 15].

MTA has been demonstrated to be biocompatible endodontic repair material. It has several clinical applications such as management of internal root resorption, immature apices (apexogenesis/apexification), pulp capping, pulpotomy, root-end filling and repair of root, and furcation perforations [16]. MTA promotes dental tissue regeneration in contact with pulp [17] or periradicular tissue [4, 8, 9, 18, 19]. Biocompatible nature of MTA suggested by its ability to form hydroxyapatite when exposed to simulated body tissue fluid [20, 21]. With some exceptions, MTA presents better microleakage protection than conventional endodontic repair materials using various methods [22]. MTA is not affected by tissue fluid or blood contamination [23], has low cytotoxicity [24], has antibacterial effects [25], and capable of promoting cementogenesis when used as root-end fillings [26].

In several in vitro or in vivo studies, MTA has proved to be superior than most of the endodontic materials (i.e., amalgam, Super-EBA, and Sealapex), when used for repairing furcal perforations [4, 9, 10]. Researcher also found some new cementum formation adjacent to MTA [4, 6, 9]. No significant differences have been reported between microleakage of white and gray MTA for furcal perforation repair [27, 28]. These features made MTA as a new gold standard for perforation repair because of its predictable periodontal ligament (PDL) regeneration [4, 6, 9].

However, extended setting time [12], poor handling [29], and relatively high price are some of its disadvantages.

Recently, a novel endodontic cement in the name of calcium-enriched mixture (CEM) cement (patent pending as endodontic filling material) has been introduced to endodontics [30, 31]. Major components of CEM cement powder are 51.75 wt.% CaO, 9.53 wt.% SO3, 8.49 wt.% P2O5, and 6.32 wt.% SiO2, and minor components are Al2O3>Na2O>MgO>Cl as essential constituents [31], which provides a bioactive calcium and phosphate-enriched material when being mixed with a water-based solution (compliant with the ISO 6876 standard for dental root canal sealing materials) [15, 31]. Results of recent studies indicate that mixed CEM cement releases calcium and phosphate ions [32] and then forms hydroxyapatite not only in simulated body tissue fluid but also in normal saline solution; the latter is unlike the MTA [21]. This material has similar pH, increased flow, but decreased working time, film thickness, and estimated price than MTA [31].

However, the chemical compositions of CEM cement are different but the clinical uses are identical to MTA [31]. CEM cement has demonstrated similar or even better results than MTA when used as pulp-capping agent [30, 33] or root-end filling material [34, 35]. It has also shown favorable results in pulpotomy of permanent molars with established irreversible pulpitis and management of internal root resorption [36]. This cement has antibacterial effects better than MTA and comparable with calcium hydroxide [37]; it has also low cytotoxic effect on different cell lines similar to MTA [30, 38]. Although several studies have used MTA for furcation perforation repair [4, 6, 9, 10], no such studies have been performed for CEM cement.

The aim of the present in vivo study was to evaluate the histologic tissue responses induced by MTA and CEM cement used for immediate furcal perforation repair in dogs' teeth.

Materials and methods

Thirty-four premolar teeth in four mature male dogs were used for the purpose of this animal study. The study protocol was according to "Principles of Laboratory Animal Care" and approved by the Shahid Beheshti University of Medical Sciences Animal Ethics Committee. The teeth were randomly divided into two experimental groups of MTA and CEM cement of 15 each and two positive (perforation without repair) and negative control (without perforation) groups of two each. Doses of 2% Bochringer Acepromazine (Ingelhim Vetmedia, Inc, St Joseph, Mo 64506, USA) were injected intramuscularly (0.1 mg/kg) for sedation. After 10 min, 10% Ketamine HCl (Hospital Products Division, Illinois, USA) was injected intravenously (25 mg/kg) for general anesthesia. The teeth and their periodontium were examined, and 0.2% chlorhexidine gluconate (Shahrdaru, Tehran, Iran) was used for oral disinfection. A dose of 2% Lidocaine containing 1:80,000 epinephrine (Daroupakhsh, Tehran, Iran) was used for anesthesia of the teeth.

Access cavities were prepared in occlusal surfaces using a no. 4 diamond fissure bur (D&Z, Wiesbaden, Germany) at a high-speed handpiece under copious water spray. The root canals were prepared using step-back technique while being irrigated with 1% sodium hypochlorite; the root canals were then filled with laterally condensed gutta-percha and AH-26 sealer (Dentsply Tulsa Dental, Switzerland).Then, 1-mm-diameter perforations were created in the furcal areas of the teeth using diamond fissure burs (D&Z, Wiesbaden, Germany). After rinsing with normal saline and providing hemostasis of the perforation sites, MTA (Pro-Root MTA, Dentsply Tulsa Dental, OK, USA) and CEM cement were prepared according to their instructions. Both materials were placed into the perforation defects and compacted with moist cotton pellets. Subsequently, the access cavities were sealed using self-curing glass ionomer (ChemFil, Superior DeTrey, Dentsply, and Konstanz, Germany).

After 3 months, the animals were sacrificed and vital perfused with 10% buffered formalin. Bone blocks with teeth included were obtained and placed in 10% formalin for 2 weeks. Then, the specimens were rinsed for 10 min and placed in 10% formic acid for decalcification at room temperature. After complete decalcification, the specimens were dehydrated in ascending concentrations of alcohol (70%, 90%, and 100%) and were then embedded in paraffin; then, 6-μm-thick serial sections were subjected to hematoxylin and eosin staining (H&E). The specimens were evaluated by an experienced oral pathologist in a blind manner under an optical microscope (Zeiss, Goettingen, Germany).

In the histologic evaluation, hard tissue formation was confirmed or rejected based on the presence/absence and continuity/discontinuity of a hard tissue bridge at the perforation site. In addition, the presence or absence of epithelium and the infiltration of inflammatory cells in the fornix were evaluated.

The parameter related to the severity of inflammatory cell infiltration was evaluated in the two following categories:

1. In integrity of the hard tissue bridge, only furcal area was evaluated;
2. In the absence of integrity of the hard tissue bridge, the inflammatory process, which had been spread beneath the test material, was also evaluated and measured.

Evaluation of the inflammatory process was carried out by counting the inflammatory cells (lymphocytes, plasma cells, and macrophages) at the center area of the inflamed tissue with ×400 of magnification. The inflammatory process was graded based on the counted cells according to studies carried out by Noetzel et al. [18] and Panzarini et al. [39]. The means of the inflammation grades were calculated in the furcal areas of all the specimens and beneath the material in the specimens in which no hard tissue integrity was observed. The inflammatory grades were as follows:

Grade 0: no inflammatory cells
Grade 1: inflammatory cells<25
Grade 2: inflammatory cells=25–50
Grade 3: inflammatory cells=51–75
Grade 4: inflammatory cells>75

The data were analyzed using chi-square and Mann–Whitney *U* tests. The significance level was set at 0.05.

Results

The results of histologic evaluation demonstrated that in the sections prepared from the positive controls (perforation without repair), there was severe infiltration of inflammatory cells with a mean grade of 4, and a prominent granulation tissue was observed between the two edges of perforation. Widespread epithelial proliferation was found in these sections. On the contrary, in the sections prepared from the negative control group (intact teeth), no inflammatory cell infiltration was observed, and a normal PDL structure with fibroblasts and collagen fibers was observed in all areas.

Evaluation of the sections related to two experimental groups revealed hard tissue bridges in all the specimens between the two edges of perforation and beneath the experimental materials. None of these specimens demonstrated epithelial infiltration in the furcation area or adjacent to the materials (Figs. 1 and 2). Therefore, no differences were observed between MTA and CEM cement in this

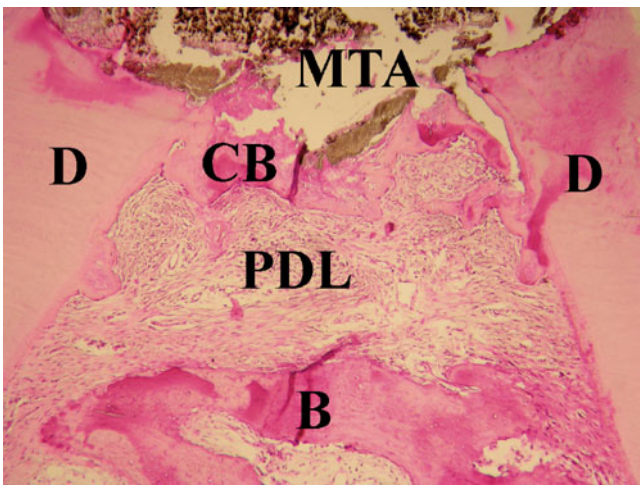

Fig. 1 Repair of furcal perforation with mineral trioxide aggregate (*MTA*). *D* dentin, *B* bone, *PDL* periodontal ligament, *CB* calcified bridge, H&E (magnification ×150)

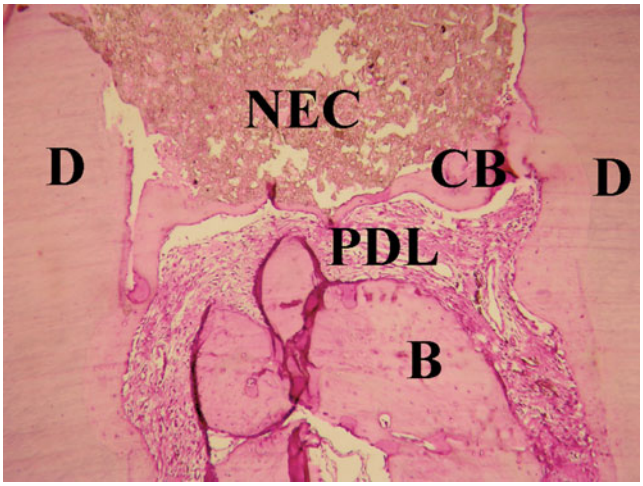

Fig. 2 Repair of furcal perforation with a new endodontic material (CEM cement). *D* dentin, *B* bone, *PDL* periodontal ligament, *CB* calcified bridge, H&E (magnification ×150)

regard, and no statistical analysis was deemed necessary to compare the two groups in this respect. Evaluation of the hard tissue integrity revealed that, in the MTA group, eight specimens had bridge integrity, while, in the CEM cement group, bridge integrity was observed in six specimens. Chi-square test was used to compare the two groups, and it was demonstrated that the differences in hard tissue bridge integrity between the two groups were not statistically significant (P=0.38).

Evaluation of the inflammatory process in the furcation area in the specimens revealed that the mean inflammation grades in the MTA and CEM cement groups were 1.83 and 1.79, respectively, in the four-grade system. In addition, means of inflammation grades beneath MTA (seven specimens) and beneath CEM cement (nine specimens) were 2.45 and 2.40, respectively, in the four-grade system. Mann–Whitney U test was used to compare means of inflammation grades since the data related to inflammation beneath the experimental materials were not normally distributed; the results did not demonstrate any statistically significant differences in the severity of inflammation in the furcation areas (P=0.89) and beneath the materials (P=0.91) between the two experimental groups.

Discussion

Dogs are demanding experimental models, having two-rooted lower premolars that often bifurcate as close as 1 to 2 mm from the cementoenamel junction (CEJ) [3, 9]. As a result, epithelialization and the formation of connective tissue at a furcation perforation are more likely than in humans, where the furcation lies deeper within the alveolus [3, 8, 9]. Thus, any technique shown to produce favorable results in dogs may have a more favorable response in humans, where the distance from the CEJ to the furcation area is greater [3, 8, 18].

In this animal study, MTA was used as a gold standard since it is a suitable sealant and highly biocompatible. Gray ProRoot MTA has proved to have less leakage and has produced a better response compared to other materials in a number of studies [8, 10, 18], prompting its use in the present study as a highly effective perforation repair material to compare its properties with CEM cement. Considering the ideal results obtained from the present study in relation to gray ProRoot MTA and the results of a study carried out by Noetzel et al. [18], the application of this material is recommended in areas in which esthetics is not of prime importance, such as furcation areas and the roots of posterior teeth.

Complete and incomplete hard tissue bridge formation in response to MTA has been reported in various studies [4, 8, 9, 18, 19]. Since CEM cement has been reported to have effects similar to MTA [21, 24, 30, 32–35, 37, 38], it was used in the present study to be meticulously compared to MTA regarding the formation of the hard tissue bridge and the continuity or discontinuity of the hard tissue bridges formed. According to the results of the present study, hard tissue bridge formation was observed between the two edges of perforations in all the specimens. In contrast, hard tissue bridge has not been reported to form in all the MTA specimens in similar studies [8–10, 18]. In addition, these studies showed that hard tissue bridge has not been reported to be formed in response to other experimental materials such as amalgam, Dycal, resin-modified glass ionomer (RMGI), Super-EBA, and tricalcium phosphate (TCP). Continuity and discontinuity of the hard tissue bridge have only been evaluated in the present study; eight of MTA specimens and six specimens of CEM cement group demonstrated complete bridge formations which were not statistically different. The results of the present study demonstrated that cementogenic properties of MTA are much better than it has been demonstrated in previous studies [8–10, 18].

The biological mechanism by which CEM cement stimulates hard tissue formation is now unclear. This quality is hypothesis to be the result of several properties, i.e., sealing ability [34, 35], biocompatibility [24, 30, 33, 36, 38], high alkalinity [31], antibacterial effect [37, 40], hydroxyapatite formation [21], and similarity to dentine [15]. Moreover, it is well accepted that the handling properties of clinically applied cements are of practical importance. Hydrophobic cements need a completely dry cavity, free of blood/saliva; in contrast, CEM cement is hydrophilic [15, 31], so that is ideal as a perforation repair material.

Regarding epithelial proliferation, no epithelial cell infiltration was observed in the experimental and negative control groups; however, severe infiltration of epithelial cells along with granulation tissue was observed in the positive control specimens. This finding is consistent with the results of studies comparing the effect of MTA with similar materials such as amalgam, Dycal, RMGI, Super-EBA, and TCP [3, 6, 8, 9, 18, 41, 42].

Researchers reported the lowest incidence of epithelium migration with MTA as an immediate perforation repair material [6, 43]. Deposition of hard tissue was observed over MTA in the majority of specimens. Furthermore, new cementum was attached to the original cementum on the root surface. These findings support the results of the present study.

Evaluation of induced inflammation of experimental materials revealed that the specimens demonstrated mild inflammatory reaction. The mean inflammation grades for MTA and CEM cement groups were 1.83 and 1.79, respectively, in the 0–4 grading system. This finding is consistent with the results of other studies in which inflammation severity in response to MTA in comparison to other experimental materials has been reported to range from mild to moderate [4, 8, 9, 18, 44].

Inflammation severity scores in the case of direct contact with experimental materials in the specimens in which the hard tissue bridge was interrupted were 2.45 in the MTA group and 2.40 in the CEM cement group in the 0–4 grading system. Statistical analysis did not demonstrate any significant differences in inflammation severity between the experimental groups, both in the furcation area and beneath the materials. According to the results of this study, both MTA and CEM cement yield acceptable results in the repair of furcal perforation in dogs' teeth. It seems that better results can be obtained in human teeth since furcation areas are more deeply located in the alveolar bone; similar results are expected in root perforations. Long-term evaluations of this material (CEM cement) are recommended before it is used for perforation repair in human teeth.

Conclusion

Based on the findings of the present in vivo study, both MTA and CEM cement, when immediately used for furcal perforation repair, induce mild inflammatory reactions and can prompt defect regeneration through hard tissue bridge formation.

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Conflict of interest The authors declare that they have no conflict of interests.

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