

A Comparative Study between Mineral Trioxide Aggregate and Calcium Hydroxide as Pulp Capping Agents in Dog's Teeth

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ABSTRACT

Introduction: This study was carried out in order to compare mineral trioxide aggregate (MTA) and dycal in dog's teeth as pulp capping agents.

Methods: After general and local anesthesia forty-two teeth of three dogs were used. In each tooth a class V cavity was prepared and the pulp was exposed using No.1 round bur. Then the exposed area was capped using either MTA or calcium hydroxide and the rest of the cavity was restored by amalgam. Radiographic image was taken before the procedure and after 4, 8, and 12 weeks. The animals were sacrificed at 4, 8, and 12 weeks and the teeth were prepared for H&E staining. The pulps of the teeth were evaluated for inflammation, necrosis, and formation and thickness of calcified bridge. Histologic data were analyzed by Kruskal Wallis, χ^2 , Cochran, and Friedman tests.

Results: Histopathologic results showed that in four weeks, MTA had significant differences with calcium hydroxide in the presence and thickness of calcified bridge ($p=0.046$) as well as inflammation ($p=0.014$) and hyperemia ($p=0.014$). Eight weeks MTA specimens showed significant difference in thickness of calcified bridge ($p=0.008$). Twelve weeks after pulp capping, the amount of necrosis and chronic inflammation were higher in dycal specimens; however, there was no significant difference between two experimental groups. Radiographic evaluation did not show any periapical lesion, internal and external resorption, or widening of periodontal ligament in none of the teeth at all time intervals.

Conclusion: Based on the result of this study, MTA showed to be a more reliable material as pulp capping agent in comparison with calcium hydroxide.

Key words: Gray MTA, Pulp capping, Calcium hydroxide.

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Introduction

Trauma and caries are two common causes of pulp necrosis¹. Pulpal vitality maintenance not only increases the survival rate of a tooth but also saves patients' and practitioners' time.

In many clinical situations, during tooth preparation and removal of decayed dentin, it is possible that the dental pulp be exposed accidentally². Several investigators have demonstrated that the exposed dental pulp has the capacity to heal when microleakage and bacterial contamination are prevented³⁻

⁵, therefore, it appears that an effective pulp capping material should be biocompatible, provide a biological seal, and prevent bacterial leakage⁶. A wide array of materials have been used⁷. Many authors believe that calcium hydroxide remains the standard material for pulp capping⁷. In fact, calcium hydroxide has been the most eligible candidate for capping of pulp exposures². It has been speculated that the high alkalinity of Ca (OH)₂ was responsible for producing a

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zone of superficial pulp necrosis just below the material interface at the exposure site. The necrotic tissue is supposedly removed by phagocytes and replaced by granulation tissue, along with an invasion of pioneer fibroblastic cells from which new odontoblastoid cells are thought to be developed. The result is production of a new dentin bridge⁸. However, calcium hydroxide shows many disadvantages when used as a pulp capping agent such as tunnel defect and inflammation of the pulp⁹.

A mineral trioxide aggregate (MTA) has been developed at Loma Linda University to seal communications between the tooth and the external surfaces¹⁰. This material was studied in a series of in vivo and in vitro investigations. MTA displays excellent low levels of microleakage when placed into extracted teeth¹¹⁻¹⁵. Furthermore, histologic findings in dogs have confirmed laboratory observations that this material has great potential to facilitate tissue healing¹⁶. MTA has been recommended for vital pulp therapy, root-end filing, apexification, and perforation repair¹⁰.

Therefore, the purpose of this study was to compare calcium hydroxide with MTA when used as pulp capping agents in dog's teeth in different time intervals.

Materials and Method

Forty-two teeth in three healthy 18-24 months old dogs were used. All experimental procedures were carried out according to protocols approved by the Ethics Committee of Research of Kerman University of Medical Sciences. Under general anesthesia with intramuscular injection of 20 mg/kg of Ketamine HCl (Alfasan-Holland) and 0.2 mg/kg of Xylazine (Alfasan-Holland), dog's teeth were rinsed by 0.2% chlorhexidine. An infiltration injection of mepivacaine 3% (HPEP-Germany) was used for local anesthesia and the teeth were isolated by rubber dam. Using a No.1 round bur in a high speed hand piece with copious water spray, class V cavities were prepared in

labial surface of the teeth and standardized pulp exposures (1mm in diameter) were obtained. Bleeding was controlled by irrigation with sodium hypochlorite and cotton pellets before placing the pulp capping materials. Quick setting calcium hydroxide paste (Dycal-Dentsply-Germany) or MTA (Pro-Root MTA Dentsply, Tulsa Dental, Tulsa, OK, USA) were prepared and alternatively were placed as pulp capping material on exposure sites. MTA was prepared according to the manufacturer's directions with MTA powder and sterile saline in a 3:1 ratio to provide putty mixture. Next, the cavities were restored with amalgam (Dentam-England). Radiographic images were taken before the procedure and after 4, 8, and 12 weeks from all capping teeth. After 4, 8, and 12 weeks intervals, vital perfusion fixation was performed with 10% formaline solution (Merck- Germany). Then teeth and their surrounding tissues were removed and the teeth were placed in 10% formaline solution for 14 days. After that all of the specimens were placed in 10% formic acid for 28 days. Following decalcification, specimens were prepared for standard pathologic processing. Six micrometer sections were cut in every 100 µm of buccolingual direction and stained with Haematoxylin and Eosin (H&E). All of the samples were observed for necrosis, calcified bridge formation, inflammation (Intensity and types), odontoblast cell layer, hyperemia, and calcified bridge thickness under a light microscope (Zeiss-Germany) and a score system was used for each of above mentioned factors as follows:

A-Inflammation

• Intensity of inflammation:

- 1+: 0-30 inflammatory cells
- 2+: 30-60 inflammatory cells
- 3+: more than 60 inflammatory cells

• Type of inflammation:

- 1+: Acute
- 2+: Chronic
- 3+: Mixture of chronic and acute

B-Hyperemia

- 1+: presence of 1 -3 vessels

2+: Presence of 4-5 vessels
 3+: Presence of 6-8 vessels
 4+: Presence of more than 8 vessels

C- Presence of calcified bridge

0: Absence of Calcified bridge
 1+: Presence of calcified bridge

D-Thickness of calcified bridge

It was measured as micrometer of thickness.

E-Odontoblastic cell layer (OCL)

0: presence of OCL
 1+: Absence of OCL

F-Necrosis

0: No apparent sign of necrosis
 1+: Sign of necrosis

All specimens were observed by an oral pathologist who was not aware of the types of capping materials and time intervals. Histologic data have been analyzed by Kruskal Wallis, χ^2 , Cochran, and Friedman tests.

Results

Radiographic findings

Radiographic evaluation showed that there was no sign of periapical lesion, internal and external resorption, or widening of periodontal ligament in none of the teeth at all time intervals.

Histologic findings

Acute inflammation was seen in none of experimental specimens. Chronic inflammation was the dominant type of inflammation which was seen after pulp capping.

A-Materials

1-MTA

Although histologic observation showed that inflammation and hyperemia were decreased from weeks 4 to 12, there was no significant difference between specimens. Kruskal Wallis test showed the thickness of calcified bridge at capping area was significantly increased during experiment ($p=0.001$). The thickness of calcified bridge was $9 \pm 6.35\mu\text{m}$ at week 4 which was increased to $37.38 \pm 2\mu\text{m}$ and $82.38 \pm 33.5\mu\text{m}$ at weeks 8 and 12, respectively.

χ^2 analysis showed that the presence of odontoblastic layer at weeks 8 and 12 had significant differences with in week 4 ($p=0.045$). Mature tall odontoblast cells could be observed under calcified bridge (figure 1, 2).

2-Calcium hydroxide

Histologic results showed that inflammation and hyperemia were decreased and the thickness of calcified bridge was increased during the experiment (Table 1).

The thickness of calcified bridge was $2.25 \pm 4.2\mu\text{m}$ at week 4 which was increased to $16.71 \pm 15.78\mu\text{m}$ and $36.86 \pm 25.64\mu\text{m}$ at weeks 8 and 12, respectively. The calcified bridge after 4 weeks had amorphous structure and was not tubular (figure 3).

B-Time intervals

1-Four weeks

There were significant differences between dycal and MTA groups in inflammation, hyperemia, and calcified bridge thickness (Table 2). MTA showed necrosis in three out of seven specimens, however, dycal showed necrosis in 6 out of 7 specimens. Cochran analysis showed no significant differences between two groups. Although two MTA specimens were free of inflammation, the rest of specimens showed chronic inflammation. However, non of dycal specimens was free of inflammation and most of them had a mixture of acute and chronic inflammation.

2-Eight weeks

There was no significant difference between dycal and MTA groups, except for calcified bridge thickness ($p=0.008$). Mid-pulp calcification could be observed in specimens that were capped with either experimental materials. The thickness of calcified bridge at the periphery of the exposure site was more than the center of capping area. Only one MTA specimen showed necrosis, however, necrosis was seen in 3 out of 7 dycal specimens. In MTA group, 5 out of 7 specimens were free of

inflammation, however, only 2 out of 7 dycal specimens were free of inflammation.

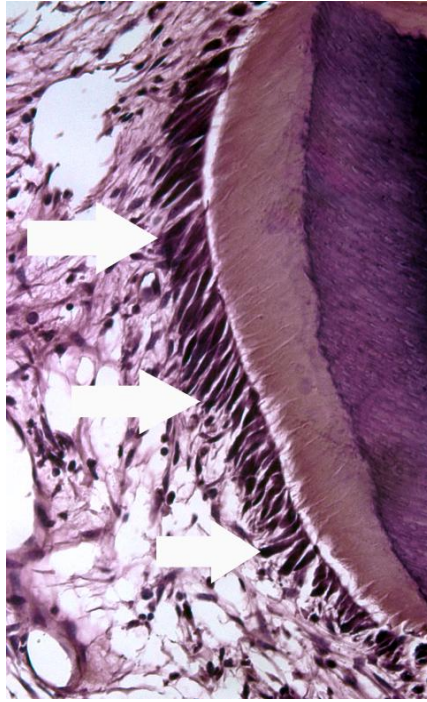


Figure 1: Mature odontoblast under calcified bridge 12 weeks after pulp capping by MTA

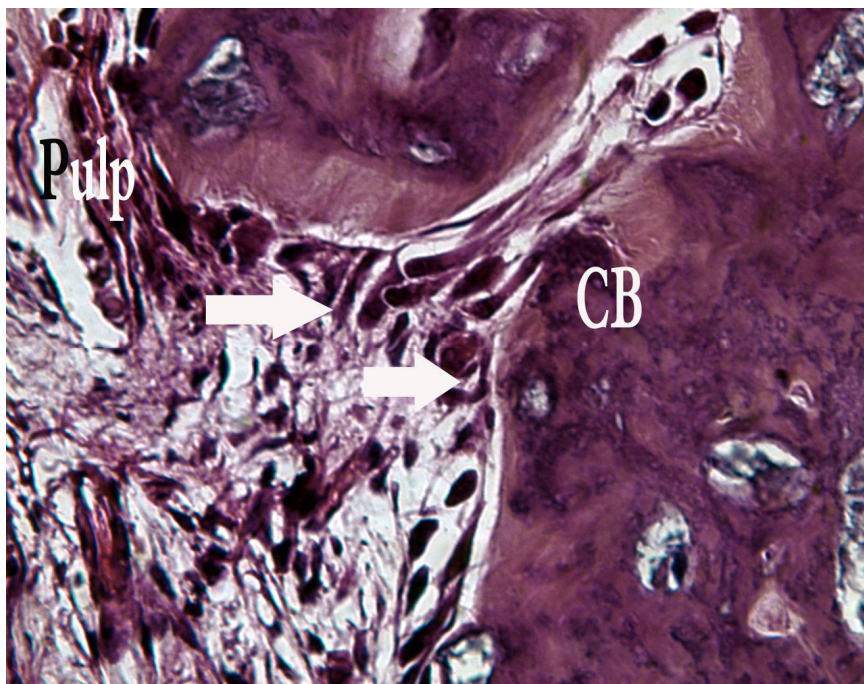
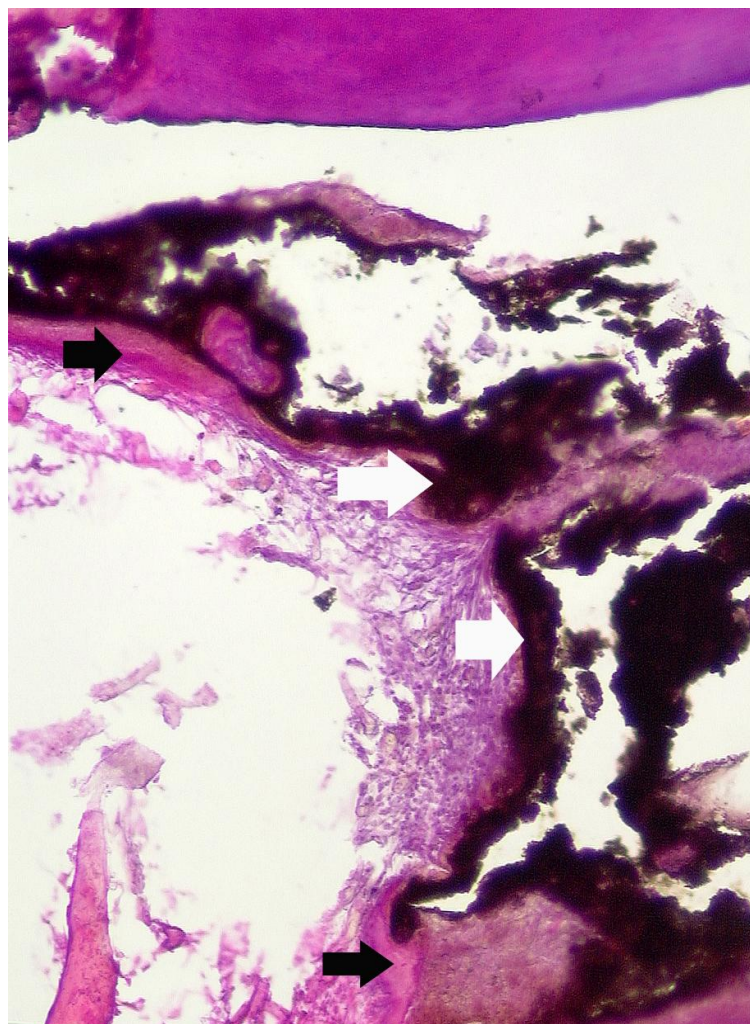


Figure 2: Calcified bridge under MTA 12 weeks after pulp capping.(CB= Calcified bridge)**Figure 3: Calcified bridge under dycal 4 weeks after pulp capping (arrows)****Table 1: A comparison of inflammation, hyperemia, and calcified bridge thickness among calcium hydroxide specimens at different time intervals**

Time interval	4 weeks	8 weeks	12 weeks	Kruskal Wallis significance
Factors	Mean Rank	Mean Rank	Mean rank	
Inflammation	15.13	7.5	11.36	P=0.049
Hyperemia	15.88	9.07	8.93	P= 0.04
Calcified bridge thickness	7.63	11.43	16	P=0.029
X± SD	2.25 ± 4.2	16.71 ± 15.78	36.86 ± 25.64	
MIN-MAX	0-10	0-32	0-62	

X: Mean

SD: Standard Deviation

MIN: Minimum thickness of calcified bridge in μm

Max: Maximum thickness of calcified bridge in μm

Table 2: A comparison of inflammation, hyperemia, and calcified bridge thickness between MTA and dycal groups at 4 weeks interval

Materials Factors	MTA	Dycal	Friedman test (Significancy)
	Mean Rank	Mean Rank	
Inflammation	1.5	2.71	P=0.014
Hyperemia	1.57	2.79	P=0.014
Calcified bridge thickness	2.21	1.29	P=0.046

3- Twelve weeks

Friedman test showed that there was no significant difference in both dycal and MTA specimens. However, the amount of hyperemia and inflammation under calcified bridge were greater in dycal than MTA specimens. The thickness of calcified bridge in MTA group was more than in calcium hydroxide group, however, there was no significant difference among those specimens ($p=0.059$). As it has been observed at 8 weeks intervals, calcified bridge in both capping material specimens had more thickness at the periphery of exposure site than the middle part. Necrosis was seen in 2 out of 7 MTA specimens, however, 4 out of 7 dycal specimens showed necrosis. All of MTA specimens were free of inflammation, where 2 out of 7 dycal specimens showed chronic inflammation.

Discussion

Histologic evaluation of pulp specimens after pulp capping is very important. Because although it is important to have a calcified bridge under capping materials, but making a dentinal bridge may be a sign of either healing or irritation¹⁷.

In this study radiographic changes could not be observed in different time intervals, however, many teeth showed necrosis and inflammation in their histologic specimens. Therefore, in judging the efficacy of a material as a pulp capping agent, it is important to determine the presence or absence of inflammation (type and severity) and necrosis, in addition to calcified bridge formation¹⁸. The concept of dentin bridges

has been questioned because of the presence of imperfections in many bridges¹⁹.

In the present study, the thickness of calcified bridge in MTA specimens was significantly different with dycal specimens at the capping area at 4 and 8 weeks intervals. Previous studies have shown that calcified bridge is more consistent and thicker when MTA has been used as the pulp capping material in comparison with calcium hydroxide^{6, 20, 21}.

Cox and associates have shown that pulp healing is more dependent on the capacity of the capping material to prevent bacterial microleakage rather than the specific properties of the material itself²². Therefore, if a tight seal is achieved and reasonable material is selected, mature dental pulp will be able to differentiate into the specific cell lineage forming tubular dentine⁹.

In the present study, amalgam has been used to cover the capping materials after pulp capping. Pitt-Ford et al have stated that the unsatisfactory pulpal response to calcium hydroxide preparation covered by amalgam could be due to the fact that amalgam did not provide a bacterial tight seal, which result in preparation's lack of biocompatibility⁶. Cox et al have shown that amalgam has shrinkage after placement in restorative cavities and the space between amalgam and the cavity walls could be a pathway for bacterial leakage²². It has been shown that MTA is an insoluble material and can provide tight seal which could prevent bacterial access to the pulp tissues^{10, 11}. However, Calcium hydroxide

is a soluble material and can not produce a tight seal against microleakage⁶.

Clarke postulated that a complete peripheral ring of dentin encircles the exposure site and that the dentin bridge will fill in toward the center²³. A research study²⁴ in which three dimensional images were used, indicated that dentin bridge formation beneath a hard set Ca(OH)₂ agent will form in 3 patterns:

- 1- from the periphery of the residual dentin chip at the wound surface,
- 2- with reparative dentin by stimulation during the cavity preparation, and
- 3- from the wound surface.

In this study, like the studies of Kitasako et al (2000) and Pairokh et al (2005) the dentin bridge formation occurs mainly from the periphery of the exposure site, as the thickness of bridge at the periphery is more than of its thickness at the middle part²⁵⁻²⁶.

Although modified pulpotomy with calcium hydroxide, called Cvek type²⁷, has shown to be more predictable than pulp capping but for many reasons using MTA is recommended for this matter^{6, 11, 28}. First, it is not important that the pulp wound bleeding be completely stopped prior to placing the MTA. As a matter of fact, the presence of a small amount of blood provides necessary moisture for curing of the material and has been shown to work as well as any other fluid²⁷. Secondly, it is not necessary to re-enter the pulpotomy site later to remove the pulp capping material; as it has been recommended for calcium hydroxide pulpotomies by Cvek^{29, 30}. MTA

does not appear to deteriorate and disintegrate with time³¹⁻³³; thus, space for microleakage does not develop as it does with calcium hydroxide. Thirdly, previous research has shown that the pulp responds favorably to the protection provided by an MTA layer^{6, 18, 28}. The reparative dentin is consistently more uniform and thicker under MTA compared with calcium hydroxide. In a recent published study it has been shown that MTA was a considerably better material than calcium hydroxide in maintaining the integrity of the pulp¹⁸.

It has been stated that MTA, because of its high PH, especially when freshly mixed, will cause denaturation of adjacent cells and tissue proteins. This denaturation includes a few bacteria that might be present in the wound area. As the materials set, the PH changes and the cell injuries subside³⁴.

Conclusion

Based on the result of this study, MTA showed to be a more reliable material as pulp capping agent in comparison with calcium hydroxide.

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References

1. Cox CF, Bogen G, Kopel HM, Ruby JD. Repair of pulpal injury by dental materials. In: Hargreaves KM, Goodis HE: Seltzer and Bender's Dental Pulp. 3rd Ed, Quintessence Publishing Co. 2002, Chapter 14, pp325-44.
2. Scarano A, Manzon L, Di Giorgio R, Orsini G, Tripodi D, Piattelli A. Direct capping with four different materials in humans: Histological analysis of odontoblast activity. *J Endod* 2003; 729-33.
3. Cvek M, Granath L, Cleaton-Jones P, Austin J. Hard tissue barrier formation in pulpotomized monkey teeth capped with cyanoacrylate or calcium hydroxide for 10 and 60 minutes. *J Dent Res* 1987; 66:1166-74.
4. Cox CF, Bergenholtz G, Fitzgerald M, Heys DR, Heys RJ, Avery SK, Baker JA. Capping

- of the dental pulp mechanically exposed to the oral microflora- a 5 week observation of wound healing in the monkey. *J Oral Pathol* 1982; 11: 327-39
5. Cox CF, Bergenholtz G, Heys DR, Syed SA, Fitzgerald M, Heys RJ. Pulp capping of dental pulp mechanically exposed to oral microflora: a 1-2 year observation of wound healing in monkey. *J Oral Pathol* 1985; 14:156-68.
 6. Pitt Ford T, Torabinejad M, Abedi HR, Bakland LK, Kariawasam SP. Using mineral trioxide aggregate as a pulp capping materials. *JADA* 1996; 127:1491-94.
 7. Camp JH, Barrett EJ, Pulver F. Pediatric endodontics: Endodontic treatment for the primary and young, permanent dentition In: Cohen S, Burns RC: *Pathways of the pulp*. 8th eds, St. Louis, Mosby Co. 2002, p 797-844.
 8. Stanley HR, Lundy T. Dycal therapy for pulp exposure. *Oral Surg Oral Med Oral Pathol* 1972; 34:818-27.
 9. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. *J Endodon* 1999; 25:197-205.
 10. Torabinejad M, Hong CU, McDonald F, Pitt Ford TR. Physical and chemical properties of a new root-end filling material. *J Endod* 1995; 21:349-53.
 11. Torabinejad M, Hong CU, Pitt Ford TR, Karyawasam SP. Dye leakage of four root end filling materials: effect of blood contamination. *J Endod* 1994; 20:159-63.
 12. Nakata TT, Bae KC, Baumgartner JC. Perforation repair comparing mineral trioxide aggregate and amalgam using an anaerobic bacterial leakage model. *J Endod* 1998; 24:184-6.
 13. Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root end filling material. *J Endod* 1995; 21:109-12.
 14. Wu M, Kantakiotis EG, Wesselink PR. Long-term seal provided by some root-end filling material. *J Endod* 1998; 24:557-60.
 15. Dominguez 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.
 16. Schuur AHB, Gruythusen RJM, Wesselink PR. Pulp capping with adhesive resin based composite versus calcium hydroxide: a review. *Endod Dent Traumatol* 2000; 16:240-5016.
 17. Cox CF, Bogen G, Kopel HM, Ruby JD. Repair of pulpal injury by dental materials. In: Hargreaves KM, Goodis HE: *Seltzer and Bender's Dental Pulp*. 3rd Ed, Quintessence Publishing Co. 2002, Chapter 14, pp325-44.
 18. Aeinehchi M, Eslami B, Ganbariha 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.
 19. Smith AJ. Dentin formation and repair In: Hargreaves K.M., Goodis H.E. Seltzer and Bender's *Dental Pulp*. 3rd Ed, Quintessence Publishing Co. 2002, Chapter 3, pp41-62.
 20. Tziafas D, Pantelidu O, Alvanou A, Belibasakis G, Papadimitrion S. The dentinogenic effect of mineral trioxide aggregate(MTA) in short term capping experiments. *Int Endod* 2002; 35:245-54.
 21. Cox CF, Keall CL, Keall HJ, Ostro E, Bergenholtz G. Biocompatibility of surface sealed dental materials against exposed pulps. *J Prosthetic Dent* 1987; 57:1-8.
 22. Cox CF, Keall CL, Keall HJ, Ostro E, Bergenholtz G. Biocompatibility of surface sealed dental materials against exposed pulps. *J Prosthetic Dent* 1987; 57:1-8.
 23. Clarke NG. The morphology of the reparative dentin bridge. *Oral Surg Oral Med Oral Pathol* 1970; 29:746-52.
 24. Kitasako Y, Shibata S, Pereira PNR, Tagami J. Short-term dentin bridging of mechanically exposed pulps capped with adhesive resin systems. *Operative Dent* 2000; 25:155-62.
 25. Kitasako Y, Shibata S, Arakawa M, Cox CF, Tagami J. A light and transmission microscopic study of mechanically exposed monkeys pulps. Dynamics of fiber elements during early dentin bridge formation. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2000; 89: 224-30.
 26. Parirokh M, Asgary S, Eghbal MJ, Eslami B, Stowe S, Eskandarizade A, Shabahang S. A comparative study of white and grey mineral trioxide aggregate as pulp capping agents in dog's teeth. *Dental Traumatol* 2005; 21:150-4.
 27. Backland LK. Endodontic consideration in dental trauma. In: Ingle J.I., Bakland L.K.

- Endodontics. 5th ed. D.C.Decker Ontario 2002; pp808.*
28. Faraco IM, Holland R. Response of the pulp of dogs to capping with mineral trioxide aggregate or a calcium hydroxide cement. *Endod Dent Traumatol* 2001; 17:163-7.
 29. Cvek M, Lundburg M. Histological appearance of pulps after exposure by a crown fracture, partial pulpotomy and clinical diagnosis of healing. *J Endod* 1983; 9:8-11.
 30. Cvek M. A clinical report on partial pulpotomy and capping with calcium hydroxide in permanent incisors with complicated crown fractures. *J Endod* 1978; 4:232-6.
 31. Torabinejad M, Hong CU, Pitt Ford TR, Karyawasam SP. Dye leakage of four root end filling materials: effect of blood contamination. *J Endod* 1994; 20:159-63.
 32. Nakata TT, Bae KC, Baumgartner JC. Perforation repair comparing mineral trioxide aggregate and amalgam using an anaerobic bacterial leakage model. *J Endod* 1998; 24:184-6.
 33. Torabinejad M, Rastegar AF, Kettering JD, Pitt Ford TR. Bacterial leakage of mineral trioxide aggregate as a root end filling material. *J Endod* 1995; 21:109-12.
 - 34-Saidon J, He J, Zhu Q, Safavi K, Spangberg LSW. Cell and tissue reactions to mineral trioxide and Portland cement. *Oral Surg Oral Med Oral Pathol Oral Radiol Oral Endod* 2003; 95:483-9.

