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Current perspectives of bio-ceramic technology in endodontics: calcium enriched mixture cement - review of its composition, properties and applications

Shivani Utneja[1]*, Ruchika Roongta Nawal[1], Sangeeta Talwar[1], Mahesh Verma[2]

[1]Department of Conservative Dentistry and Endodontics, [2]Department of Prosthodontics, Maulana Azad Institute of Dental Sciences, New Delhi, India

Advancements in bio-ceramic technology has revolutionised endodontic material science by enhancing the treatment outcome for patients. This class of dental materials conciliates excellent biocompatibility with high osseoconductivity that render them ideal for endodontic care. Few recently introduced bio-ceramic materials have shown considerable clinical success over their early generations in terms of good handling characteristics. Calcium enriched mixture (CEM) cement, Endosequence sealer, and root repair materials, Biodentine and BioAggregate are the new classes of bio-ceramic materials. The aim of this literature review is to present investigations regarding properties and applications of CEM cement in endodontics. A review of the existing literature was performed by using electronic and hand searching methods for CEM cement from January 2006 to December 2013. CEM cement has a different chemical composition from that of mineral trioxide aggregate (MTA) but has similar clinical applications. It combines the biocompatibility of MTA with more efficient characteristics, such as significantly shorter setting time, good handling characteristics, no staining of tooth and effective seal against bacterial leakage. (*Restor Dent Endod* 2015;40(1):1-13)

Key words: Biological and physical properties; Calcium enriched mixture cement; Clinical applications; Composition; Leakage; Mechanism of action

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[1]Utneja S; Nawal RR; Talwar S, Department of Conservative Dentistry and Endodontics, Maulana Azad Institute of Dental Sciences, New Delhi, India
[2]Verma M, Department of Prosthodontics, Maulana Azad Institute of Dental Sciences, New Delhi, India
***Correspondence to**
Shivani Utneja, MDS.
Senior Research Associate, Department of Conservative Dentistry and Endodontics, Maulana Azad Institute of Dental Sciences, Bahadur Shah Zafar Marg, New Delhi, India 110002
TEL, +91-9711459961; FAX, +91-1-23217081; E-mail, shivaniutneja81@yahoo.co.in

Introduction

In the recent past we have witnessed significant changes in endodontic material science. Bio-ceramic materials have been seen as the dawn of a new era in dentistry. Although used mainly for dental implants and coatings for implants, their introduction into endodontics as mineralising materials has brought about enormous productive changes. The applications vary from their use for Pulp Capping, to apexogenesis, apexification, and furcation repair.[1] Bio-ceramics are biocompatible ceramic materials applicable for use in medicine and dentistry. They include alumina and zirconia, bioactive glass, glass ceramics, calcium silicates, hydroxyapatite and resorbable calcium phosphates, and radiotherapy glasses.[2]

The unique capabilities of bio-ceramics make them an attractive option for orthopaedic applications (such as joint or tissue replacements), for coatings to improve the biocompatibility of metal implants, and can function as resorbable lattices which provide a framework that is eventually dissolved as the body rebuilds tissue.[3]

Bio-ceramics can be classified as:

- Bioinert: non-interactive with biological systems
- Bioactive: durable tissues that can undergo interfacial interactions with surrounding tissue
- Biodegradable, soluble or resorbable: eventually replaced or incorporated into tissue.[4]

The physical properties associated with bio-ceramics are very attractive to dentistry. Absolute biocompatibility, osseoconductivity, ability to achieve excellent hermetic seal, formation of chemical bond with the tooth structure, insolubility in tissue fluids, good radiopacity and easy handling characteristics have lead to the widespread use of these materials in the area of endodontic science.[1] There are numerous bio-ceramics currently in use in endodontics.

Hydroxyapatite (HA) has been used successfully in clinical and animal studies for endodontic treatments including pulp capping, repair of mechanical bifurcation perforation, apical barrier formation, and repair of peri-apical defects.[5-7] It is also employed as a scaffold in regenerative endodontics.[8]

Calcium phosphate is another biocompatible material useful for inducing hard tissue formation, pulp capping, apical barrier formation, and apexification and as regenerative scaffold.[8-12] Calcium phosphate based sealers have been found to be less cytotoxic than AH26 and Zinc Oxide Eugenol (ZOE) sealers and have the potential to promote bone regeneration.[13]

Bioglass is a new bioactive material, and has been recently developed. It was reported to be able to produce reparative dentin formation with no evidence of tissue necrosis, significantly better than that produced by calcium hydroxide.[14] It also has the potential to induce root formation through apexification procedure. The reparative activity of Bioglass in apical closure and periapical bone formation was found to be superior to that of Tricalcium Phosphate.[15]

Glass-Ionomer cements (GIC) have a variety of applications in endodontics.[16] Use of these materials as a temporary restoration during endodontic therapy has been investigated in a number of studies with favourable results.[17] Since GICs show low shrinkage on setting and possess the virtually unique ability to bond directly to dentine and enamel, these materials make good root-canal sealers. In a confocal microscopic study, the adaptation and sealing ability of light cured glass ionomer as a retrograde root filling material was found to be better than those of amalgam and conventional GIC.[18] Other applications of GIC in endodontics include luting of posts and pulp capping.[19,20]

Mineral trioxide aggregate (MTA) is a biomaterial that has been investigated for endodontic applications since the early 1990s. It is a promising material for root-end filling, perforation repair, vital pulp therapy and apical barrier formation for teeth with necrotic pulps and open apices. MTA has also been successfully used for the treatment of internal and external resorptions, horizontal root fractures, sealing communications between the root canal space and external root surfaces, filling root canals of teeth with mature and open apices, as well as management of dens invaginatus.[21] Recently MTA based sealers have opened up the horizon for root canal sealers. Sealers based on MTA have been reported to be biocompatible, stimulate mineralization and encourage apatite-like crystalline deposits along the apical- and middle-thirds of canal walls.[22,23]

More recently, calcium enriched mixture (CEM) cement, Biodentin, Bioaggregate, and EndoSequence Root Repair Material (ERRM) and EndoSequence BC Sealer have been introduced to the market. So far, none of the articles published present a comprehensive review of these newer bio-ceramic materials with endodontic applications. This paper is an attempt to bring to light the uses of newly introduced bio-ceramic materials in endodontics. The aim of this literature review is to summarize brief history, composition, mode of action, properties and clinical applications of CEM cement in experimental animals and humans.

Review

Search methodology

A review of the literature from peer reviewed journals published in English was performed by using electronic and hand-searching methods for the bio-ceramic materials in endodontics until December 2013. Appropriate MeSH headings and key words related to different aspects of CEM cement in endodontics were searched in PubMed database from January 2006 to December 2013. A hand-search was conducted of the last 2 years' worth of issues of the following major endodontic journals, International Endodontic Journal; Journal of Endodontics; Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology. The process of cross-referencing continued until no new articles were identified. 62 relevant articles on CEM cement were identified after search which formed the basis of this review.

Calcium enriched mixture cement

1. Overview

A novel endodontic cement named calcium-enriched mixture (CEM) cement was introduced to dentistry in 2006 as an endodontic filling material.[24] The physical properties of this biomaterial, such as flow, film thickness, and primary setting time are favorable.[25] It has the ability to promote hydroxyapatite formation in saline solution and

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might promote the process of differentiation in stem cells and induce hard tissue formation.[26-28] It also possesses ability to set in aqueous environments with shorter setting time than MTA and sealing ability comparable to MTA.[25,29]

The clinical uses of the CEM cement are similar to MTA. CEM cement has demonstrated similar results to MTA when used as pulp capping agent or furcation perforation repair.[30,31] It has also shown favorable results in pulpotomy of permanent molar teeth with established irreversible pulpitis, and in management of internal root resorption.[32,33] Furthermore, this material has an antibacterial effect comparable to calcium hydroxide and better than MTA or Portland cement (PC).[34]

2. Composition and mechanism of action

CEM cement is composed of different calcium compounds. The major components of the powder are CaO (51.75%), SO_3 (9.53%), P_2O_5 (8.49%), SiO_2 (6.32%) and minor components are Al_2O_3 > Na_2O > MgO > Cl.[26] The important constituents of CEM are alkaline earth metal oxides and hydroxides (for example, calcium oxide and calcium hydroxide [CH]), calcium phosphate, and calcium silicate.[25] CEM differs chemically from MTAs and PCs, phosphorous is the major component of CEM, whereas this element is close to the detection limit in MTAs and PCs.[35] In contrast with MTA, CEM shows surface composition similar to surrounding dentin. Since HA is the main component of dentin, similarity between CEM cement and dentin might help the cementogenesis over it.[26]

When mixed with water-based solution, bioactive calcium and phosphate enriched materials are formed, which is compliant with the International Standard Organization (ISO) 6876 standard for dental root canal sealing materials. During and after mixing with its liquid, hydration reactions take place, producing CH. This production is mostly because of the reactions involving calcium silicates, calcium phosphate, and calcium oxide in addition to the presence of CH. CH dissociates into calcium and hydroxyl ions, increasing the pH and calcium concentration.[25] Additionally, this novel cement releases calcium and phosphorus ions from indigenous sources that result in a rich pool of OH^-, Ca^{2+}, and $PO4^-$ ions. These elements are used in the process of HA production.[36] Studies have shown HA formation not only in simulated body tissue fluid, but also in normal saline solution.[26]

3. Properties of calcium enriched mixture

1) Physical properties

The physical properties of CEM were found to be acceptable and met the ISO 6876:2001 standard.[25] CEM showed slight expansion (0.075 ± 0.032 mm) on setting which was not significantly different from MTA (0.085 ± 0.042 mm). The material also exhibited reasonable film thickness (174 ± 25 μm) and flow (14 ± 1 mm), which were statistically different from MTA (452 ± 63 μm and 10 ± 0.79 mm, respectively). The slight expansion and reasonable flow and film thickness of CEM can ensure an effective seal after setting, and reduce the subsequent leakage. The setting time of CEM was found to be less than an hour (50 minutes), and shows alkaline pH of 10.71 ± 0.19. This novel endodontic cement appeared to fulfil the physical requirements of a root-end filling material from the point of consistency, workability, adaptability, and setting time.[25]

2) Biological properties

(1) Antibacterial effects

① Antibacterial and antifungal properties of CEM

Numerous studies have examined the antimicrobial activity of various materials used in endodontics. CH is well known for its wide range of antimicrobial activity against common endodontic pathogens, but is less effective against *Enterococcus faecalis* (*E. faecalis*) and *Candida albicans* (*C. albicans*).[37] MTA has been examined as a potential antibacterial material since 1995. However, several investigations reported that it has limited antimicrobial effect against some microorganisms.[38,39]

CEM cement introduced in 2006 has demonstrated antibacterial effect comparable to CH and better than MTA or PC. In an agar diffusion test on CEM, MTA, and CH against *Pseudomonas aeruginosa*, *E. faecalis, Staphylococcus aureus* and *Escherichia coli*, both CEM and CH caused greater growth inhibited zones of tested bacteria than MTA. The favoured results of CEM cement and CH in comparison with MTA indicated the potentiality of CEM cement usage as antibacterial agent.[40] Another similar investigation compared the antimicrobial activities of CH, gray MTA (GMTA), white MTA (WMTA), PC and CEM on the same species of microorganisms used by Asgary *et al.*[34] Highest growth inhibited zone diameters were observed around CEM and CH. There was a significant difference between CH and CEM in comparison with MTA and PC groups.[34]

Zarrabi *et al.* compared the antibacterial and antifungal effects of CEM, MTA, and PC on some selected oral microorganisms and found that the antimicrobial action of CEM on all the microorganisms tested was superior to that of MTA and PC.[41] The authors suggested that CEM contains more potent antibacterial inhibitors than MTA. Alkaline earth metal oxide and hydroxides (for example, calcium oxide and CH), calcium phosphate, and calcium silicate are important constituents of CEM. When CEM is transferred to agar plates and makes contact with medium, $Ca(OH)_2$ dissociates into calcium and hydroxyl ions which increases the pH and calcium concentration. These mechanisms may partly explain the more favourable antibacterial activity of

this material. An alternative explanation is also put forth stating that the antimicrobial components of CEM have better diffusion properties than those of MTA and PC.[41]

One experiment showed that freshly mixed and set CEM cement and ProRoot MTA were effective in killing *C. albicans* at 24 and 48 hours observations. CEM cement in concentration of 50 mg/mL was found to have an effective antifungal activity, comparable to MTA.[42] A recent study evaluated the effect(s) of dentin powder on antibacterial properties of CEM cement against *E. faecalis* in an aqueous solution before and after setting, in comparison with MTA.[43] In contrast to previous studies, the results of this study revealed similar antibacterial property of CEM to MTA, and showed that their antibacterial properties increased in presence of dentin. Freshly mixed powder from set materials, and blocks of uncrushed set of both cements killed > 95% of the bacteria in 1 minunte duration in presence of dentin. It was assumed to be due to increased silica dissociation. The greatest proportion in MTA is calcium oxide followed by silica (21.20%) which is also contained in CEM (6.32%). The increased antibacterial activity might also be the result of the osmolarity changes obtained from dissolution of various mixtures of CEM and MTA, and the complex ionic flow which takes place in the interface between cements and dentin particles.[43]

The literature shows that CEM has an antibacterial and antifungal effect. The antimicrobial effect of CEM is enhanced with incubation time and with increase in cement concentration. However, ineffectiveness of CEM has been reported against *E. Faecalis*.[41]

(2) Biocompatibility

Biocompatibility and non-toxicity are important qualities of endodontic materials, especially when used for pulp capping, perforation repair, coronal obturation as well as for root-end filling. Biocompatibility of materials is evaluated by various techniques, including *ex vivo* cytotoxicity and *in vivo* subcutaneous or intraosseous implantation procedures.[44] The biocompatibility of CEM has been associated with its ability to release calcium ions during setting, and the subsequent binding of calcium with phosphorus to form hydroxyapatite crystals. This new biomaterial is more likely to cause alterations in cellular enzymic activity than to change permeability, which facilitates healing.[25,35]

① Cell cultures

Several investigations using different cell culture systems have shown that CEM has low cytotoxicity. A study on L929 mouse fibroblasts compared the cytotoxicity of different dilutions (Neat, 1/2, 1/10, 1/100) of fresh and set CEM and MTA using optical microscopy and methyl-tetrazolium bromide (MTT) assay in three time intervals (24, 48, and 72 hours after mixing). The results indicated that there were no significant difference in cytotoxicity among the test materials, and between them and the control group. However, there was statistically significant difference between different time intervals within each group, and between different concentrations of test materials. In all samples, set materials showed better viability than fresh ones, and cytotoxicity of fresh CEM equalled fresh MTA.[45]

Another recent study compared the cytotoxicity of CEM with IRM and MTA. The materials were tested in fresh and set states on L929 fibroblasts with MTT assay and enzyme-linked immunosorbent assay (ELISA) reader at 1, 24, and 168 hours (7 days).[46] CEM cement demonstrated favourable cell viability values when set in all three time intervals. Fresh CEM also demonstrated good cell viability values, though lower than MTA. In this study, both set and fresh MTA and CEM had greater cytotoxicity at 7 days compared with 1 hour, possibly because of the calcium hydroxide produced as a by-product of their hydration reaction.[25,47] The gradual release of hydroxyl ion may decrease cell viability *ex vivo*. However this may be neutralised by the body tissue fluid under *in vivo* conditions. The results of an *ex vivo* study assessing the adhesion of human gingival fibroblast (HGF) to MTA and CEM cement using a scanning electronic microscope (SEM) found no statistical differences between these two experimental groups. HGF cells displayed favourable biological response in contact with MTA and CEM.[48]

② Subcutaneous and intra-osseous implantation

Histological evaluation of skin reactivity of rabbits to MTA and CEM showed that the highest inflammation was observed in MTA, followed by CEM and control groups. The results demonstrated that the biocompatibility of CEM cement is higher than MTA.[49] A recent study comparing the subcutaneous tissue response to CEM and MTA in rats showed that unlike MTA, CEM did not induce any cellular necrosis after one week. After 60 days, levels of inflammation in the CEM group were significantly lower than the white/gray MTA groups. Another significant finding was the presence of dystrophic calcification adjacent to the biomaterials, which is an indication of their osteo-inductive potential.[50] A study evaluated the bone tissue reaction of rat femur to CEM and compared it with MTA. The severity of inflammatory processes and the extent of bone formation adjacent to the biomaterials were evaluated at intervals of 1, 4, and 8 weeks. The results indicated that both the biomaterials initially elicited severe inflammatory reaction, which subsided by the end of the eighth week. The higher inflammation grades in the first week might be attributed to high pH value, production of heat during the setting reaction, and the release of IL-1 and IL-6. New bone formation had increased around the experimental groups, and at the end of the eighth week complete coverage of the material surfaces with bone or

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the formation of an osseous bridge around the materials was observed. This process might be attributed to calcium-containing components in both MTA and CEM cements.[51]

③ Neurologic effects

The electrophysiological effects of WMTA and CEM on F_1 neuronal excitability in a garden snail *Helix aspersa* were assessed using intracellular recording techniques.[52] Both WMTA and CEM reduced the cell excitability and altered the action potential characteristics suggesting the possible involvement of Ca^{2+} release from the applied dental materials, although WMTA was more effective than CEM. The increase in the after hyperpolarization amplitude (AHP) and decrease in neuronal excitability was also speculated to be due to the extracellular alkaline shift caused by both WMTA and CEM, which in turn modulated voltage-gated Ca^{2+} channels function. These properties suggest the possible analgesic and regenerative effects of both biomaterials.[52]

④ Periradicular tissue reactions

Studies on CEM cement reveal that this material is capable of inducing hard tissue formation, in particular, cementogenesis. In an *in vivo* investigation on dog's teeth, histological evaluation demonstrated that CEM cement and MTA have similar favourable biological effects in furcation perforation repair cases, especially in inducing the formation of cementum-like hard tissue bridges.[31] The biological mechanism by which CEM cement stimulates hard tissue formation is thought to be the result of several properties, i.e., sealing ability, biocompatibility, high alkalinity, antibacterial effect, hydroxyapatite formation, and similarity to dentine.[24-26,29,30,32,34,35,40,45,48,53]

A randomized controlled animal study demonstrated that both CEM cement and MTA induced periradicular tissue healing regeneration, including the production of cementum and new bone, when used as root-end filling biomaterials. CEM cement has the ability to promote cementogenesis over both the root-end dentinal surface and the material. A remarkable feature was that the newly formed eosinophilic cementum containing entrapped cementocytes and periodontal ligament (PDL) fibers insertions.[27] A case of furcal perforation in a mandibular first molar accompanied by furcal lesion was managed with CEM. Regeneration of the PDL around the site of injury and complete resolution of furcal lesion at two-year follow up was noted.[54] A prospective outcome study of periradicular surgery using CEM revealed complete healing of periradicular lesions, *i.e.* regeneration of PDL and lamina dura in 13 out of 14 permanent teeth during a mean time of 18 months.[55]

⑤ Pulpal reactions

Several animal studies have shown that in various forms of vital pulp therapy (VPT), the induction of dentin bridge formation in CEM was comparable with that in MTA, and superior to that in CH.[30,56] Studies of complete pulpotomy treatment using CEM, MTA, and CH have shown that samples in the CEM group exhibited lower inflammation, improved quality/thickness of calcified bridge, superior pulp vitality status and morphology of odontoblast cells, compared to CH. However, no significant differences were identified in comparison to MTA.[56]

A few case reports and a randomized clinical trial study of permanent molars with open apices that were treated by pulpotomy using CEM have shown dentin bridge formation beneath CEM and closure of the tooth apex.[57-59] Direct Pulp Capping (DPC) treatment of human deciduous and permanent teeth with CEM exhibited similar and acceptable outcomes compared to MTA.[60-62] And in one of the prospective randomized controlled trials, thickness of dentinal bridge beneath CEM was higher than MTA.[63] Pulp inflammation was also lower in the CEM groups.[63] Indirect Pulp Capping (IPC) treatment with CEM has also demonstrated favourable clinical and radiographic outcomes.[64] In addition, expression of fibronectin/tenascin in the CEM groups were higher than the MTA groups during both time intervals (2 and 8 weeks) although the differences were not statistically significant. This is suggestive of its role as a suitable biomaterial for DPC.[62]

(3) Microleakage studies

Penetration of microorganisms and their by-products into filled root canal systems causes failure in root canal treatments. Therefore, a repair material should provide a good seal to an otherwise unobturated root canal or improve the seal of existing filling material. An adequate apical seal is one of the major factors for improving endodontic success.[65] Microleakage is a well established indicator that assesses sealing ability of root-end filling materials. Different methods may be used to measure microleakage. Methods such as fluid filtration and dye extraction techniques are more reproducible when compared to SEM and capillary-flow porometry.[66-69] Leakage investigations on CEM have evaluated the sealing ability of the material as root-end filling material, root canal filling, perforation repair and coronal barrier material.

① Leakage of CEM as a root-end and root canal filling material

The sealing ability of CEM and other root-end filling material has been tested using dye, fluid filtration, and bacterial leakage methods.

i) Dye leakage

Methylene blue and Indian ink dye have been used to evaluate CEM's sealing ability. Results from these investigations indicated that CEM exhibits similar sealing

ability compared to other commonly used Root-end filling materials such as MTA.[29,70-72] CEM cement has shown lower mean dye leakage than commercial types of MTA and IRM in dry root-end preparations.[24] Another study investigated the sealing ability of CEM as a root end filling material in comparison to MTA and IRM by using the methylene blue dye penetration method. The results showed no statistical difference in apical seal between CEM and MTA, considering that the lowest mean apical microleakage value was obtained for CEM. Good sealing property of CEM cement was hypothesized to be due to its handling characteristics and chemical properties.[29] However, dye leakage studies are known to have certain disadvantages such as dissolution during demineralization and clearing process. In addition, its molecule size, pH, and chemical reactivity affect the degree of its penetration and hence the dye molecule is not considered to be a reliable parameter due to its small size.

When the apical sealing ability of CEM cement was compared to MTA in the various conditions (dry, saliva or blood-contaminated root-end cavities), it was shown to have lower mean dye penetration than MTA even when contaminated with saliva.[70] The seal, however, was found similar to MTA in dry and blood contaminated environment. The excellent seal of CEM cement, particularly in saliva contaminated environment, was thought to be due to several physical and chemical characteristics of this novel material.[70] First, CEM cement provides good handling characteristics. Once mixed, this cement does not adhere to the applicator and is easily adaptable. Second, saliva increases the wetting of the dentinal walls, enabling adaptation of CEM cement within irregularities of root canal walls, and also facilitates its penetration into the dentinal tubules. Slight setting expansion of CEM cement also contributes to much better adaptation of this material to the root-end cavity walls.[25] High percentage of small particles (0.5 - 2.5 μm) in this material supports this cement's access to dentinal tubules with inner diameter range of 2 - 5 μm.[73] Furthermore, in the presence of an aqueous environment, this biomaterial produces large amount of hydroxyl, calcium, and phosphate ions which leads to HA formation and thus provides an additional seal at the interface of the material and cavity walls.[26]

Milani *et al.* compared the sealing abilities of resected roots filled with MTA or CEM cement.[71] CEM cement showed less microleakage compared with MTA in the resected or retrofilled state although the differences were not statistically significant. This study revealed that MTA and CEM had similar sealing abilities after resection, and resection increased the microleakage of CEM cement. Therefore, if limited access and isolation impede retrofill placement, both materials can be used to fill the canal prior to root-end resection.[71]

ii) Bacterial leakage studies

Kazem *et al.* compared the apical sealing of WMTA, GMTA, PC, and CEM by dye and bacterial leakage methods and found comparable microleakage of CEM cement with other test materials.[72] E. faecalis and methylene blue dye were used for determination of bacterial and dye leakage respectively. Poor agreement was obtained between the two test methods.[72] Another investigation using bacterial leakage method suggested that apical sealing ability of orthograde MTA and CEM plugs after root-end resection did not differ from the conventional MTA retrofillings.[74] Although the results of these studies indicate comparable results of CEM with MTA, more data is required comparing sealing ability of different thicknesses of CEM as apical plug.

iii) Fluid filtration

An investigation evaluated the microleakage of CEM cement in two different media, including phosphate buffer solution (PBS) and distilled water. Sealing ability of CEM cement was found significantly superior in PBS compared to distilled water. This was attributed to the promotion of mineralization and hydroxyapatite formation that CEM cement induces by the presence of exogenous sources of phosphorous provided by PBS.[75]

② Leakage of CEM cement as furcal perforation repair material

A dye leakage model compared the sealing ability of CEM cement and MTA in repair of furcal perforation of primary molars. The results did not reveal any statistically significant difference in dye penetration between MTA and CEM.[76]

③ Leakage of CEM cement as intra-orifice sealing material

Yavari *et al.* compared the coronal microleakage of four dental materials (CEM cement, MTA, amalgam, and composite resin) using polymicrobial analysis. The results indicated that CEM cement's potential as an intra-orifice barrier against bacterial penetration is comparable with that of MTA and higher than that of amalgam and composite resin.[77]

4. Clinical applications of CEM

1) Animal studies

(1) Direct pulp capping

The continuity, morphology, and thickness of dentinal bridge, presence of inflammatory cells and preservation of the pulp are the considered evaluation criteria after direct pulp capping (DPC) in the following investigations.

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① Histologic study

In an investigation on dog's teeth, Asgary *et al*. used CH, MTA, and CEM as pulp capping agents and reported complete dentin bridge formation in 75% of the samples capped with CEM and MTA after eight weeks.[30] None of the samples showed inflammation, and in all samples the pulp was vital. Also, in 50% of the cases capped with CEM, a well-organized odontoblast-like cell layer was formed adjacent to the dentinal bridge with tubular dentin. In addition, in 75% of the cases, the dentinal bridge had sufficient thickness (> 0.25 mm). Although MTA group did not differ significantly in each measure from CEM group, Dycal capped teeth showed less favourable biological response to pulp cap treatment. On the basis of these results, the researchers concluded that CEM and MTA showed similar favourable results, better than Dycal, when used as pulp capping materials. The pulp response indicated similar biocompatibility for CEM, compared with MTA, by inducing the formation of a complete dentinal bridge at its interface with the pulp tissue.[30]

② Scanning electron microscope observation

In a SEM investigation of dog's teeth Asgary *et al*. evaluated the effects of MTA, CH, and CEM as pulp capping materials on dental pulp tissues.[53] They reported complete dentinal bridge formation in all the cases of direct pulp capping with CEM. The bridges consisted of thee different aspects. The outer aspect was composed of CEM in direct contact with newly formed hard tissue. In the middle portion, a dentin-like bridge with irregular dentinal tubules was identified. The pulpal or inner aspects exhibited predentin layer, which was similar to normal condition. Young odontoblasts-like cells were differentiated and they elaborated collagen matrix and predentin layer. Based on the results of this study it was concluded that all test materials were effective pulp capping materials and able to stimulate hard tissue bridge. Also, CEM cement was found to have identical biologic effects with MTA.[53]

(2) Pulpotomy

Tabarsi *et al*. compared CEM with MTA and CH as cervical pulpotomy agent on dog's teeth.[56] They reported similar favourable biological results of MTA and CEM, and also demonstrated a more effective induction of dentinal bridge formation compared to CH. The results of this histological observation showed that both MTA and CEM cement were significantly better than CH in terms of dentine bridge formation, pulp vitality, and intensity of inflammation. The pulp tissue underneath CEM cement and MTA specimens was very similar to healthy pulp tissue containing odontoblast-like cells. CH specimens more often showed necrosis in comparison with both white MTA and CEM cement.[56]

(3) Root-end filling

In an investigation, the response of periradicular tissues to MTA and CEM cement as root-end fillings was compared, and hard tissue healing after periradicular surgery was analysed.[27] The results demonstrated complete healing and absence of inflammation in 11 of 12 roots in the MTA group and 10 of 12 in CEM cement group. Cementum formation was observed adjacent to MTA and CEM cement in healed samples, whereas cementogenesis occurred over the dentinal surface of the resected root ends in all samples. Newly formed eosinophilic cementum showed entrapped cementoblasts and insertion of PDL fibers. In addition, bone cavities were filled with newly formed bone tissue in all of the experimental samples. Favourable sealing ability, comparable biocompatibility, and greater alkalinity than MTA might explain CEM's ability to induce cementogenesis.[24,25,27,29-31,45,48,56]

(4) Furcation perforation

Samiee *et al*. compared the healing of furcation perforations repaired with CEM cement versus MTA in dogs' teeth.[31] Their findings revealed hard tissue bridges in every specimen between the two edges of perforation and beneath the experimental materials after an interval of three months. Eight of MTA specimens and six specimens of CEM cement group demonstrated complete bridge formations, which were not statistically different. None of these specimens demonstrated epithelial infiltration in the furcation area or adjacent to the materials. Additionally, statistical analysis did not show any significant differences in inflammation severity between CEM and MTA, both in the furcation area and beneath the materials.

According to the results of this study, CEM cement yielded acceptable results in the repair of furcal perforation in dogs' teeth. However, long-term evaluations of this material are recommended before it is used for perforation repair in human teeth.[31]

2) Human studies

CEM has been proposed as a potent bio-ceramic material and an alternative to MTA for numerous clinical applications like pulp capping, pulpotomy for primary teeth, root-end filling, apical barrier formation for teeth with necrotic pulps and open apexes, perforation repair, and apexification.[28,54,55,57,58,60,78-81]

(1) Vital Pulp Therapy

Evidence-based success in various VPT in human subjects using CEM cement has been documented.[60,78,82,83] A recent evidence-based review has revealed that CEM cement is a suitable endodontic biomaterial for VPT treatments of primary molars as well as mature/immature permanent teeth with reversible/irreversible pulpitis.[84]

① Primary teeth

i) Pulp capping

In a randomized controlled prospective clinical trial of pulp capped primary molar teeth, either with CEM or MTA, Ghajari *et al.* reported clinical and radiographic success rates after six months for both materials.[60] Pain, swelling, tenderness to percussion, or pathologic luxation was not observed in any of the studied teeth, except one tooth treated with CEM cement showing a sinus tract. No radiographic failure was observed in both groups at 6 months.[60] A recent split mouth quadruple-blind randomized clinical trial has shown that CEM and MTA exhibit similar and acceptable outcomes in DPC treatment of human deciduous molars.[78]

ii) Pulpotomy

Pulpotomy is one of the most commonly used treatments for retaining pulpally involved primary molar teeth in order to prevent tooth extraction, and to maintain space within the jaws. A randomised clinical trial found that CEM cement demonstrated favourable 2 year treatment outcomes for pulpotomy of carious primary molars comparable with MTA.[79] A recent case report has shown successful outcome after the use of CEM for pulpotomy in a maxillary first primary molar using cone beam computed tomography **(**CBCT) and histologic evaluation method.[85]

② Permanent teeth

i) DPC with CEM cement

DPC is one of the best known clinical treatments available; whereby connection between the exposed pulp and oral cavity is eliminated using appropriate materials.[86] A case report of a mature first mandibular molar with symptomatic irreversible pulpitis/apical periodontitis demonstrated favourable clinical/radiographic outcomes, such as complete resolution of the apical lesion at a 15 months follow-up.[61]

ii) Indirect pulp capping with CEM cement

Currently, the concept of complete caries removal is being challenged for permanent teeth. IPC decreases the risk of pulp exposure, reduces the substrate for bacteria, prevents lesion development, and promotes a physiological reaction in the pulp-dentin complex. Once cariogenic flora is isolated from their nutritional supply by an effective coronal seal, they perish/become inactive. An interesting case report of IPC treatment with CEM of a mature symptomatic first mandibular molar with irreversible pulpitis associated with apical periodontitis demonstrated favorable clinical and radiographic outcomes, such as complete resolution of symptoms and healing of the apical lesion within a 15 month follow-up period.[64]

iii) Pulpotomy with CEM cement

Open apex

Apexogenesis is considered to be the treatment of choice in vital permanent teeth with incomplete root formation. Nosrat and Asgary reported a rare case of a maxillary incisor with an open apex and traumatic pulp exposure that was treated by pulpotomy using CEM.[57] Acceptable clinical/radiographic results were achieved, including formation of a dentin bridge beneath CEM and closure of the tooth apex.[57] Another case report of a permanent molar with an open apex and signs of irreversible pulpitis showed that complete pulpotomy using CEM resulted in formation of a calcified bridge beneath the CEM cement after 12 months along with continuation of root development.[58]

Harandi *et al.* compared CEM, MTA, and ZOE as pulpotomy agents in decayed immature molar teeth with established irreversible pulpitis that were indicated for apexogenesis procedures.[87] Eighteen months clinical and radiographic follow-up revealed successful preservation of pulpal vitality with continued root development in every treated teeth.[87] A recent randomized clinical trial study of extensively carious permanent molars with open apices and signs of reversible/irreversible pulpitis was carried out on 51 subjects. The outcome of the pulpotomy treatment with both CEM and MTA was clinically successful at all follow-up appointments without any side effects / complications. Radiographically, complete apical closure occurred in 78.9% and 81.5% of treated roots in CEM and MTA groups, respectively at 12 months follow-up.[59]

Mature molars

A case report of a mature mandibular molar with irreversible pulpitis and condensing apical periodontitis indicated that acceptable clinical/radiographic results, such as formation of normal trabecular bone structure around the root apices had occurred two years after pulpotomy.[88] In a case series study of 12 permanent mature molars with irreversible pulpitis, CEM was used for pulpotomy, and resulted in complete success at a 16 months follow-up. It was also shown that the pulp-dentin complex had isolated itself by forming a calcified bridge to enable improved regeneration.[32] In a multicenter randomized clinical trial in 23 medical centers linked to five medical universities in Iran, pulpotomy treatments of mature permanent molar teeth with irreversible pulpitis using CEM and MTA were examined. The results of this trial indicated that pulpotomy treatment carried out by trained dentists can result in successful control of pain.[83]

Another randomized clinical trial also found both CEM cement and MTA material are equally successful statistically when used as pulpotomy dressings in human permanent molars with irreversible pulpitis.[89] A recent 2 year prospective multi-center clinical trial reported superior clinical and radiographic success rates as well as cost-

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effectiveness of vital pulp therapy using CEM cement compared to root canal treatment in mature permanent molar teeth with established irreversible pulpitis.[82]

iv) Root end filling

In a prospective outcome study of CEM as root-end filling material on 14 permanent teeth with persistent apical periodontitis, Asgary and Ehsani reported complete healing of periradicular lesions, *i.e.* regeneration of PDL and lamina dura in 13 teeth (93% success) during a mean time of 18 months.[55] CEM cement has been successfully used to fill retrograde cavity in a vertically fractured root of maxillary incisor that was re-implanted after treatment with adhesive resin cement.[90]

v) Perforation

CEM cement has been found to be an appropriate material for furcal perforation repair in human subjects after its successful outcome was demonstrated in dog's teeth. A case report illustrated a mandibular first molar with bifurfal perforation that was successfully treated by application of CEM cement. A 24 months recall showed no evidence of periodontal breakdown and no symptoms, in addition to complete healing of furcal lesion.[54] More cases are needed to substantiate the effectiveness of CEM cement for repair of furcal perforations, but early indications are promising enough to suggest its use.

vi) Resorption

Asgary *et al.* reported successful management of inflammatory external root resorption (IERR) using CEM cement in an avulsed tooth of a young male patient.[91] Healing of a progressive IERR occurred within 40 months with re-establishment of normal periodontal condition.[91] Another case report describes the management of an endodontically failed molar that was severely affected by combined external and internal root resorption (ERR/IRR). Favourable treatment outcomes were reported after 12 months of reobturation of entire distal root canal with CEM cement.[33] On the basis of biological properties of CEM cement, the authors believe that this cement might be an appropriate biomaterial in treatment of IERR and also in obturation of immature teeth. However, further clinical studies with longer follow-up periods and larger samples are recommended.

vii) CEM cement as an apical barrier for teeth with necrotic pulps and open apices

Apexification is the induction of a calcified apical barrier or creation of an artificial apical barrier across the open apex after the elimination of necrotic tissues and bacteria from root canal space.[92] The conventional apexification uses densely packed CH as an intra-canal medicament for the induction of calcified apical barrier. The main drawbacks of this procedure include its multiple scheduled visits and susceptibility of treated roots to fracture.[93] Currently, MTA as apical plug is a promising material in obturation of the open apex teeth indicated by several studies carried out on human subjects.[94-97]

Recently, animal studies have revealed that, like MTA, PDL regeneration, cementogenesis, and dentinogenesis occur in contact with CEM cement.[27,56] A few case series have also described clinical procedures with CEM cement as an apical barrier in teeth with necrotic pulps and open apices. In one study, 13 single-rooted teeth with necrotic pulps and open apices were successfully treated by CEM cement apical plug insertion with an average follow-up time of 14.5 months.[80] A recent study showed that medication with calcium hydroxide had no adverse effect on marginal adaptation of the CEM cement apical plug.[80] Milani *et al.* showed that CEM cement exhibit distinct reinforcing effect on immature teeth.[98]

viii) Regenerative endodontic treatment with CEM cement

Revascularization is a valuable treatment in immature necrotic teeth that allows the continuation of root development. Several case reports, case series, and clinical studies have been published demonstrating successful results for this technique and material in treating immature necrotic teeth.[99-101] Two cases of successful revascularization in necrotic immature molars by using CEM cement as new endodontic biomaterial with a modified approach have been reported by Nosrat *et al.*[28]

Conclusions

CEM cement combines the biocompatibility of MTA with more efficient characteristics, such as significantly shorter setting time, good handling characteristics, and no tooth staining. The cement is able to induce hard tissue formation, has antibacterial effect, and forms an effective seal against entrance of microorganisms. CEM cement has demonstrated similar results to MTA when used for VPT, furcation perforation repair, and management of internal and external root resorption. However, future investigations with a high level of evidence are needed to evaluate the actual effect of CEM in various clinical applications, and to confirm its efficacy compared with other materials.

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References

1. Swarup S, Rao A. Bioceramics in pediatric endodontics. 1st ed. Trivandrum: Lambert Academic Publishing; 2013. p53-68.

2. Nasseh A. The rise of bioceramics. *Endodontic Practice* 2009;2:17-22.
3. Koch K, Brave D. A new day has dawned: the increased use of bioceramics in endodontics. *Dentaltown* 2009; 10(4):39-43.
4. Best SM, Porter AE, Thian ES, Huang J, Bioceramics: past, present and for the future. *J Eur Ceram Soc* 2008; 28:1319-1327.
5. Jean A, Kerebel B, Kerebel LM, Legeros RZ, Hamel H. Effects of various calcium phosphate biomaterials on reparative dentin bridge formation. *J Endod* 1988;14: 83-87.
6. Pissiotis E, Spangberg LS. Biological evaluation of collagen gels containing calcium hydroxide and hydroxyapatite. *J Endod* 1990;16:468-473.
7. Liu C, Wang W, Shen W, Chen T, Hu L, Chen Z. Evaluation of the biocompatibility of a nonceramic hydroxyapatite. *J Endod* 1997;23:490-493.
8. Dhingra A, Chopra V, Raj S. Challenges of bioengineering and endodontics. *J Dev Biol Tissue Eng* 2011;3:80-84.
9. Harbert H. Generic tricalcium phosphate plugs: an adjunct in endodontics. *J Endod* 1991;17:131-134.
10. Boone ME 2nd, Kafrawy AH. Pulp reaction to a tricalcium phosphate ceramic capping agent. *Oral Surg Oral Med Oral Pathol* 1979;47:369-371.
11. Coviello J, Brilliant JD. A preliminary clinical study on the use of tricalcium phosphate as an apical barrier. *J Endod* 1979;5:6-13.
12. Metsger DS, Driskell TD, Paulsrud JR. Tricalcium phosphate ceramic-a resorbable bone implant: review and current status. *J Am Dent Assoc* 1982;105:1035-1038.
13. Bae WJ, Chang SW, Lee SI, Kum KY, Bae KS, Kim EC. Human periodontal ligament cell response to a newly developed calcium phosphate-based root canal sealer. *J Endod* 2010;36:1658-1663.
14. Oguntebi B, Clark A, Wilson J. Pulp capping with Bioglass and autologous demineralised dentin in miniature swine. *J Dent Res* 1993;72:484-489.
15. Abu Zeid ST, Ezzat KM, Kamal SM. Reparative activity of bioglass and tricalcium phosphate to induce apical closure compared to normal apexogensis (histological and histochemical study). Available from: http://www.kau.edu.sa/Files/0052394/Researches/28671_Reparative%20Activity%20of%20Bioglass%20And%20Tricalcium%20Phosphate.pdf(updated 2014 Mar 30).
16. Friedman S. Application of glass-ionomer in endodontics. In: Davidson CL, Mjor IA, editors. Advances in glass-ionomer cements. Chicago: Quintessence Books; 1999. p183-200.
17. Naoum HJ, Chandler NP. Temporization for endodontics. *Int Endod J* 2002;35:964-978.
18. Chong BS, Pitt Ford TR, Watson TF. The adaptation and sealing ability of light-cured glass ionomer retrograde root fillings. *Int Endod J* 1991;24:223-232.
19. Pameijer CH. A review of luting agents. *Int J Dent* 2012;2012:752861. doi: 10.1155/2012/752861. Epub 2012 Feb 22.
20. Hilton TJ. Keys to Clinical Success with pulp capping: a review of the literature. *Oper Dent* 2009;34:615-625.
21. Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review-Part III: clinical applications, drawbacks, and mechanism of action. *J Endod* 2010;36:400-413.
22. Gomes-Filho JE, Watanabe S, Bernabé PF, de Moraes Costa MT. A mineral trioxide aggregate sealer stimulated mineralization. *J Endod* 2009;35:256-260.
23. Tyagi S, Mishra P, Tyagi P. Evolution of root canal sealers: an insight story. *Eur J Gen Dent* 2013;2:199-218.
24. Asgary S, Eghbal MJ, Parirokh M, Torabzadeh H. Sealing ability of three commercial mineral trioxide aggregate and an experimental root-end filling material. *Iran Endod J* 2006;1:101-105.
25. Asgary S, Shahabi S, Jafarzadeh T, Amini S, Kheirieh S. The properties of a new endodontic material. *J Endod* 2008;34:990-993.
26. Asgary S, Eghbal MJ, Parirokh M, Ghoddusi J. Effect of two storage solutions on surface topography of two root-end fillings. *Aust Endod J* 2009;35:147-152.
27. Asgary S, Eghbal MJ, Ehsani S. Periradicular regeneration after endodontic surgery with calcium-enriched mixture cement in dogs. *J Endod* 2010;36:837-841.
28. Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: a review and report of two cases with a new biomaterial. *J Endod* 2011;37:562-567.
29. Asgary S, Eghbal MJ, Parirokh M. Sealing ability of a novel endodontic cement as a root-end filling material. *J Biomed Mater Res A* 2008;87:706-709.
30. Asgary S, Eghbal MJ, Parirokh M, Ghanavati F, Rahimi H. A comparative study of histologic response to different pulp capping materials and a novel endodontic cement. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; 106:609-614.
31. Samiee M, Eghbal MJ, Parirokh M, Abbas FM, Asgary S. Repair of furcal perforation using a new endodontic cement. *Clin Oral Investig* 2010;14:653-658.
32. Asgary S, Ehsani S. Permanent molar pulpotomy with a new endodontic cement: a case series. *J Conserv Dent* 2009;12:31-36.
33. Asgary S, Ahmadyar M. One-visit endodontic retreatment of combined external/internal root resorption using a calcium-enriched mixture. *Gen Dent* 2012;60:e244-248.
34. Asgary S, Kamrani FA. Antibacterial effects of five different root canal sealing materials. *J Oral Sci* 2008; 50:469-474.
35. Asgary S, Eghbal MJ, Parirokh M, Ghoddusi J, Kheirieh

http://dx.doi.org/10.5395/rde.2015.40.1.1

S, Brink F. Comparison of mineral trioxide aggregate's composition with Portland cements and a new endodontic cement. *J Endod* 2009;35:243-250.
36. Amini Ghazvini S, Abdo Tabrizi M, Kobarfard F, Akbarzadeh Baghban A, Asgary S. Ion release and pH of a new endodontic cement, MTA and Portland cement. *Iran Endod J* 2009;4:74-78.
37. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. *Int Endod J* 2011;44:697-730.
38. Estrela C, Bammann LL, Estrela CR, Silva RS, Pécora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. *Braz Dent J* 2000;11:3-9.
39. Torabinejad M, Hong CU, Pitt Ford TR, Kettering JD. Antibacterial effects of some root end filling materials. *J Endod* 1995;21:403-406.
40. Asgary S, Akbari Kamrani F, Taheri S. Evaluation of antimicrobial effect of MTA, calcium hydroxide, and CEM cement. *Iran Endod J* 2007;2:105-109.
41. Hasan Zarrabi M, Javidi M, Naderinasab M, Gharechahi M. Comparative evaluation of antimicrobial activity of three cements: new endodontic cement (NEC), mineral trioxide aggregate (MTA) and Portland. *J Oral Sci* 2009; 51:437-442.
42. Kangarlou A, Sofiabadi S, Yadegari Z, Asgary S. Antifungal effect of calcium enriched mixture cement against Candida albicans. *Iran Endod J* 2009;4:101-105.
43. Razmi H, Aminsobhani M, Bolhari B, Shamshirgar F, Shahsavan S, Shamshiri AR. Calcium enriched mixture and mineral trioxide aggregate activities against *Enterococcus Faecalis* in presence of dentin. *Iran Endod J* 2013;8:191-196.
44. Murray PE, García Godoy C, García Godoy F. How is the biocompatibilty of dental biomaterials evaluated? *Med Oral Patol Oral Cir Bucal* 2007;12:e258-266.
45. Ghoddusi J, Tavakkol Afshari J, Donyavi Z, Brook A, Disfani R, Esmaeelzadeh M. Cytotoxic effects of new endodontic cement and mineral trioxide aggregate on L929 line culture. *Iran Endod J* 2008;3:17-23.
46. Mozayeni MA, Milani AS, Marvasti LA, Asgary S. Cytotoxicity of calcium enriched mixture cement compared with mineral trioxide aggregate and intermediate restorative material. *Aust Endod J* 2012; 38:70-75.
47. Camilleri J, Pitt Ford TR. Mineral trioxide aggregate: a review of the constituents and biological properties of the material. *Int Endod J* 2006;39:747-754.
48. Asgary S, Moosavi SH, Yadegari Z, Shahriari S. Cytotoxic effect of MTA and CEM cement in human gingival fibroblast cells. Scanning electronic microscope evaluation. *N Y State Dent J* 2012;78:51-54.
49. Tabarsi B, Pourghasem M, Moghaddamnia A, Shokravi M, Ehsani M, Ahmadyar M, Asgary S. Comparison of skin test reactivity of two endodontic biomaterials in rabbits. *Pak J Biol Sci* 2012;15:250-254.
50. Parirokh M, Mirsoltani B, Raoof M, Tabrizchi H, Haghdoost AA. Comparative study of subcutaneous tissue responses to a novel root-end filling material and white and grey mineral trioxide aggregate. *Int Endod J* 2011;44:283-289.
51. Rahimi S, Mokhtari H, Shahi S, Kazemi A, Asgary S, Eghbal MJ, Mesgariabbasi M, Mohajeri D. Osseous reaction to implantation of two endodontic cements: mineral trioxide aggregate (MTA) and calcium enriched mixture (CEM). *Med Oral Patol Oral Cir Bucal* 2012;17: e907-911.
52. Abbasipour F, Akheshteh V, Rastqar A, Khalilkhani H, Asgary S, Janahmadi M. Comparing the effects of mineral trioxide aggregate and calcium enriched mixture on neuronal cells using an electrophysiological approach. *Iran Endod J* 2012;7:79-87.
53. Asgary S, Parirokh M, Eghbal MJ, Ghoddusi J. SEM evaluation of pulp reaction to different pulp capping materials in dog's teeth. *Iran Endod J* 2006;1:117-123.
54. Asgary S. Furcal perforation repair using calcium enriched mixture cement. *J Conserv Dent* 2010;13:156-158.
55. Asgary S, Ehsani S. Periradicular surgery of human permanent teeth with calcium-enriched mixture cement. *Iran Endod J* 2013;8:140-144.
56. Tabarsi B, Parirokh M, Eghbal MJ, Haghdoost AA, Torabzadeh H, Asgary S. A comparative study of dental pulp response to several pulpotomy agents. *Int Endod J* 2010;43:565-571.
57. Nosrat A, Asgary S. Apexogenesis treatment with a new endodontic cement: a case report. *J Endod* 2010;36: 912-914.
58. Nosrat A, Asgary S. Apexogenesis of a symptomatic molar with calcium enriched mixture. *Int Endod J* 2010; 43:940-944.
59. Nosrat A, Seifi A, Asgary S. Pulpotomy in caries-exposed immature permanent molars using calcium-enriched mixture cement or mineral trioxide aggregate: a randomized clinical trial. *Int J Paediatr Dent* 2013;23: 56-63.
60. Fallahinejad Ghajari M, Asgharian Jeddi T, Iri S, Asgary S. Direct pulp-capping with calcium enriched mixture in primary molar teeth: a randomized clinical trial. *Iran Endod J* 2010;5:27-30.
61. Asgary S, Nosrat A, Homayounfar N. Periapical healing after direct pulp capping with calcium-enriched mixture cement: a case report. *Oper Dent* 2012;37:571-575.
62. Zarrabi MH, Javidi M, Jafarian AH, Joushan B. Immunohistochemical expression of fibronectin and tenascin in human tooth pulp capped with mineral trioxide aggregate and a novel endodontic cement. *J Endod* 2011;37:1613-1618.

63. Zarrabi MH, Javidi M, Jafarian AH, Joushan B. Histologic assessment of human pulp response to capping with mineral trioxide aggregate and a novel endodontic cement. *J Endod* 2010;36:1778-1781.
64. Torabzadeh H, Asgary S. Indirect pulp therapy in a symptomatic mature molar using calcium enriched mixture cement. *J Conserv Dent* 2013;16:83-86.
65. Torabinejad M, Eby WC, Naidorf IJ. Inflammatory and immunological aspects of the pathogenesis of human periapical lesions. *J Endod* 1985;11:479-488.
66. Pommel L, Camps J. Effects of pressure and measurement time on the fluid filtration method in endodontics. *J Endod* 2001;27:256-258.
67. Veríssimo DM, do Vale MS. Methodologies for assessment of apical and coronal leakage of endodontic filling materials: a critical review. *J Oral Sci* 2006;48:93-98.
68. Xavier CB, Weismann R, de Oliveira MG, Demarco FF, Pozza DH. Root-end filling materials: apical microleakage and marginal adaptation. *J Endod* 2005; 31:539-542.
69. De Bruyne MA, De Bruyne RJ, De Moor RJ. Long-term assessment of the seal provided by root-end filling materials in large cavities through capillary flow porometry. *Int Endod J* 2006;39:493-501.
70. Hasheminia M, Loriaei Nejad S, Asgary S. Sealing ability of MTA and CEM cement as root-end fillings of human teeth in dry, saliva or blood-contaminated conditions. *Iran Endod J* 2010;5:151-156.
71. Milani AS, Shakouie S, Borna Z, Sighari Deljavan A, Asghari Jafarabadi M, Pournaghi Azar F. Evaluating the effect of resection on the sealing ability of MTA and CEM Cement. *Iran Endod J* 2012;7:134-138.
72. Kazem M, Mahjour F, Dianat O, Fallahi S, Jahankhah M. Root-end filling with cement-based materials: an *in vitro* analysis of bacterial and dye microleakage. *Dent Res J (Isfahan)* 2013;10:46-51.
73. Soheilipour E, Kheirieh S, Madani M, Akbarzadeh Baghban A, Asgary S. Particle size of a new endodontic cement compared to Root MTA and calcium hydroxide. *Iran Endod J* 2009;4:112-116.
74. Moradi S, Disfani R, Ghazvini K, Lomee M. Sealing ability of orthograde MTA and CEM cement in apically resected roots using bacterial leakage method. *Iran Endod J* 2013;8:109-113.
75. Ghorbani Z, Kheirieh S, Shadman B, Eghbal MJ, Asgary S. Microleakage of CEM cement in two different media. *Iran Endod J* 2009;4:87-90.
76. Haghgoo R, Arfa S, Asgary S. Microleakage of CEM cement and ProRoot MTA as furcal perforation repair materials in primary teeth. *Iran Endod J* 2013;8:187-190.
77. Yavari HR, Samiei M, Shahi S, Aghazadeh M, Jafari F, Abdolrahimi M, Asgary S. Microleakage comparison of four dental materials as intra-orifice barriers in endodontically treated teeth. *Iran Endod J* 2012;7:25-30.
78. Fallahinejad Ghajari M, Asgharian Jeddi T, Iri S, Asgary S. Treatment outcomes of primary molars direct pulp capping after 20 months: a randomized controlled trial. *Iran Endod J* 2013;8:149-152.
79. Malekafzali B, Shekarchi F, Asgary S. Treatment outcomes of pulpotomy in primary molars using two endodontic biomaterials. A 2-year randomised clinical trial. *Eur J Paediatr Dent* 2011;12:189-193.
80. Nosrat A, Asgary S, Eghbal MJ, Ghoddusi J, Bayat-Movahed S. Calcium-enriched mixture cement as artificial apical barrier: a case series. *J Conserv Dent* 2011;14:427-431.
81. Bidar M, Disfani R, Asgary S, Forghani M, Gharagozlo S, Rouhani A. Effect of calcium hydroxide premedication on the marginal adaptation of calcium-enriched mixture cement apical plug. *Dent Res J (Isfahan)* 2012;9:706-709.
82. Asgary S, Eghbal MJ, Ghoddusi J. Two-year results of vital pulp therapy in permanent molars with irreversible pulpitis: an ongoing multicenter randomized clinical trial. *Clin Oral Investig* 2014;18:635-641.
83. Asgary S, Eghbal MJ. The effect of pulpotomy using a calcium-enriched mixture cement versus one-visit root canal therapy on postoperative pain relief in irreversible pulpitis: a randomized clinical trial. *Odontology* 2010; 98:126-133.
84. Asgary S, Ahmadyar M. Vital pulp therapy using calcium-enriched mixture: an evidence-based review. *J Conserv Dent* 2013;16:92-98.
85. Mehrdad L, Malekafzali B, Shekarchi F, Safi Y, Asgary S. Histological and CBCT evaluation of a pulpotomised primary molar using calcium enriched mixture cement. *Eur Arch Paediatr Dent* 2013;14:191-194.
86. Aguilar P, Linsuwanont P. Vital pulp therapy in vital permanent teeth with cariously exposed pulp: a systematic review. *J Endod* 2011;37:581-587.
87. Harandi A, Forghani M, Ghoddusi J. Vital pulp therapy with three different pulpotomy agents in immature molars: a case report. *Iran Endod J* 2013;8:145-148.
88. Asgary S. Calcium-enriched mixture pulpotomy of a human permanent molar with irreversible pulpitis and condensing apical periodontitis. *J Conserv Dent* 2011; 14:90-93.
89. Asgary S, Eghbal MJ. Treatment outcomes of pulpotomy in permanent molars with irreversible pulpitis using biomaterials: a multi-center randomized controlled trial. *Acta Odontol Scand* 2013;71:130-136.
90. Moradi Majd N, Akhtari F, Araghi S, Homayouni H. Treatment of a vertical root fracture using dual-curing resin cement: a case report. *Case Rep Dent* 2012;2012:985215. doi: 10.1155/2012/985215. [Epub

2012 Dec 18]
91. Asgary S, Nosrat A, Seifi A. Management of inflammatory external root resorption by using calcium-enriched mixture cement: a case report. *J Endod* 2011;37:411-413.
92. Camp J, Fuks A. Pediatric endodontics: endodontic treatment for the primary and young permanent dentition. In: Cohen S, Hargreaves K, Keiser K, editors. Pathways of the Pulp. 9th ed. St Louis: Mosby Elsevier; 2006. p822-882.
93. Rafter M. Apexification: a review. *Dent Traumatol* 2005;21:1-8.
94. Giuliani V, Baccetti T, Pace R, Pagavino G. The use of MTA in teeth with necrotic pulps and open apices. *Dent Traumatol* 2002;18:217-221.
95. Hayashi M, Shimizu A, Ebisu S. MTA for obturation of mandibular central incisors with open apices: case report. *J Endod* 2004;30:120-122.
96. Gaitonde P, Bishop K. Apexification with mineral trioxide aggregate: an overview of the material and technique. *Eur J Prosthodont Restor Dent* 2007;15:41-45.
97. Hiremath H, Gada N, Kini Y, Kulkarni S, Yakub SS, Metgud S. Single-step apical barrier placement in immature teeth using mineral trioxide aggregate and management of periapical inflammatory lesion using platelet-rich plasma and hydroxyapatite. *J Endod* 2008; 34:1020-1024.
98. Milani AS, Rahimi S, Borna Z, Jafarabadi MA, Bahari M, Deljavan AS. Fracture resistance of immature teeth filled with mineral trioxide aggregate or calcium-enriched mixture cement: an *ex vivo* study. *Dent Res J (Isfahan)* 2012;9:299-304.
99. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? *J Endod* 2004;30:196-200.
100. Ding RY, Cheung GS, Chen J, Yin XZ, Wang QQ, Zhang CF. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. *J Endod* 2009;35:745-749.
101. Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: a case series. *J Endod* 2010;36:536-541.