

# Histologic Assessment of Human Pulp Response to Capping with Mineral Trioxide Aggregate and a Novel Endodontic Cement

Mobammad Hassan Zarrabi, DDS,\* Maryam Javidi, DDS,\* Amir Hossein Jafarian, MD,<sup>†</sup> and Babareh Joushan, DDS\*

## Abstract

**Introduction:** This study was conducted to compare human pulp response to mineral trioxide aggregate (MTA) and a novel endodontic cement (NEC) when used as pulp capping materials after a time period of 2 and 8 weeks. **Methods:** Thirty-two premolar teeth that were scheduled for extraction for orthodontic reasons were exposed and capped with either MTA or NEC. Half of the specimens underwent extraction and histologic analysis after 2 weeks, and the remaining half were assessed after 8 weeks. Each slide was graded histologically according to the morphology of the dentinal bridge, thickness of the dentinal bridge, presence of odontoblast cells, and inflammation of the pulp. **Results:** Both MTA and NEC showed significantly better pulp response after 8 weeks compared with 2 weeks, with a thicker and more tubular pattern of the dentinal bridge, less pulp inflammation, and a palisade pattern of odontoblast cells. Although MTA and NEC groups had no significant difference in each measure in both time intervals, NEC induced a thicker dentinal bridge with less pulp inflammation at both 2 weeks and 8 weeks, compared with MTA. **Conclusions:** MTA and NEC showed similar biocompatibility and favorable response in pulp capping treatment and inducing the formation of the dentinal bridge. (*J Endod* 2010;36:1778–1781)

## Key Words

Direct pulp cap, histology, MTA, NEC

From the \*Department of Endodontics, Faculty of Dentistry and Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, and Iranian Endodontic Research Center, Tehran, Iran; and <sup>†</sup>Department of Pathology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.

Address requests for reprints to Dr Amir Hossein Jafarian, Department of Pathology, Ghaem Hospital, Mashhad University of Medical Sciences, Ahmad Abad St. Parastar Av., Mashhad, Khorasan, Iran. E-mail address: jafarianah@mums.ac.ir. 0099-2399/\$ - see front matter

Copyright © 2010 American Association of Endodontists. doi:10.1016/j.joen.2010.08.024

Conservative pulp therapy is indicated whenever the remaining pulp exhibits reversible pulpitis and can induce a reparative barrier that protects the tissue from microbial challenges. In particular, direct pulp capping can be performed for teeth with deep caries, mechanical exposures, and traumatic injuries to maximize pulp preservation. The goal of treating the exposed pulp with an appropriate pulp capping material is to promote the dentinogenic potential of pulp cells (1, 2).

Dentin bridge formation can occur under a variety of pulp capping materials. Direct pulp capping with mineral trioxide aggregate (MTA) has proved to be effective in stimulating tertiary dentin formation in canine models and primates (3). However, the mechanism by which this occurs has not been explained. MTA has been found to induce a significantly greater frequency of dentinal bridge formation, less pulp inflammation, and bridges with greater mean thickness compared with calcium hydroxide (3–5). However, MTA has a delayed setting time, poor handling characteristics, an off-white color, and is more expensive (6).

Recently, a novel endodontic cement (NEC) with different chemical composition from MTA (7, 8), but with identical applications, has been developed (9, 10). NEC consisting of different calcium compounds (ie, calcium oxide, calcium phosphate, calcium carbonate, calcium silicate, calcium sulfate, and calcium chloride) combines the biocompatibility of MTA with more efficient characteristics such as significantly shorter setting time, good handling characteristics, and no tooth staining (7, 10, 11). This cement has been evaluated in several research projects. Asgary et al (10) showed that NEC can result in better pulp response compared with MTA when used as a pulp capping agent and also can result in higher mean thickness of the dentinal bridge than MTA.

The purpose of the present study was to evaluate the histomorphologic response of human dental pulp to capping with MTA and NEC in 2-week and 8-week intervals after pulp capping.

## Material and Methods

This prospective randomized controlled clinical trial was performed *in vivo* and was approved by the Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran, in 2009, and the protocol was registered at clinical [trials.gov](http://trials.gov) (Clinicaltrials.gov ID: NCT01066533). First premolars that were scheduled for extraction for orthodontic reasons were selected. Inclusion criteria included the following: (1) patients who were 15–25 years old; (2) patients with no systemic disease; (3) teeth with clinically normal pulps, with closed apex, no caries either clinically or radiographically, and without any restoration; and (4) teeth with no periodontal involvement.

A total of 32 teeth were used, and according to the material used for pulp capping and the duration of follow-up, they were divided into 4 groups: NEC with 2-week follow-up (group 1), NEC with 8-week follow-up (group 2), MTA with 2-week follow-up (group 3), and MTA with 8-week follow-up (group 4). Teeth were randomly assigned to treatment groups by using a statistical randomized treatment table. Two intact teeth were selected as the control group. Signed consent was given by patients and their parents after they had received a thorough explanation related to the study. After local anesthesia, operative procedures were performed with rubber dam placement and

**TABLE 1.** Histologic Features and the Grading System to Evaluate the Samples

Feature	Grade I (worst)	Grade II	Grade III (best)
Morphology of dentinal bridge	No tubules present	Irregular pattern of tubules	Regular pattern of tubules
Thickness of dentinal bridge	<0.1 mm	0.1–0.25 mm	>0.25 mm
Intensity of pulp inflammation	Severe inflammation or abscess formation	Minimal to moderate inflammation	No inflammation
Odontoblast layer	Absent	Presence of odontoblast cells	Palisade pattern of cells

disinfected with 2% chlorhexidine gluconate. Class I occlusal cavities were prepared with ½ round carbide burs under air-distilled water cooling. At the exposure site hemorrhage was controlled by sterile cotton pellets, saline, and 5.25% NaOCl. NEC cement was mixed with its liquid to provide a dense creamy mixture, and it was applied as pulp capping material in groups 1 and 2. MTA (Angelus, Londrina, Brazil) was mixed according to the manufacturer’s instructions until it had the consistency of wet sand. In experimental groups 3 and 4, each group had 8 teeth receive MTA as pulp capping material. All procedures were performed by the same operator, and the test materials were applied in a blinded fashion. The cavities were sealed immediately by sandwich technique, with a layer of Fuji II glass ionomer (GC International Corp, Tokyo, Japan) and composite resin (Helomolar HB; Ivoclar Vivadent, Schaan, Liechtenstein).

After 2 weeks teeth in groups 1 and 3 were extracted, and after 8 weeks teeth in groups 2 and 4 were extracted. After extraction, the apical third of the root was sectioned under water cooling, allowing better formalin fixation. The specimens were fixed in 10% formalin for 24 hours and then were decalcified in 14% ethylenediaminetetraacetic acid (4–6 months). They were then embedded in paraffin and cut at a microtome setting for 5 µm. The sections were stained with hematoxylin-eosin (H&E). Samples were evaluated microscopically in a blinded manner by a pathologist by using a light microscope equipped for histometry. The pathologist was not aware of the types of capping material. Each slide was graded from I to III (Table 1) according to criteria that were based on the standards defined by earlier studies (10, 12, 13). The data were analyzed with Mann-Whitney tests. Statistically significant differences were set at  $P < .05$ .

**Results**

The control intact teeth showed normal pulp tissue with no inflammatory cells, with columnar odontoblast cells. A summary of the results is presented in Table 2.

In the NEC group after 2 weeks (group 1), there was fibrous tissue with no calcification at the exposure site in 62.5% of specimens, and only 37.5% showed some evidence of calcified tissues in the fibrous matrix. In 50% of specimens the mean thickness of barrier was 0.1–0.25 mm, and the others were less. In this group, 50% of samples had no inflammation. Although 75% showed the presence of onto-

blast cells beneath the barrier, 25% showed an absence of these cells (Fig. 1A, B).

In the NEC group after 8 weeks (group 2), 50% of samples showed a hard tissue barrier at the exposure site with regular tubules, and 75% showed sufficient thickness of the bridge (>0.25 mm). In this group 87.5% showed no inflammation, and 37.5% of specimens showed palisade pattern of odontoblast cells beneath the dentinal bridge (Fig. 1C–E).

In the MTA group after 2 weeks (group 3), 37.5% of samples showed fibrous tissue formed at the exposure site with no calcification. Also, in 62.5% the mean thickness of the formed tissue was <0.1 mm. Minimum to moderate inflammation was observed in 62.5% of samples, and the odontoblast cells were present in 75% of specimens (Fig. 1F, G).

In the MTA group after 8 weeks (group 4), 75% of samples showed a calcified dentinal bridge with irregular tubules, although regular tubules were present in 25%. In this group 37.5% of specimens showed adequate thickness of the dentinal bridge (>0.25 mm). Also 75% showed no inflammation, and in 50% of samples a palisade pattern of odontoblast cells was observed adjacent to the dentinal bridge (Fig. 1H–J). The Mann-Whitney test revealed that according to the morphology of the dentin bridge, there was a significant difference between groups 1 and 2 ( $P = .003$ ) and between groups 3 and 4 ( $P = .029$ ). The difference between other groups was not significant.

According to the thickness of the dentinal bridge, there was also a significant difference between groups 1 and 2 ( $P = .002$ ) and between groups 3 and 4 ( $P = .005$ ).

Evaluating the odontoblast-like cell layer showed that there was a significant difference between groups 1 and 2 ( $P = .029$ ) and between groups 3 and 4 ( $P = .015$ ). However, the difference was not significant between other groups. These findings showed that the morphology and thickness of the dentinal bridge and odontoblast-like cell alignment were improved in NEC and MTA groups after 8 weeks, compared with 2 weeks.

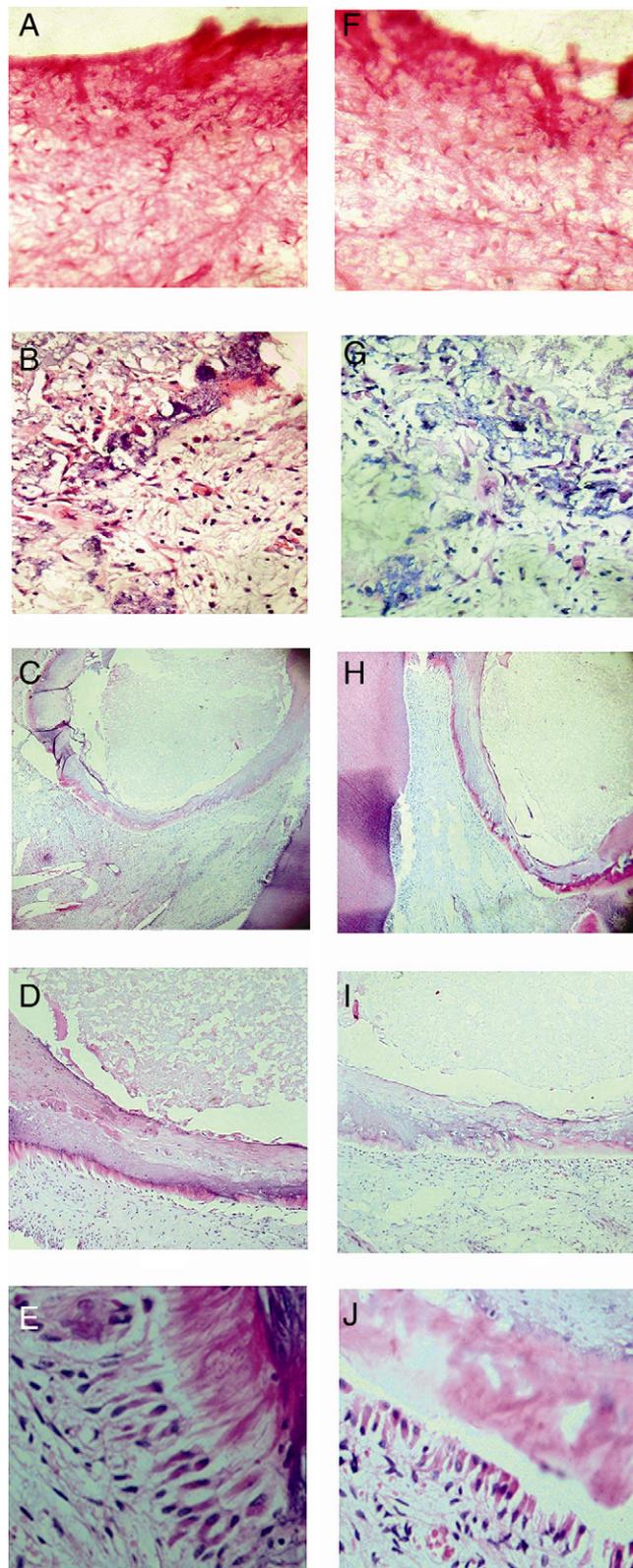
This test showed that there was no significant difference between groups according to intensity of inflammation, except between groups 3 and 4 ( $P = .011$ ), which showed less inflammation in group 4 compared with group 3.

**Discussion**

Preserving the vitality of exposed pulp, particularly in immature teeth, is the ultimate goal in vital pulp therapy. Stanley (12) advocated that pulp capping procedures could be performed successfully on

**TABLE 2.** Number (%) of Different Categories of Histologic Features for Each Group According to the Scores (Table 1)

Feature	Group 1			Group 2			Group 3			Group 4		
	I	II	III	I	II	III	I	II	III	I	II	III
Morphology of dentinal bridge	5 (62.5)	3 (37.5)	0	0	4 (50)	4 (50)	3 (37.5)	5 (62.5)	0	0	6 (75)	2 (25)
Thickness of dentinal bridge	4 (50)	4 (50)	0	0	2 (25)	6 (75)	5 (62.5)	3 (37.5)	0	0	5 (62.5)	3 (37.5)
Intensity of pulp inflammation	1 (12.5)	3 (37.5)	4 (50)	0	1 (12.5)	7 (87.5)	2 (25)	5 (62.5)	1 (12.5)	0	2 (25)	6 (75)
Odontoblast layer	2 (25)	6 (75)	0	0	5 (62.5)	3 (37.5)	2 (25)	6 (75)	0	0	4 (50)	4 (50)



**Figure 1.** (A) Formation of fibrous bridge after pulp capping with NEC in 2 weeks (H&E; original magnification,  $\times 400$ ). (B) Calcification in fibrous bridge (H&E; original magnification,  $\times 400$ ). (C) Formation of dentinal bridge after pulp capping with NEC in 8 weeks (H&E; original magnification,  $\times 100$ ). (D) Magnification of (C) showing mineralization of dentinal bridge (H&E; original magnification,  $\times 400$ ). (E) Odontoblast-like cells beneath calcified bridge (H&E; original magnification,  $\times 1000$ ). (F) Formation of fibrous bridge

asymptomatic carious exposures. Haskell et al (13) proved that asymptomatic carious exposures could survive an average of 12 years after pulp capping. MTA has been introduced with reasonable properties for direct pulp capping such as less inflammation and greater dentinal bridge formation, compared with traditional materials like Ca(OH)<sub>2</sub> (3–5). According to the appropriate characteristics of this material, in the present study, MTA was selected as a pulp capping material. However, there have been some disadvantages with the clinical application of MTA. Recently, researchers have shown other materials to have the same biocompatibility as MTA and with advantages over it. In a study evaluating the rat subcutaneous tissue response to a fast endodontic cement (CER) and Angelus MTA, the CER response was similar to that of Angelus MTA, characterized by organized connective tissue and presence of some chronic inflammatory cells, and CER was biocompatible and stimulated mineralization (14). In another study comparing physical and chemical properties of white MTA with 3 experimental root-end filling materials (Generex-A, Capasio, and Ceramicrete-D), Porter et al (15) claimed that the washout resistance, radiopacity, and compressive strength of Generex-A and Capasio make them suitable alternatives for MTA. However, these materials need to be further studied.

According to previous studies on an NEC, researchers have claimed that NEC has the same biocompatible characteristics of MTA, the same favorable results for pulp capping, but with better handling characteristics and significantly shorter setting time (7, 10). Thus in the present study NEC was selected as the other pulp capping material to compare its effectiveness against MTA.

In the present study a period of 8 weeks was used to evaluate pulp response to capping materials. The same time interval was used in a recent study by Asgary et al (11) and in many other studies (16–21). Moreover, it was programmed to analyze pulp response in another earlier time interval to find whether there is any difference in response compared with 8 weeks. The 2-week interval was selected on the basis of the study by Tziafas et al (22), which showed the initiation of hard tissue formation after 2 weeks.

The present study demonstrated that pulp capping with MTA induces the formation of fibrodentin and reparative dentin at the surface in direct contact with MTA. These findings are in agreement with other studies, which demonstrated the high success rate of MTA in pulp capping procedures (11, 16, 21, 22). The initial effect of MTA on the surface of exposed pulp is the formation of a superficial layer of crystalline structures onto the pulpal surface. Columnar odontoblast-like cells demonstrating good cytoplasmic organization are further arranged along the crystalline structures. Although MTA does not include Ca(OH)<sub>2</sub> in its composition, after it hardens, it contains calcium oxide that could react with tissue fluids to form calcium hydroxide (11). Therefore, it seems that the MTA mechanism of action is similar to calcium hydroxide. Hard tissue bridge formation next to MTA might be due to its high alkalinity (23), sealing ability (9, 24), and biocompatibility (25). According to the present study, pulps capped with MTA, after 2 weeks, showed a thin layer of fibrous matrix with a few calcified areas under the MTA. Most of them showed minimum to moderate inflammation, and also a few odontoblast-like cells could be seen under this matrix, whereas none of these cells were in a palisade

after pulp capping with MTA in 2 weeks (H&E; original magnification,  $\times 400$ ). (G) Calcification in fibrous bridge (H&E; original magnification,  $\times 400$ ). (H) Formation of dentinal bridge after pulp capping with NEC in 8 weeks (H&E; original magnification,  $\times 100$ ). (I) Magnification of (C) showing mineralization of dentinal bridge (H&E; original magnification,  $\times 400$ ). (J) Odontoblast-like cells beneath calcified bridge (H&E; original magnification,  $\times 1000$ ).

pattern. These findings were in agreement with the findings by Tziafas et al (22), showing the initiation of dentinal bridge formation at 2 weeks.

Formation of the dentinal bridge at the interface of pulp and pulp capping material is a controversial issue, because dentinal bridge presence does not necessarily prove a healthy status of the pulp and does not protect the pulp from microbial challenges. However, it can be a sign of healing or a reaction to irritation (2, 10). In the present study, formation of the dentinal bridge was interpreted as a sign of healing.

There was a significant difference between pulp response to MTA after 2 weeks and 8 weeks. After 8 weeks there was less inflammation, a palisade pattern of odontoblast-like cells, and a thicker hard tissue barrier with a more tubular pattern. These findings confirmed that after 2 weeks, pulps capped with MTA will show initiation of reparative dentin formation. Gradually this matrix would be calcified with tubular pattern, and the odontoblast-like cells get a palisade pattern, forming an effective dentinal bridge.

Regarding the studies evaluating the properties of NEC (7–11), this material was used as a pulp capping material to evaluate its efficiency against MTA. Although there was less pulp inflammation in teeth capped with NEC after 8 weeks compared with 2 weeks, the difference was not significant. However, the dentinal bridge was thicker, with a more tubular pattern and a more palisade pattern of odontoblast-like cells after 8 weeks, which demonstrated that the dentinal bridge under NEC would gradually be calcified and have sufficient thickness (>0.25 mm).

Comparing MTA and NEC, NEC showed less pulp inflammation and a thicker dentinal bridge at both time intervals; also there was a more tubular pattern of formed hard tissue barrier under NEC after 8 weeks, but these differences were not significant. These data suggest the biocompatibility and efficiency of NEC as a pulp capping material. This cement contains several calcium compounds that result in a rich pool of calcium and phosphorous ions. These elements are used in the process of hydroxyapatite production, which is a natural product of dental pulp cells (26). This process might be responsible for its biocompatibility.

The present study was performed on normal healthy pulps. Therefore, these results do not necessarily reflect what will happen with both MTA and NEC if they are used on inflamed pulps. Most of the previously published studies for evaluation of pulp response to pulp capping materials were performed in healthy intact teeth of animals (3, 16, 22). Although the present investigation is an *in vivo* evaluation, careful consideration is necessary to adapt these results with clinical situations. Therefore, the authors suggest that further assessment is required for evaluation of pulp response to these pulp capping materials in inflamed pulp. Moreover, further research with immunohistochemistry, scanning electron microscopy, and transmission electron microscopy would provide better understanding of the mechanism of action of NEC.

In conclusion, the results of the present *in vivo* study demonstrated that NEC and MTA have favorable outcomes when used as pulp capping materials. Both materials showed biocompatibility and induced the formation of a complete dentinal bridge at its interface with the pulp tissue. However, these results apply to pulp capping in healthy pulps with no inflammation. Therefore, their application on inflamed pulps needs to be studied. Regarding the appropriate characteristics and advantages of NEC over MTA, such as easier handling, shorter setting time, and no tooth staining, NEC could be considered as an alternative to MTA in pulp capping treatment.

## Acknowledgments

*This study was supported by Vice Chancellor for Research of Mashhad University of Medical Sciences and Endodontic Research Center of Shabid Beheshti University M.C., Tebran, Iran. The authors wish to thank Dr Hamid Jafarzadeh for assistance in preparing this manuscript and D. Saeed Asgary for providing NEC cement.*

*The authors deny any conflicts of interest.*

## References

- Ingle J, Bakland M, Baumgartner J. Endodontics. 6th ed. Hamilton, Canada: BC Decker Inc; 2008. 1313.
- Schröder U. Effects of calcium hydroxide-containing pulp-capping agents on pulp cell migration, proliferation and differentiation. *J Dent Res* 1985;64:541–8.
- Ford TR, Torabinejad M, Abedi HR, Bakland LK, Kariyawasam SP. Using mineral trioxide aggregate as a pulp-capping material. *J Am Dent Assoc* 1996;127:1491–4.
- Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS. Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. *Int Endod J* 2003;36:225–31.
- Junn DJ. Quantitative assessment of dentin bridge formation following pulp capping with mineral trioxide aggregate [master's thesis]. Loma Linda, CA: Loma Linda University; 2000.
- Chng HK, Islam I, Yap AU, Tong YW, Koh ET. Properties of a new root end filling material. *J Endod* 2005;31:665–8.
- Asgary S, Shahabi S, Jafarzadeh T, Amini S, Kheirieh S. The properties of a new endodontic material. *J Endod* 2008;34:990–3.
- Asgary S, Eghbal MJ, Parirokh M, Ghodduji J, Kheirieh S, Brink F. Comparison of mineral trioxide aggregate's composition with Portland cements and a new endodontic cement. *J Endod* 2009;35:243–50.
- Asgary S, Eghbal MJ, Parirokh M. Sealing ability of a novel endodontic cement as a root end filling material. *J Biomed Mater Res* 2008;87:706–9.
- 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;106:609–14.
- Asgary S, Parirokh M, Eghbal MJ, Ghodduji J. SEM evaluation of pulp reaction to different pulp capping materials in dog's teeth. *Iran Endod J* 2006;4:117–22.
- Stanley HR. Pulp capping: conserving the dental pulp—can it be done? is it worth it? *Oral Surg Oral Med Oral Pathol* 1989;68:628–39.
- Haskell EW, Stanley HR, Chellemi J, Stringfellow H. Direct pulp capping treatment: a long term follow up. *J Am Dent Assoc* 1978;97:607–12.
- Gomes-Filho JE, Rodrigues G, Watanabe S, et al. Evaluation of the tissue reaction to fast endodontic cement (CER) and Angelus MTA. *J Endod* 2009;35:1377–80.
- Porter ML, Bertó A, Primus CM, Watanabe I. Physical and chemical properties of new-generation endodontic materials. *J Endod* 2010;36:524–8.
- Domínguez MS, Witherspoon DE, Gutmann JL, Opperman LA. Histological and scanning electron microscopy assessment of various vital pulp therapy materials. *J Endod* 2003;29:324–33.
- Briso AI, Rahal V, Mestreneur SR, Dezan Junior E. Biological response of dogs submitted to different capping materials. *Braz Oral Res* 2006;20:219–25.
- Ishizaki NT, Matsumoto K, Kimura Y, Wang X, Yamashita A. Histopathological study of dental pulp tissue capped with enamel matrix derivative. *J Endod* 2003;29:176–9.
- Kolinitou-Koumpia E, Tziafas D. Pulpal responses following direct pulp capping of healthy dog teeth with dentin adhesive systems. *J Dent* 2005;33:639–47.
- Accorinte M de L, Holland R, Reis A, et al. Evaluation of mineral trioxide aggregate and calcium hydroxide cement as pulp capping agents in human teeth. *J Endod* 2008;34:1–6.
- Faraco IM Jr, Holland R. Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement. *Dent Traumatol* 2001;17:163–6.
- Tziafas D, Pantelidou O, Alvanou A, Belibasakis G, Papadimitriou S. The dentinogenic effect of mineral trioxide aggregate (MTA) in short term capping experiments. *Int Endod J* 2002;35:245–54.
- Torabinejad M, Hong CU, McDonald F, Pittford TR. Physical and chemical properties of a new root end filling material. *J Endod* 1995;21:349–53.
- Torabinejad M, Watson TF, Pittford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J Endod* 1993;19:591–5.
- Mitchell PJ, Pittford TR, Torabinejad M, McDonald F. Osteoblast biocompatibility of mineral trioxide aggregate. *Biomaterials* 1999;20:167–73.
- Smith AJ. Dentin formation and repair. In: Hargreaves KM, Goodis HE, eds. *Seltzer and Bender's dental pulp*. Chicago: Quintessence; 2002:41–62.