International Endodontic Journal

doi:10.1111/j.1365-2591.2011.01916.x

A histological study of pulp reaction to various water/powder ratios of white mineral trioxide aggregate as pulp-capping material in human teeth: a double-blinded, randomized controlled trial

A. Shahravan[1], S. P. Jalali[2,*], M. Torabi[3], A. A. Haghdoost[4] & H. Gorjestani[1]

[1]Kerman Oral and Dental Diseases Research Center, Kerman University of Medical Sciences, Kerman; [2]Iranian Endodontic Research Center, Kerman; [3]Oral and Maxillofacial Pathology Department, Kerman University of Medical Sciences, Kerman; and [4]Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran

Abstract

Shahravan A, Jalali SP, Torabi M, Haghdoost AA, Gorjestani H. A histological study of pulp reaction to various water/powder ratios of white mineral trioxide aggregate as pulp-capping material in human teeth: a double-blinded, randomized controlled trial. *International Endodontic Journal*, **44**, 1029–1033, 2011.

Aim To compare the histological pulp reaction to various water/powder ratios of white mineral trioxide aggregate (MTA) as a pulp-capping material in healthy human teeth.

Methodology Twenty-nine disease-free maxillary and mandibular third molar teeth, scheduled for extraction, were exposed mechanically and then capped with 0.28, 0.33 and 0.40 water/powder ratios of white MTA (ProRoot; Dentsply Maillefer, Ballaigues, Switzerland) and restored with glass ionomer. After 30 days, the teeth were extracted, resected apically and immersed in 10% formalin. For histological processing, the teeth were sectioned buccolingually in 5-μm-thick slices, stained with Haematoxylin and Eosin and evaluated by a light microscope. Samples were evaluated for intensity and type of inflammation, presence of necrosis, as well as continuity, morphology and thickness of calcified bridges. The data were analysed by Kruskal–Wallis and Mann–Whitney tests.

Results There were no significant differences in the diameter, morphology and continuity of the calcified bridges, intensity and type of inflammation or presence of necrosis ($P > 0.05$) in the pulps covered by MTA with various water/powder ratios. Two teeth failed to display a calcified bridge, and one had a pulp necrosis.

Conclusion Water-to-powder ratios of MTA had no significant influence on the histological outcome of direct pulp capping on healthy pulps.

Keywords: direct pulp cap, mineral trioxide aggregate.

Received 17 May 2010; accepted 4 June 2011

Introduction

Mineral trioxide aggregate (MTA) has been used as a capping material on mechanically exposed pulps (Torabinejad & Chivian 1999). Several laboratory and *in vivo* studies have reported that MTA has characteristics such as high pH, high calcium ion release and low solubility (Torabinejad *et al.* 1995, Parirokh & Torabinejad 2010a,b) and is biocompatible (Kettering & Torabinejad 1995, Torabinejad & Parirokh 2010). It also provides a good seal (Barrieshi-Nusair & Hammad 2005), has excellent marginal adaptation (Torabinjejad *et al.* 1995) and maintains a high PH for a long period of time (Fridland & Rosado 2005). Therefore, MTA has been suggested as a suitable material to be used in vital pulp therapies (Torabinejad & Chivian 1999). However, relatively few published studies have been conducted to evaluate MTA histologically as a pulp-capping agent in humans (Aeinehchi *et al.* 2003,

Correspondence: Arash Shahravan, Kerman Oral and Dental Diseases Research Center, Kerman University of Medical Sciences, PO Box 7618759689, Shafah Street, Kerman, Iran (fax: +983412118073; e-mail: arashahravan@gmail.com).

*Correction added after online publication, 30 June 2011: initial added to second author name.

Iwamoto *et al.* 2006, Bogen *et al.*2008, Min *et al.* 2008, Nair *et al.* 2008); therefore, there is little information on the detailed healing mechanism and the effects of MTA on pulp tissues in humans.

Al-Hezaimi *et al.* (2005) revealed that water/powder ratios of white MTA had different inhibition effects on *Candida albicans* in a laboratory study. Fridland & Rosado (2003, 2005) revealed that the degree of solubility and porosity increased as the water/powder ratios of MTA increased. However, no published studies have yet evaluated various water/powder ratios of MTA as a pulp-capping agent on human teeth. The various water/powder MTA ratios may have different consequences in human tooth pulp-capping treatment such as its effects on intensity and type of inflammation, presence of necrosis, as well as the continuity and morphology of calcified bridge formation because of its physical and chemical properties. The purpose of this study was to compare the histological outcomes of direct pulp capping on healthy human pulps using different water/powder MTA ratios.

Materials and methods

In this double-blinded, randomized controlled trial, 36 intact maxillary and mandibular third molars (one up to four teeth in each patient), which had a vital pulp, free of restorations and disease and required extraction were included from 18 volunteers between the ages of 20 and 24 years. The sample size calculation showed that at least 10 teeth per group were required to detect at least a 1.5 effect size difference with type one and two errors of 5% and 10%, respectively. This sample size was similar to that in other relevant studies (Aeinehchi *et al.* 2003, Faraco & Holland 2004).

Percussion and pulp sensibility tests were performed, and radiographs were examined. Only teeth that could be extracted nonsurgically and with minor trauma were included. All subjects were informed of the possible complications of the procedure. Ethical approval was sought and granted (NO. K/87/22, Ethics Committee of Kerman Medical University). Informed consent was obtained from all subjects.

Prilocaine (Prilonest; DFL, RJ, Brazil) was used to anaesthetize the teeth. The teeth were disinfected by irrigation with chlorhexidine solution (0.2%; Iran-Najo, Tehran, Iran). A conventional Class I cavity of approximately one millimetre width was prepared on the occlusal surface, until the pulp was exposed. Cotton rolls were used to isolate the teeth, making all possible efforts to avoid salivary contamination. Haemostasis was achieved by irrigating the cavity with sterile saline and compaction with small pieces of moist cotton pellet before applying white MTA (ProRoot; Dentsply Maillefer). Three different water/powder proportions (0.28, 0.33 and 0.40) were selected according to the studies by Fridland & Rosado (2003, 2005). The ratios of MTA were prepared in a scale (GF-300; A&D Company, Tokyo, Japan) with an accuracy of 0.001 g.

Water/powder ratios were selected for each tooth by block randomization. Using a stiff metal spatula, MTA powder was mixed with the liquid and then placed over the exposure site with a plastic instrument and then a light pressure was applied with moist cotton pellets. Light-cured glass ionomer (Fuji II LC; GC Corp, Tokyo, Japan) was used as the filling material at the same visit. After 1 month, the teeth were extracted by an Oral and Maxillofacial surgeon. The apex of each tooth was immediately removed using a bur in a high-speed handpiece with water coolant to allow penetration of 10% formalin (Merck, Darmstadt, Germany) for the purpose of tissue fixation. The teeth were kept in formalin for 3 days and subsequently decalcified in 10% nitric acid (Merck) for 1–2 weeks. After histological processing, the teeth were embedded in paraffin (Mojallali, Tehran, Iran) and serially sectioned in a buccolingual direction every 5 μm. Haematoxylin and Eosin (H&E) staining was used.

Two Oral and Maxillofacial pathologists, who were blinded in respect of the specimen source, evaluated the specimens under a microscope (Nikon Corp., Tokyo, Japan) at ×40 magnification, according to grades and definitions (Faraco & Holland 2004) listed in Table 1. The thickness of the dentine bridge formed was registered in micrometres. All samples were also evaluated for intensity and type of inflammation, presence of necrosis, as well as the continuity and morphology of the calcified bridge.

All statistical analyses were run in SPSS (SPSS Inc, Chicago, IL, USA) version 11.2. The main outcome variables were measured in an ordinal scale (hard tissue: continuity, morphology, thickness; dental pulp: intensity of inflammation, type of inflammation and necrosis). Therefore, the nonparametric Kruskal–Wallis and Mann–Whitney tests were used to compare the histological outcomes of pulp capping using various liquid/power ratios of MTA: P-values <0.05 were considered significant.

Results

The results attributed to all specimens and various ratios of MTA are provided in Table 2. The pulp-capping procedure was completed on 36 teeth, two teeth were

Table 1 Grading and definitions of histopathologic evaluation of the pulp

Hard tissue bridge
Continuity
1. Complete
2. Little communication of capping material with dental pulp
3. Only lateral deposition of hard tissue on the walls of the cavity of pulp exposition
4. Absence of hard tissue bridge and absence of lateral deposition of hard tissue
Morphology
1. Dentine with or without irregular hard tissue
2. Only irregular hard tissue deposition
3. Only a slight layer of hard tissue deposition
4. No hard tissue deposition
Thickness (evaluated by ocular in three different points of the bridge)
1. Up to 250 μm
2. From 150 to 249 μm
3. From 1 to 149 μm
4. Partial or absent bridge
Dental pulp
Intensity of inflammatory reaction (acute and chronic processes)
1. Absent or very few cells
2. Mild: average number <10 cells
3. Moderate: average number 10–25 cells
4. Severe: average number >25 cells
Type of inflammation
1. Acute
2. Chronic
3. None
Necrosis
1. Yes
2. No

lost to follow-up and five were lost during histological preparation. Eventually, 29 teeth were evaluated. There was no statistically significant difference between the 0.28, 0.33 and 0.40 MTA water/powder ratios in relation to the calcified bridge thickness, morphology and uniformity nor the severity and type of inflammation, and the presence of necrosis.

A dentine bridge was formed directly underneath the capping materials at the pulp exposure site in all of the samples except two (93%). Eleven (38%) formed a complete calcified bridge. There were no signs of inflammation in 23 (79%) of the samples, and the remainder had mild inflammation. Only one (3%) sample was necrotic (Figs 1 and 2).

Discussion

In the present randomized trial, histological outcome of direct pulp capping in 29 healthy human teeth using

Table 2 Histopathologic reaction of pulp when capped with different water/powder ratios of MTA

	Type of inflammation			Continuity of the calcified bridge				Calcified bridge diameter				Morphology of calcified bridge				Inflammation intensity				Necrosis	
Ratio	Acute (%)	Chronic (%)	Non (%)	Complete (%)	Little (%)	Lateral deposition (%)	Non (%)	>250 (%)	150< × <249 (%)	<149 (%)	Non (%)	Dentin (%)	Irregular hard tissue (%)	Slight layer (%)	Non (%)	Non (%)	Mild (%)	Moderate	Severe	Yes (%)	No (%)
0.28	0 (0)	1 (10)	9 (90)	4 (40)	6 (60)	0 (0)	0 (0)	3 (30)	2 (20)	5 (50)	0 (0)	5 (50)	2 (20)	3 (30)	0 (0)	9 (90)	1 (10)	0	0	1 (10)	9 (90)
0.33	1 (11.1)	1 (11.1)	7 (77.8)	3 (33.3)	5 (55.6)	0 (0)	1 (11.1)	1 (11.1)	1 (11.1)	6 (66.7)	1 (11.1)	6 (66.7)	1 (11.1)	1 (11.1)	1 (11.1)	7 (77.8)	2 (22.2)	0	0	0 (0)	9 (100)
0.40	0 (0)	3 (30)	7 (70)	4 (40)	2 (20)	3 (30)	1 (10)	2 (20)	1 (10)	6 (60)	1 (10)	5 (50)	1 (10)	3 (30)	1 (10)	7 (70)	3 (30)	0	0	0 (0)	10 (100)
Total	1 (3.4)	5 (17.2)	23 (79.3)	11 (37.9)	13 (44.8)	3 (10.3)	2 (6.9)	6 (20.7)	4 (13.8)	17 (58.6)	2 (6.9)	16 (55.2)	4 (13.8)	7 (24.1)	2 (6.9)	23 (79.3)	6 (20.7)	0 (0%)	0 (0%)	1 (3.4)	28 (96.6)

MTA, mineral trioxide aggregate.

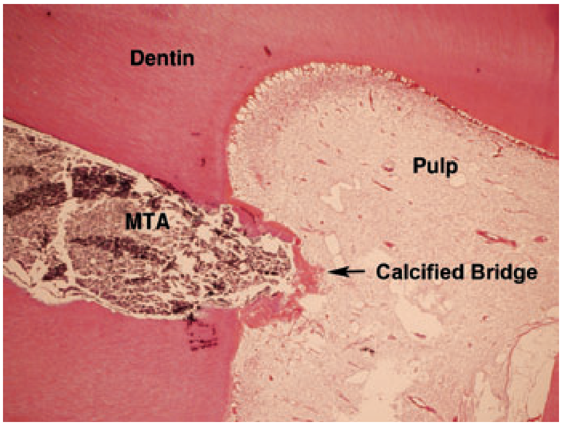

Figure 1 Direct pulp cap with mineral trioxide aggregate water/powder ratio of 0.33 at 30 days showing bridge formation and healthy pulp under the bridge (×40).

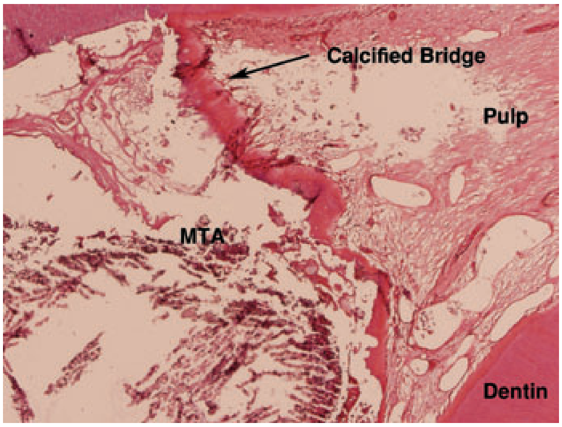

Figure 2 Calcified bridge formation under an mineral trioxide aggregate water/powder ratio of 0.28 (×100).

various water/powder MTA ratios was compared. Mineral trioxide aggregate in 0.28, 0.33 and 0.40 water/powder ratios did not reveal any significant differences in calcified bridge diameter, morphology of the calcified bridge, continuity of the calcified bridge, inflammation intensity, type of inflammation or necrosis. In contrast, Al-Hezaimi *et al.* (2005) and Fridland & Rosado (2003, 2005) reported that various MTA water/powder ratios affected its properties such as solubility and porosity. As these were laboratory studies, the reported differences caused by various ratios of MTA may not be clinically relevant during pulp capping.

Vital pulp therapy is defined as a treatment initiated to preserve healthy pulp tissue to stimulate the formation of reparative dentine. This is particularly important in the young adult tooth where apical root development is incomplete (Kakehashi *et al.* 1965).

The formation, quality and thickness of a calcified bridge, presence of inflammatory cells and preservation of the pulp are indicators of the outcome of vital pulp therapy (Parirokh & Torabinejad 2010a,b).

Several laboratory and *in vivo* studies have reported that MTA has good physical and clinical characteristics for use as a capping material on mechanically exposed pulps (Kettering & Torabinejad 1995, Torabinjejad *et al.* 1995, Barrieshi-Nusair & Hammad 2005, Parirokh & Torabinejad 2010a,b, Torabinejad & Parirokh 2010). The present study was undertaken on healthy pulps and not as usual on pulps with reversible pulpitis. Thus, to compare possible differences of pulp reactions, repeating the study on teeth with caries and reversible pulpitis may be helpful. The 0.28, 0.33 and 0.40 MTA water/powder ratios suggested by Fridland & Rosado (2003, 2005) were tested in the present study to allow direct comparisons with their findings. Secondly, the 0.33 ratio is usually suggested by the manufacturer (Dentsply Maillefer). Fridland & Rosado (2003, 2005) revealed that the degree of solubility and porosity increased as the water/powder ratios of MTA increased; however, these variations in physical properties did not cause any histological differences in the present study. The MTA reaction that produces a hydroxyapatite-like layer on the surface of MTA (Asgari *et al.* 2009) may explain similar pulp responses to various MTA ratios in the present study.

All samples were isolated with cotton rolls. Rubber dam was not used because the position of the third molars proved difficult to isolate. However, every attempt was made to prevent saliva contamination.

The present study revealed that human pulp reactions to various ratios of MTA were acceptable. Of the 29 teeth, six had mild inflammation and one had necrosis. Aeinehchi *et al.* (2003) and Min *et al.* (2008), respectively, found mild inflammation in 50% and 77% of their samples, whilst 10% and 1.1% of their teeth had necrosis.

The results revealed that the number of mildly inflamed samples appeared to increase at higher water/powder ratios, although differences were not statistically significant. One, two and three teeth were found to have mild inflammation, in groups with 0.28, 0.33 and 0.40 water/powder ratios of MTA, respectively (Table 1). Increasing solubility and porosity as a result of increasing water/powder ratios of MTA may be an explanation as revealed by Al-Hezaimi *et al.* (2005).

In 27 teeth that formed a calcified bridge, 11 had a complete bridge after 1 month. All of the nine teeth in the study by Min *et al.* (2008) formed a full calcified bridge over a period of 2 months. Further studies with longer duration of follow-up are required to confirm the present findings because 30-day follow-up may not be sufficient for evaluating pulp inflammation, necrosis and complete calcified bridge formation.

Conclusion

The water-to-powder ratios of MTA had no significant influence on the histological outcome of direct pulp capping on healthy pulps at 2 month. All three MTA preparations were found to be suitable for direct pulp capping under the condition of this study.

Acknowledgement

This project was supported financially by the Kerman Research Center, Kerman University of Medical Sciences, Kerman, Iran.

References

Aeinehchi M, Eslami B, Ghanbariha M, Saffar SD (2003) Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. *International Endodontic Journal* **36**, 225–31.

Al-Hezaimi K, Al-Hamdan K, Naghshbandi J, Oglesby S, Simon JH, Rostein I (2005) Effect of white colored mineral trioxide aggregate in different concentrations on Candida albicans *in vitro*. *Journal of Endodontics* **31**, 640–6.

Asgari S, Eghbal MJ, Parirokh M, Ghoddusi J (2009) Effect of two storage solutions on surface topography of two root-end fillings. *Australian Endodontic Journal* **35**, 147–52.

Barrieshi-Nusair KM, Hammad HM (2005) Intracoronal sealing comparison of mineral trioxide aggregate and glass ionomer. *Quintessence Interntional* **36**, 539–45.

Bogen G, Kim Js, Bakland LK (2008) Direct pulp capping with mineral trioxide agregate an observational study. *The Journal of the American Dental Association* **139**, 305–14.

Faraco IM, Holland R (2004) Histomorphological Response of dog's Dental pulp capped with mineral trioxide aggregate. *Brazilian Dental Journal* **15**, 104–8.

Fridland M, Rosado R (2003) Mineral trioxide aggregate (MTA) Solubility and porosity with different water–to–powder Ratios. *Journal of Endodontics* **29**, 814–7.

Fridland M, Rosado R (2005) MTA solubility: a long term study. *Journal of Endodontics* **31**, 376–9.

Iwamoto CE, Adachi E, Pameijer CH, Barnes D, Romberg EE, Jefferies S (2006) Clinical and histological evaluation of white Proroot MTA in direct pulp capping. *American Journal of Dentistry* **19**, 85–90.

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 and Oral Pathology* **20**, 340–9.

Kettering JD, Torabinejad M (1995) Investigation of mutagenicity of mineral trioxide aggregate and other commonly used root-end filling materials. *Journal of Endodontics* **21**, 537–9.

Min KS, Park HJ, Lee SK *et al.* (2008) Effect of mineral trioxide aggregate on dentin Bridge formation and expression of dentin sialoprotein and heme oxygenase-1in human Dental pulp. *Journal of Endodontics* **34**, 666–70.

Nair PN, Duncan HF, Pitt Ford TR, Luder HU (2008) 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* **41**, 128–50.

Parirokh M, Torabinejad M (2010a) Mineral trioxide aggregate: a comprehensive literature review- Part I: chemical, physical, and antibacterial properties. *Journal of Endodontics* **36**, 16–27.

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

Torabinejad M, Chivian N (1999) Clinical applications of mineral trioxide aggregate. *Journal of Endodontics* **25**, 197–205.

Torabinejad M, Parirokh M (2010) Mineral trioxide aggregate: a comprehensive literature review- Part II: sealing ability and biocompatibility properties. *Journal of Endodontics* **36**, 190–202.

Torabinejad M, Wilder Smith P, Pitt Ford TR (1995) Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. *Journal of Endodontics* **21**, 295–9.

Torabinjejad M, Hong CU, Pitt Ford TR (1995) Physical properties of a new root end filling material. *Journal of Endodontics* **21**, 349–54.