INTERNATIONAL
ENDODONTIC JOURNAL

doi:10.1111/iej.12231

Comparison of pulp response to mineral trioxide aggregate and a bioceramic paste in partial pulpotomy of sound human premolars: a randomized controlled trial

S. Azimi, M. Fazlyab, D. Sadri, M. A. Saghiri, B. Khosravanifard & S. Asgary

Department of Endodontics, Islamic Azad University, Dental Branch, Tehran, Iran

Abstract

Azimi S, Fazlyab M, Sadri D, Saghiri MA, Khosravanifard B, Asgary S. Comparison of pulp response to mineral trioxide aggregate and a bioceramic paste in partial pulpotomy of sound human premolars: a randomized controlled trial. *International Endodontic Journal*.

Aim This randomized clinical trial evaluated clinical sign/symptoms as well as histological pulp reactions in terms of inflammation and mineralized bridge formation after partial pulpotomy of sound human premolars and placement of a bioceramic paste (iRoot BP) or tooth-colored ProRoot MTA as pulp-covering biomaterials.

Methodology Twenty-four human sound premolars were randomly allocated into two experimental groups ($n = 12$) treated either with iRoot BP or MTA subsequent to partial pulpotomy. Six weeks after treatment, clinical sign/symptoms and radiographic changes were evaluated. The teeth were then extracted and examined histologically for inflammatory status of the pulp, formation of hard tissue bridge and appearance of the bridge. In terms of pulp inflammation and dentinal bridge formation, the Mann–Whitney *U*, and for clinical signs, the chi-square test was used ($\alpha = 0.05$).

Results In terms of pulp inflammation, formation of hard tissue bridge and its appearance, the differences between the two experimental groups were not significant. However, clinical sensitivity to cold was significantly less for teeth treated with MTA ($P < 0.05$). All cases had formed a hard tissue bridge, and none of the specimens in either group had pulpal necrosis.

Conclusion When treating teeth with healthy pulps, the response to partial pulpotomy treatment with both MTA and iRoot BP was favourable. However, pulps covered with iRoot BP were more sensitive to cold stimuli.

Keywords: bioceramics, Cvek pulpotomy, iRoot BP, mineral trioxide aggregate, MTA, partial pulpotomy.

Received 25 April 2013; accepted 7 December 2013

Introduction

Bioceramic-based materials have a long history of use in tissue regeneration and medicine (Marcacci *et al.* 2007, Koch & Brave 2012). These materials were introduced to dentistry, specifically endodontics, in the form of root and perforation repair materials or sealers (De-Deus *et al.* 2009, Yuan *et al.* 2010, Damas *et al.* 2011, De-Deus *et al.* 2012). Bioceramics are homogenous materials consisting of nanosphere particles, with the maximum dimension not exceeding 1×10^{-3} μm (De-Deus *et al.* 2009) and mainly composed of tricalcium silicate, dicalcium silicate, calcium phosphate monobasic, amorphous silicon dioxide and tantalum pentoxide (Zhang *et al.* 2013). Due to their ability to penetrate dentinal tubules and to interact with dentine moisture, an optimum

Correspondence: Mahta Fazlyab, Shahid Beheshti Dental School, Evin, Tehran, 1983963113, Iran (Tel.: +98 21 22413897; Fax: +98 21 22427753; e-mail: dr.mfazlyab@gmail.com).

dimensional stability and the least amount of shrinkage can be expected. Compared to white MTA, bioceramics offer the advantage of being aluminium-free (De-Deus *et al.* 2009) and contain tantalum pentoxide as an opacifier (Zhang *et al.* 2009, Park *et al.* 2010). In recent studies, bioceramics have proved to be as effective as MTA in terms of antibacterial activity (Zhang *et al.* 2013), biocompatibility (De-Deus *et al.* 2009) and sealing ability (Leal *et al.* 2011). Bioceramics can induce differentiation of human PDL fibroblasts (Yan *et al.* 2010), and their bioactivity is comparable with that of MTA (Shokouhinejad *et al.* 2012). They also have an alkaline pH (12.8), which can be responsible for antibacterial activity (Damas *et al.* 2011).

iRoot BP (Innovative BioCeramix, Inc., Vancouver, BC, Canada) is a water-based pre-mixed bioceramic cement (De-Deus *et al.* 2009) with the main chemical composition based on another bioceramic-based product, BioAggregate (iRoot BP stands for 'Injectable Root BioAggregate Paste') (Zhang *et al.* 2013). According to the manufacturer, it requires the presence of moisture to set (De-Deus *et al.* 2012).

Mineral trioxide aggregate (MTA) (ProRoot MTA, Dentsply Tulsa Dental, Tulsa, OK, USA) is a commonly used material for vital pulp therapy, but can cause tooth discoloration (Parirokh & Torabinejad 2010, De-Deus *et al.* 2012) and has handling difficulties, long setting time and difficulty in maintaining consistency of mixture (Nosrat & Asgary 2010, Paranjpe *et al.* 2010, Parirokh & Torabinejad 2010, Ma *et al.* 2011). Some authors believe that MTA releases hazardous substances (De-Deus *et al.* 2012).

Many studies have shown biocompatibility and nontoxicity of endodontic bioceramics in culture medium (De-Deus *et al.* 2009, Yuan *et al.* 2010, De-Deus *et al.* 2012). Considering that usage testing offers the advantage of allowing complex interactions between the host and the examined material, additional investigations are needed to establish a more general outlook on the real potential of these materials. To date, no clinical trial has been performed on human vital pulps to assess the clinical effects of bioceramics. Studies on the effects of different biomaterials on dental pulp have used direct pulp capping (Asgary *et al.* 2006, Lu *et al.* 2008, Accorinte *et al.* 2009, Nair *et al.* 2009, Paranjpe *et al.* 2010, Parirokh *et al.* 2011) and partial pulpotomy techniques (Barrieshi-Nusair & Qudeimat 2006, Kiatwateeratana *et al.* 2009). The concept behind this treatment is to remove 2–3 mm of inflamed superficial pulp tissue. The wound surface is treated with a dressing agent to promote healing and maintain the health of the remaining pulp tissue (Cvek 1978).

Amongst studies that have assessed the effect(s) of a specific intervention on human pulp, many have used sound teeth to be able to justify any probable failure by the biomaterial (Lu *et al.* 2008, Accorinte *et al.* 2009, Kiatwateeratana *et al.* 2009, Nair *et al.* 2009, Parirokh *et al.* 2011). Considering the relatively wide contact surface of dressing material with pulp tissue in this technique besides the need for differentiation of new cells (Barrieshi-Nusair & Qudeimat 2006), the present study was based on comparing the effect(s) of a bioceramic paste on partially pulpotomized teeth to that of mineral trioxide aggregate.

The aim of this randomized controlled clinical trial was to evaluate clinical symptoms, radiographic changes and histological pulp tissue reactions in terms of inflammation, hard tissue bridge formation and its appearance, subsequent to placing iRoot BP or tooth-colored ProRoot MTA as the capping materials on partially pulpotomized caries-free human premolars after a 6-week follow-up period. The null hypothesis was that the dental pulp would respond similarly to both biomaterials in terms of inflammation and deposition of hard tissue bridges.

Materials and methods

Case selection

The study was approved by the Ethics Committee of Azad University, Dental Branch, Tehran, Iran. After performing a pilot study on four cases for each material, using a two-proportion submenu from the sample size calculation menu of Minitab 14, considering $\alpha = 0.05$, $\beta = 0.2$, $P_1 = 0.3$ and $P_2 = 0.8$, for clinical sensitivity to cold, the minimum estimated sample size for each group was 12. As a result, this study was performed on twelve pairs of contra-lateral maxillary or mandibular first premolars from twelve healthy volunteers aged 12 to 16 years (mean 14 years). The teeth were scheduled for orthodontic extraction at the Orthodontic Clinic, Azad University, Dental Branch, Tehran, Iran. The project was registered at www.clinicaltrial.gov (ClinicalTrials.gov Identifier: NCT01420718).

Strict inclusion and exclusion criteria were applied to determine whether the patient was a potential participant. The inclusion criteria for the study were:

1. No systemic disease and no medication consumption.
2. Participants, with healthy first premolars in either of the jaws, assigned for orthodontic extraction (scheduled extraction of the maxillary/mandibular first premolars that needed to be free from caries, restoration, crack, radiographic PDL widening or pathosis).
3. The premolar teeth needed to be fully erupted (to be able to provide precise rubber dam isolation).
4. The premolar teeth needed to respond within the normal range to cold testing with ENDO-ICE frozen gas (Coltene/Whaledent, Inc., Mahwah, NJ, USA) and heat testing with a hot burnisher that were applied for 5 s on the buccal surface of the tooth. An uncomfortable sensation or pain that had the tendency to linger as a dull ache after the stimulus was removed (Sigurdsson 2004) was considered abnormal.
5. The patients' parents had read, signed and thoroughly understood the informed consent.

Volunteers would be excluded if they fell into any of the following categories:

1. Presence of systematic disease and medication consumption of any type.
2. Anti-inflammatory medicine taken before and during the time of study.
3. History of allergy to anaesthetics, latex, etc.
4. Premolars with caries, restoration or any abnormality on periapical radiographs.
5. If the premolar teeth were not fully erupted so that the rubber dam isolation was impossible.
6. If the premolar teeth revealed a lingering pain [a pain sensation that had the tendency to linger as a dull ache after the stimulus had been removed (Sigurdsson 2004)], upon cold testing with ENDO-ICE frozen gas and heat testing with hot burnisher.

If the volunteers met all the inclusion criteria, they were invited to participate in the study. An informed consent was given to the patients and their parents to assure them in case of any spontaneous pain or discomfort or even a change of mind, they would be excluded from the study and their teeth would be extracted immediately to continue with the orthodontic treatment. Sixteen patients providing 32 sound premolars matched the criteria. Four patients did not agree to participate in the study, so a total number of twelve patients, providing twenty-four first premolars, signed the consent form outlining the procedure and its possible risks (Fig. 1).

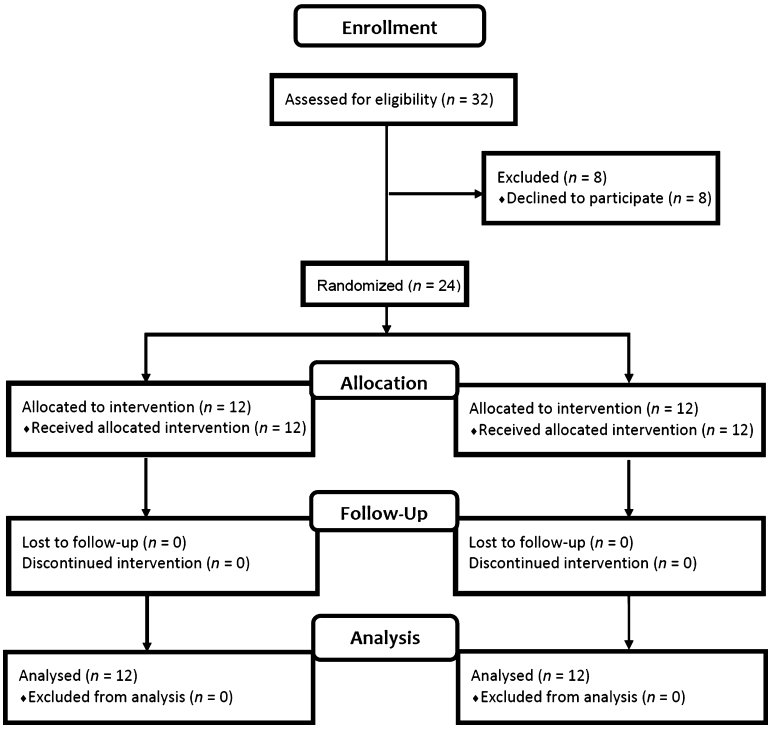

Figure 1 The diagram of patients' randomization, allocation and follow-up.

Randomization

After partial pulpotomy, the teeth were randomly allocated to two groups. For each selected patient, one premolar was randomly allocated to iRoot BP and the other with tooth-colored ProRoot MTA by a toss of coin, with the aid of a computer algorithm (http://www.random.org/coins/?num=1&cur=60-usd.0001c). The main operator gave each patient a numerical code (from 101 to 112) whilst the teeth had an alphabetical coding (e.g. for the patient coded as 101, left and right premolars were coded 101-a, and 101-b, respectively). In all patient documents, the teeth were labelled in the same way so that the pathologist was blind to the material used for each tooth whilst the main operator who completed the patient charts and performed pulpotomy knew which material was used for each premolar because of the need for mixing the MTA.

Partial pulpotomy and restoration

There is a standard protocol suggested by the International Organization for Standardization (ISO), ISO7405, that clarifies the testing methods for evaluation of biocompatibility of materials used in dentistry at different testing levels, from *in vitro* cellular testing to usage studies (Wataha 2012). In this study, the process of tooth extraction and fixation, histological preparation of the specimen and microscopic evaluation of the slides were set according to this standard protocol.

After local anaesthesia with 2% lidocaine containing epinephrine 1 : 80 000 (DarouPakhsh-Tehran, Iran), a cavity was prepared on the occlusal surface with a cylindrical high-speed diamond bur 835.010 (D+Z Germany) under air–water spray. The occlusal depth of the cavity was 3 mm. Before pulp exposure, the teeth were isolated with rubber dam and cavities were disinfected with 0.12% chlorhexidine gluconate (Behsa Pharmaceutical Company, Tehran, Iran). A superficial pulp amputation (similar to the maximum bur diameter of 1.4 mm) was performed using a high-speed sterile round diamond bur D+Z 801.014 (Germany) under air–water spray. The pulp wound was irrigated with sterile saline solution to remove debris. Any bleeding was then arrested by pressing moist sterile cotton pellets on the pulp wound for 1–2 min. Subsequently, the exposed pulp was dressed with either tooth-coloured ProRoot MTA (Dentsply) or iRoot BP (Innovative) according to the manufacturer's instruction. In the MTA group, a sterile saline wet cotton pellet was placed over the MTA to provide primary setting hydration which was removed after 30 min. Then, the cavity floor was covered with a resin-modified glass–ionomer cement (Fuji II LC; GC Corp, Tokyo, Japan), and finally, in both groups, the tooth was restored with resin composite (Spectrum and Prime & Bond NT, Dentsply).

Follow-up

Before dismissal, each patient was asked to record any clinical signs or symptoms associated with the treated teeth, such as sensitivity to cold or heat, sensitivity upon chewing, spontaneous pain or swelling. Each subject was interviewed individually on the first, third and seventh day after the operation and then weekly up to the forty-second day (sixth week). All responses were recorded. Any spontaneous pain or prolonged lingering dull ache in response to cold or hot stimulus was interpreted as failure. In case the patients wished to change their mind about participation in the study or in case of spontaneous pain, they were excluded from the study. The teeth in these patients would be extracted immediately and sent for histological evaluation.

Histological assessment

The patients' documents were kept by the dental assistant who called patients to be evaluated by the main operator. The teeth were extracted under local anaesthesia after 6 weeks. At the time of extraction, the patients were asked about the presence or absence of postoperative sensitivity and periapical radiograph of the teeth was taken to evaluate the periapical status of the teeth by the main operator. The main operator was blind to the type of used material during the pre-extraction clinical evaluation. As suggested by ISO7405 (Wataha 2012), after extraction, 5 mm of the most apical portion of the roots were removed to facilitate fixation in 10% buffered formalin solution for 72 h. The teeth were decalcified in 10% formic acid for 6–8 weeks, prepared according to routine histological techniques and embedded in paraffin. Five-micron-thick bucco-lingual sections were cut parallel to the vertical axis of the tooth. The sections, mounted on glass slides, were stained with haematoxylin and eosin (H&E) and were blindly evaluated twice by a pathologist by means of light microscopy with magnification of ×4, ×20, ×40 and ×100

(Nikon, Plan Flour, Japan). The pathologist was calibrated according to ISO7405 modified criteria (Wataha 2012) to evaluate the formation of hard tissue bridge, its histological appearance and pulp inflammatory response. Evaluation of pulp inflammatory status was based on a 1 – 4 scoring system: 0 – no inflammation, 1 – mild inflammation, 2 – moderate inflammation, 3 – severe inflammation and 4 – abscess formation or extended lesions not localized to the tissue beneath the material. In addition, the degree of bridging over the capped area of the pulp with tertiary dentine was scored as none, partial or complete. The appearance of dentinal bridge was classified as resembling natural dentine, atubular and presence of tunnel defects.

In terms of pulp inflammation, dentinal bridge formation and its appearance, the overall scores attributed to each group were evaluated by Mann–Whitney *U*-test. Chi-square test was used to compare the clinical signs. The comparisons between averages were performed by comparing the ranks with appropriately computed critical values ($\alpha = 0.05$).

Results

During the time of study, none of the patients complained of severe pain or any discomfort that could cause preterm extraction of the teeth. By the end of sixth week, twelve patients providing a total number of 24 teeth, 12 in each group (the tooth-colored ProRoot MTA or iRoot BP), were available for microscopic evaluation (Figs 2–5).

Formation of hard tissue bridge and its appearance

In all of the specimens, a hard tissue bridge was formed. A layer of flat cells were aligned parallel to the hard tissue bridge (Figs 3–5).

In the ProRoot MTA group, a complete hard tissue bridge was formed in eight specimens (~67%), whilst in 4 teeth (~33%), it was incomplete. Meanwhile, tunnel defects, tubular and atubular bridges were detected in two (~17%), eight (~67%) and two (~17%) teeth, respectively. In the iRoot BP group, complete and incomplete bridge formation was evident in seven (~58%) and five (~42%) specimens, respectively. Tubular and atubular appearance and tunnel defects were present in three (25%), eight (~67%) and one (~8%), respectively (Tables 1 and 2).

As shown by the Mann–Whitney test, the difference between the groups was not significant in terms of hard tissue formation ($P = 0.317$) and appearance of the bridge ($P = 0.414$).

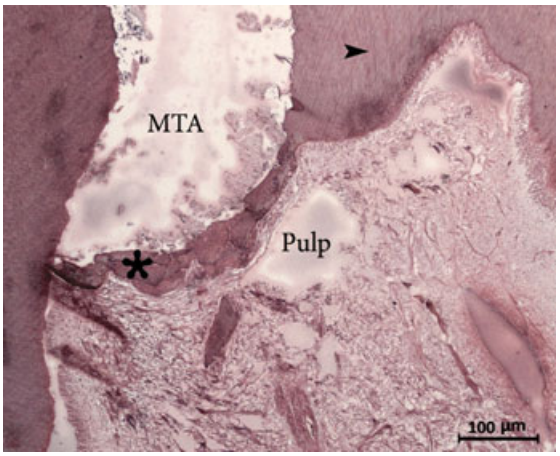

Figure 2 A light microscopic view of Cvek pulpotomy with MTA, the buccal pulp horn and a small part of the coronal pulp are removed. Note the hard tissue bridge (black star) formed by the newly differentiated cells and also the secondary dentine (black arrow head). (H&E, magnification 4 ×).

Pulp inflammation

In terms of inflammatory infiltration, none of the specimens in either group scored 0 (no inflammation) or 4 (abscess formation). In the ProRoot MTA group, seven specimens (~58%) had mild inflammation, whilst four (~33%) and one (~8%) showed moderate and severe inflammation, respectively. In the iRoot BP group, eight teeth (~67%) were diagnosed with mild inflammation whilst three (25%) had moderate inflammation. Like the ProRoot MTA group, one case (~8%) was severely inflamed (Table 3).

In both groups, inflammatory infiltration revealed the absence of poly-morphonuclear cells (PMNs) with mononuclear white blood cells (WBCs) being dominant. The Mann–Whitney test revealed the difference between the two groups was not significant ($P = 0.366$).

Sensitivity to thermal stimuli

Amongst the 24 specimens, only five had sensitivity to cold stimuli, which did not exceed 1–2 s. All of these teeth were in the iRoot BP group. Four of these teeth were scored as mildly inflamed, whilst one had moderate inflammation. The chi-square test revealed a significant difference between the groups

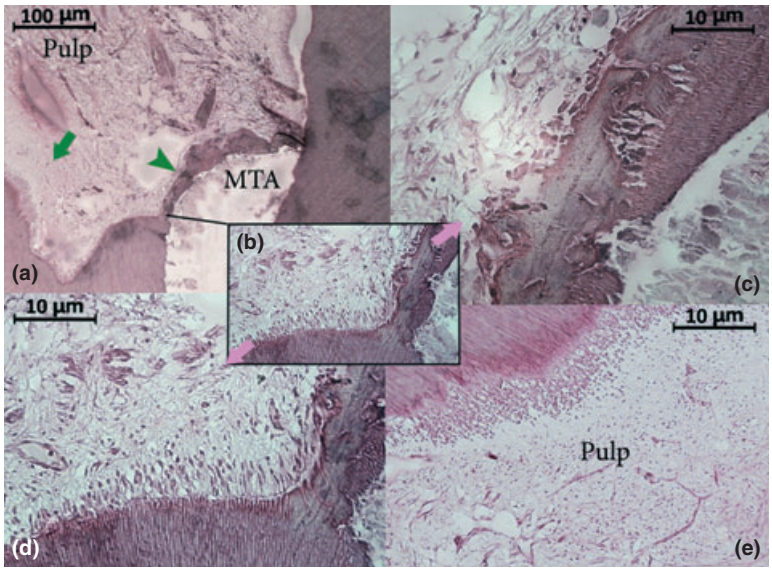

Figure 3 (a) A 4 × view of the same tooth in Fig. 1 treated with ProRoot MTA. Note the hard tissue bridge (green arrow head). (b) A 20 × view of contact point between secondary dentine and the hard tissue bridge. Compare the secondary dentine and primary odontoblasts (bottom) with hard tissue bridge created by newly differentiated cells (right). (c) A 40 × view of hard tissue bridge and its producing cells. Note the flat shape of the new cells and globular appearance of the formed bridge. (d) A 40 × view of primary odontoblasts and secondary dentine with tubular appearance. Note the columnar shape of the odontoblasts and palisading look of their nuclei and also the tubular appearance of the dentine. (e) A 40 × view of the underlying pulp with collagen fibres, spindle shape nuclei of the fibroblasts and infiltration of WBCs (H&E).

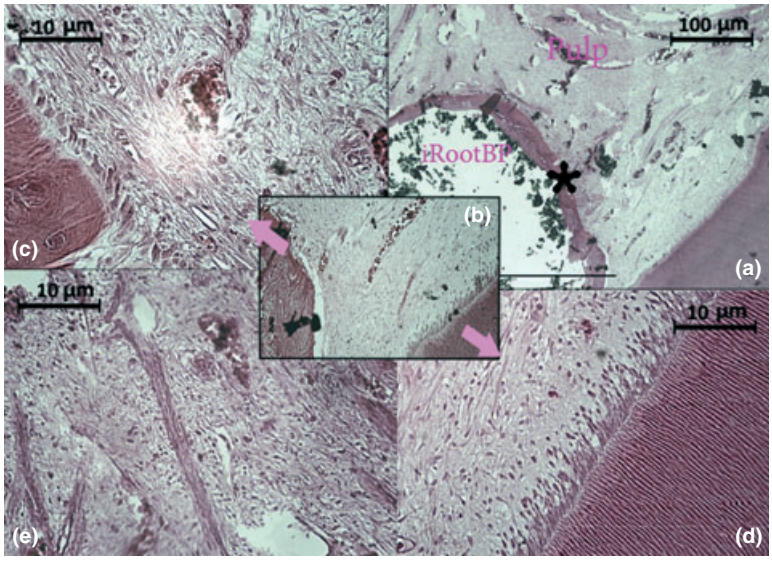

Figure 4 (a) A 4 × view of a tooth treated with iRoot BP. Note the hard tissue bridge (black star). (b) A 20 × view of contact point between secondary dentine and the hard tissue bridge. (c) A 40 × view of hard tissue bridge and its producing cells. Note the flat shape of the newly differentiated cells and globular appearance of the formed bridge. (d) A 40 × view of primary odontoblasts and primary dentine with tubular appearance. Note the columnar shape of the odontoblasts and palisading look of the nuclei and also the tubular appearance of the dentine. (e) A 40 × view of the underlying pulp with collagen fibres, spindle shape nuclei of the fibroblasts and infiltration of white blood cells (H&E).

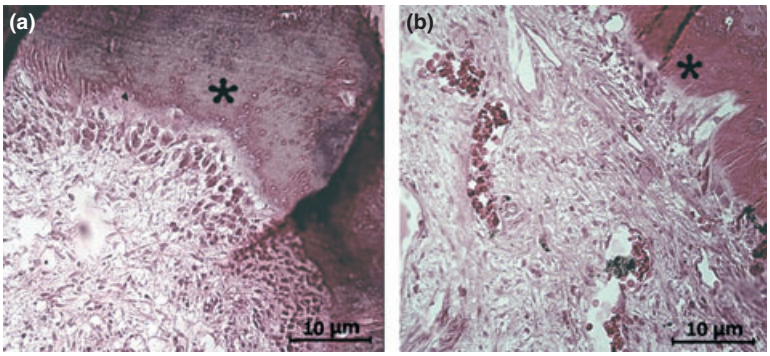

Figure 5 (a) A 40 × view of the hard tissue bridge in a right maxillary premolar treated with ProRoot MTA. (b) A 40 × view of a left maxillary premolar in the same patient treated with iRoot BP. Note the newly differentiated hard tissue producing cells in both images and also the appearance of the hard tissue bridge (H&E).

Table 1 Number (per cent) of hard tissue formation

	Hard tissue formation			
	None	Partial	Complete	Total
iRoot BP	0 (0)	5 (41.7)	7 (58.3)	12 (100)
MTA	0 (0)	4 (33.3)	8 (66.7)	12 (100)
Total	0 (0)	9 (37.5)	15 (62.5)	24 (100)

Table 2 Appearance (per cent) of hard tissue bridge

	Appearance of hard tissue bridge			
	Tubular	Atubular	Tunnel defects	Total
iRoot BP	3 (25)	8 (66.7)	1 (8.3)	12 (100)
MTA	2 (16.7)	8 (66.7)	2 (16.7)	12 (100)
Total	5 (20.8)	16 (66.7)	3 (12.5)	24 (100)

($P < 0.05$). None of the patients complained of sensitivity upon chewing or palpation, swelling, spontaneous pain or stimulated discomfort, and there were no signs of PDL widening on periapical radiographs.

Discussion

This was a randomized controlled clinical trial, on healthy human premolars randomly treated with tooth-coloured ProRoot MTA or iRoot BP, as partial pulpotomy materials. Clinical symptoms were evaluated and teeth were extracted after 6 weeks to histologically compare the pulp inflammation, formation of hard tissue bridges and their structure.

Numerous studies designed to evaluate the effect(s) of different materials on pulp tissue have been conducted on human or experimental animal teeth with *sound* teeth (Lu *et al.* 2008, Accorinte *et al.* 2009, Kiatwateeratana *et al.* 2009, Nair *et al.* 2009, Parirokh *et al.* 2011). The dental pulp has a compromised ability to respond to external irritants due to its encasement in a rigid chamber and lack of collateral circulation (Bender & Bender 2003). Thus, the more severely the pulp is inflamed, the less will be its ability to respond to further irritation. Every effort should be made to minimize additional irritation because it can convert the pulpal inflammatory status from reversible to irreversible. Under the circumstances of the present study, pulps were expected to form a hard tissue barrier in contact with the biomaterials (Kakehashi *et al.* 1965). A Cvek pulpotomy was chosen because it represents a substantial area of contact between the pulp tissue and the biomaterial (Kiatwateeratana *et al.* 2009), and the hard tissue bridge is likely to be formed by newly differentiated cells (Barrieshi-Nusair & Qudeimat 2006). In

Table 3 Number (per cent) of pulp inflammatory state

	Inflammation grade					
	0- None	1-Mild	2-Moderate	3-Severe	4- Abscess	Total
iRoot BP	0(0)	8(66.7)	3(25.0)	1(8.3)	0(0)	12(100)
MTA	0(0)	7(58.3)	4(33.3)	1(8.3)	0(0)	12(100)
Total	0(0)	15(62.5)	7(29.2)	2(8.3)	0(0)	24(100)

this way, it was possible to assess the effects of the biomaterials on pulp tissue and differentiation of new cells capable of producing a mineralized bridge (Figs 3–5).

During a time period of 42 days, all cases had formed a hard tissue bridge (Figs 3 and 4). Hard tissue bridges are permeable, and although this feature reduces over time as the bridge gets older and thicker, they never become impermeable (Matsuo *et al.* 1996). Known as 'tunnel defects', this porous feature is the result of cellular inclusions and empty vascular spaces; it indicates severe pulp injury at the time of exposure (Asgary *et al.* 2006, Cox *et al.* 1996, Matsuo *et al.* 1996). Thus, the cavity needs to be completely sealed so that the dentine bridge acts as a secondary barrier to protect the pulp. Preventing pulp injury and sealing the MTA with glass–ionomer were the important aspects of the present study. The results revealed that most of the hard tissue bridges were free from tunnel defects (Fig. 5). In addition, placement of glass–ionomer over partially set MTA did not affect its hydration, and the glass–ionomer setting was not disrupted by the presence of MTA (Ballal *et al.* 2008).

In the iRoot BP group, five patients reported sensitivity to cold, which lasted for few seconds. In microscopic evaluation, four of these cases had mild and one moderate inflammation. It is possible that host-derived factors can cause the difference between clinical symptoms and the histopathologic status of the pulp, a situation known as 'painless pulpitis' (Michaelson & Holland 2002). The inflammatory infiltration in all cases was composed of mononuclear WBCs, and the absence of polymorphonuclear (PMN) cells was evident (Fig. 5), which rules out acute inflammation (Hahn & Liewehr 2007). Previous immuno-histochemistry studies have shown that these mononuclear cells are T-lymphocytes, B-cells and macrophages (Hahn & Liewehr 2007).

Previous studies aimed to evaluate the nature of hard tissue bridges formed subsequent to pulp capping with MTA (Asgary *et al.* 2006). Scanning electron microscope (SEM) and electron probe microanalysis revealed that the hard tissue bridge is composed of collagen bundles containing centres of calcification (calcium phosphate compounds) whilst the bridge close to the pulpal surface exhibited irregular *dentinal* tubules (Asgary *et al.* 2006). Many studies have evaluated the quality of the hard tissue bridge by histological assessment (Barrieshi-Nusair & Qudeimat 2006, Cox *et al.* 1996, Accorinte *et al.* 2009, Kiatwateeratana *et al.* 2009). It should be noted, however, that the results of this clinical and histological assessment do not reveal the true effects of bioceramics in a clinically relevant context when the pulp is already inflamed due to caries.

Conclusion

No significant difference between ProRoot MTA and iRoot BP in terms of pulp inflammation, formation of hard tissue bridge and its appearance was detected. The teeth treated with MTA had less sensitivity to cold. Performing large-scale clinical trials with longer follow-up periods on carious teeth is recommended.

Acknowledgement

The authors would like to appreciate Iranian Center for Endodontic Research (ICER), Shahid Beheshti University of Medical Sciences, Tehran, Iran, for preparation of the microscopic photography. We are also thankful to Dr. Mohammad Javad Kharazifard and all the patients and their parents without whom the study would be impossible. The authors deny any conflict of interest.

References

Accorinte ML, Loguercio AD, Reis A, *et al.* (2009) Evaluation of two mineral trioxide aggregate compounds as pulp-capping agents in human teeth. *International Endodontic Journal* **42**, 122–8.

Asgary S, Parirokh M, Eghbal MJ, Ghoddusi J, Eskandarizadeh A (2006) SEM evaluation of neodentinal bridging after direct pulp protection with mineral trioxide aggregate. *Australian Endodontic Journal* **32**, 26–30.

Ballal S, Venkateshbabu N, Nandini S, Kandaswamy D (2008) An *in vitro* study to assess the setting and surface crazing of conventional glass ionomer cement when layered over partially set mineral trioxide aggregate. *Journal of Endodontics* **34**, 478–80.

Barrieshi-Nusair KM, Qudeimat MA (2006) A prospective clinical study of mineral trioxide aggregate for partial pulpotomy in cariously exposed permanent teeth. *Journal of Endodontics* **32**, 731–5.

Bender IB, Bender AB (2003) Diabetes mellitus and the dental pulp. *Journal of Endodontics* **29**, 383–9.

Cox CF, Subay RK, Ostro E, Suzuki S, Suzuki SH (1996) Tunnel defects in dentin bridges: their formation following direct pulp capping. *Operative Dentistry* **21**, 4–11.

Cvek M (1978) A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fracture. *Journal of Endodontics* **4**, 232–7.

Damas BA, Wheater MA, Bringas JS, Hoen MM (2011) Cytotoxicity comparison of mineral trioxide aggregates and EndoSequence bioceramic root repair materials. *Journal of Endodontics* **37**, 372–5.

De-Deus G, Canabarro A, Alves G, Linhares A, Senne MI, Granjeiro JM (2009) Optimal cytocompatibility of a bioceramic nanoparticulate cement in primary human mesenchymal cells. *Journal of Endodontics* **35**, 1387–90.

De-Deus G, Canabarro A, Alves GG, Marins JR, Linhares AB, Granjeiro JM (2012) Cytocompatibility of the ready-to-use bioceramic putty repair cement iRoot BP Plus with primary human osteoblasts. *International Endodontic Journal* **45**, 508–13.

Hahn CL, Liewehr FR (2007) Update on the adaptive immune responses of the dental pulp. *Journal of Endodontics* **33**, 773–81.

Kakehashi S, Stanley HR, Fitzgerald RJ (1965) The Effects of Surgical Exposures of Dental Pulps in Germ-Free and Conventional Laboratory Rats. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology* **20**, 340–9.

Kiatwateeratana T, Kintarak S, Piwat S, Chankanka O, Kamaolmatyakul S, Thearmontree A (2009) Partial pulpotomy on caries-free teeth using enamel matrix derivative or calcium hydroxide: a randomized controlled trial. *International Endodontic Journal* **42**, 584–92.

Koch KA, Brave DG (2012) Bioceramics, part I: the clinician's viewpoint. *Dentistry Today* **31**, 130–5.

Leal F, De-Deus G, Brandao C, Luna AS, Fidel SR, Souza EM (2011) Comparison of the root-end seal provided by bioceramic repair cements and White MTA. *International Endodontic Journal* **44**, 662–8.

Lu Y, Liu T, Li H, Pi G (2008) Histological evaluation of direct pulp capping with a self-etching adhesive and calcium hydroxide on human pulp tissue. *International Endodontic Journal* **41**, 643–50.

Ma J, Shen Y, Stojicic S, Haapasalo M (2011) Biocompatibility of two novel root repair materials. *Journal of Endodontics* **37**, 793–8.

Marcacci M, Kon E, Moukhachev V, *et al.* (2007) Stem cells associated with macroporous bioceramics for long bone repair: 6- to 7-year outcome of a pilot clinical study. *Tissue Engineering* **13**, 947–55.

Matsuo T, Nakanishi T, Shimizu H, Ebisu S (1996) A clinical study of direct pulp capping applied to carious-exposed pulps. *Journal of Endodontics* **22**, 551–6.

Michaelson PL, Holland GR (2002) Is pulpitis painful? *International Endodontic Journal* **35**, 829–32.

Nair PN, Duncan HF, Pitt Ford TR, Luder HU (2009) Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with Mineral Trioxide Aggregate: a randomized controlled trial. *International Endodontic Journal* **42**, 422–44.

Nosrat A, Asgary S (2010) Apexogenesis of a symptomatic molar with calcium enriched mixture. *International Endodontic Journal* **43**, 940–4.

Paranjpe A, Zhang H, Johnson JD (2010) Effects of mineral trioxide aggregate on human dental pulp cells after pulp-capping procedures. *Journal of Endodontics* **36**, 1042–7.

Parirokh M, Torabinejad M (2010) Mineral trioxide aggregate: a comprehensive literature review–Part III: Clinical applications, drawbacks, and mechanism of action. *Journal of Endodontics* **36**, 400–13.

Parirokh M, Asgary S, Eghbal MJ, Kakoei S, Samiee M (2011) A comparative study of using a combination of calcium chloride and mineral trioxide aggregate as the pulp-capping agent on dogs' teeth. *Journal of Endodontics* **37**, 786–8.

Park JW, Hong SH, Kim JH, Lee SJ, Shin SJ (2010) X-Ray diffraction analysis of white ProRoot MTA and Diadent BioAggregate. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology* **109**, 155–8.

Shokouhinejad N, Nekoofar MH, Razmi H, *et al.* (2012) Bioactivity of EndoSequence root repair material and bioaggregate. *International Endodontic Journal* **45**, 1127–34.

Sigurdsson A (2004) Pulpal diagnosis. *Endodontic Topics* **5**, 12–25.

Wataha JC (2012) Predicting clinical biological responses to dental materials. *Dental Materials* **28**, 23–40.

Yan P, Yuan Z, Jiang H, Peng B, Bian Z (2010) Effect of bioaggregate on differentiation of human periodontal ligament fibroblasts. *International Endodontic Journal* **43**, 1116–21.

Yuan Z, Peng B, Jiang H, Bian Z, Yan P (2010) Effect of bioaggregate on mineral-associated gene expression in osteoblast cells. *Journal of Endodontics* **36**, 1145–8.

Zhang H, Pappen FG, Haapasalo M (2009) Dentin enhances the antibacterial effect of mineral trioxide aggregate and bioaggregate. *Journal of Endodontics* **35**, 221–4.

Zhang S, Yang X, Fan M (2013) BioAggregate and iRoot BP Plus optimize the proliferation and mineralization ability of human dental pulp cells. *International Endodontic Journal* **46**, 923–9.