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Evaluation of two mineral trioxide aggregate compounds as pulp-capping agents in human teeth

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Abstract

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Aim The present randomized, controlled prospective study evaluated the histomorphological response of human dental pulps capped with two grey mineral trioxide aggregate (MTA) compounds.

Methodology Pulp exposures were performed on the occlusal floor of 40 human permanent pre-molars. The pulp was capped either with ProRoot (Dentsply) or MTA-Angelus (Angelus) and restored with zinc oxide eugenol cement. After 30 and 60 days, teeth were extracted and processed for histological examination and the effects on the pulp were scored. The data were subjected to Kruskal–Wallis and Conover tests ($\alpha = 0.05$).

Results In five out of the 40 teeth bacteria were present in pulp tissue. No significant difference was observed between the two materials ($P > 0.05$) in terms of overall histological features (hard tissue bridge, inflammatory response, giant cells and particles of capping materials). Overall, 94% and 88% of the specimens capped with MTA-Angelus and ProRoot, respectively, showed either total or partial hard tissue bridge formation ($P > 0.05$).

Conclusions Both commercial materials ProRoot (Dentsply) and MTA-Angelus (Angelus) produced similar responses in the pulp when used for pulp capping in intact, caries-free teeth.

Keywords: hard tissue bridge, inflammatory response, mineral trioxide aggregate, pulp-capping human teeth.

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Introduction

Direct pulp capping is a well-established method of treatment in which the exposed dental pulp is covered with a material that protects the pulp from additional injury and permits healing and repair (American Association of Endodontists, 1981). Despite calcium hydroxide being considered the gold standard for vital pulp therapy, considerable confusion and condemnation of its use persists (Stanley 1989). Recent attempts to develop different pulp capping materials have resulted in the development of the so-called mineral trioxide aggregate (MTA; grey ProRoot MTA; Dentsply, Tulsa, OK, USA) (Tziafas *et al.* 2000), which was first proposed for pulp capping in 1996 (Pitt Ford *et al.* 1996).

Initially, MTA was used for endodontics to seal off all pathways of communication between the root canal system and the internal tissues (Torabinejad & Chivian 1999); however this material may also be applied in several other clinical scenarios, such as internal root resorption, apexification, endodontic surgery, sealing of

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perforations and as a pulp-capping material (Torabinejad & Chivian 1999). Although several case reports and clinical studies have evaluated the use of MTA in permanent human teeth for pulp capping (Aeinehchi *et al.* 2003, Witherspoon *et al.* 2006), few studies have evaluated the histological response in pulp tissue (Iwamoto *et al.* 2006, Nair *et al.* 2008). A recent literature review reported that there were insufficient clinical studies on this subject (Roberts *et al.* 2008), and encouraged further evaluation of MTA as a pulp capping material.

There are two products available on the market: ProRoot (ProRoot MTA; Dentsply) and MTA-Angelus (Angelus; Prod. Odont. Ltda, Londrina, PR, Brazil). It was recently demonstrated that the composition, as well as, particle size and shape of these materials are different (Camilleri *et al.* 2005, Song *et al.* 2006, Oliveira *et al.* 2007, Hashem & Hassanien 2008, Komabayashi & Spångberg 2008). According to Duarte *et al.* (2003), the pH of MTA-Angelus is higher than that of ProRoot; whether or not this has any effect on the performance of these materials in pulp healing is yet to be addressed. The studies of Iwamoto *et al.* (2006) and Nair *et al.* (2008) compared the effect of calcium hydroxide liner with white but not grey MTA (ProRoot). No study has so far evaluated the performance of Grey MTA-Angelus and Grey ProRoot for pulp capping.

The purpose of this clinical study was to compare the histomorphological features of ProRoot and MTA-Angelus after 30 and 60 days when used for pulp capping. The null hypothesis tested showed that no significant difference could be observed in pulps capped with ProRoot and MTA-Angelus in the two periods of evaluation.

Materials and methods

A consent form and the research protocol were reviewed and approved by the Human Subject Review Committee from the University of Oeste of Santa Catarina, SC, Brazil. Patient screening and pre-treatment selection of teeth were performed by two clinical investigators. The investigators screened the patients initially to determine whether they met the study entry criteria (described below). They enrolled the qualified patients in the study for the evaluation visit. Qualified patients were recruited in the order in which they reported for the screening session. As this study was not performed on a population basis, no randomization was used for patient selection.

Forty healthy human pre-molar teeth scheduled to be extracted for orthodontic reasons were selected from patients ranging from 25 to 42 years. All teeth were examined clinically and radiographically to ensure absence of occlusal and proximal caries and periapical pathosis. All subjects were treated in accordance with the Helsinki declaration. The patients considered for this randomized controlled study read and signed consent forms after receiving a detailed explanation about the experimental rationale, clinical procedures and possible risks. Patient participation was voluntary and they were not financially remunerated for participation in the study.

The sensitivity of all teeth was tested using thermal testing. ENDO-ICE frozen gas (Coltène/Whaledent Inc., Mahwah, NJ, USA) was applied for 5 s on the buccal surface of the teeth scheduled for the pulp therapy and adjacent teeth. After local anaesthesia (Citanest 3%; Merrel Lepetit, São Paulo, Brazil) the tooth was isolated with rubber dam and cleaned with pumice on a rubber cup at low speed. The teeth were washed with 0.12% chlorexidine (Dentscare, Joinville, SC, Brazil). Occlusal cavities were prepared with a sterile diamond bur (Number 1095; KG Sorensen, Barueri, Brazil) at high speed under a water spray coolant. The dimensions of the cavity were: occlusal depth: 3.0 ± 0.2 mm; mesiodistal width: 4.0 ± 0.5 mm and buccolingual width: 3.0 ± 0.2 mm. Pulp exposure was performed in the centre of the floor of the cavity with a round diamond bur under water-coolant (Number 1014, diameter 1.2; KG Sorensen). Only one bur was used for the entire preparation of each cavity. The teeth were then divided into four experimental groups (n = 10). Randomization of the groups was performed on each patient by tossing a coin.

Haemostasis was established with a sterile cotton pellet soaked in saline solution. The grey MTA from Angelus (Angelus Prod. Odont.) was applied in groups 1 and 2, whilst in groups 3 and 4, the grey ProRoot (Dentsply Caulk, Milford, DE, USA) was placed. The composition of both MTA products is shown in Table 1.

Table 1 Composition of the MTA products evaluated

Material	Composition [a]
ProRoot (Dentsply)	Portland cement (75%); bismuth oxide (20%) and calcium sulphate dehydrate (5%)
MTA-Angelus (Angelus)	Portland cement (80%) and bismuth oxide (20%)

[a]Material safety data sheet instructions for each manufacturer (Dentsply Tulsa Dental 1998; Angelus Ind. Prod. Odont. Ltda 2005).

The cavities were then filled with zinc oxide eugenol (IRM; Dentsply Caulk). When necessary, material excess was removed using an ultrafine diamond bur at high speed under a water coolant (KG Sorensen). All materials were manipulated and applied according to the manufacturer's instructions.

After 30 (groups 1 and 3) and 60 days (groups 2 and 4) teeth were extracted under local anaesthesia. At the time of extraction, the patients were asked about the presence or absence of postoperative sensitivity. The roots of all teeth were reduced by 5 mm in the apical area in order to facilitate fixation in 10% buffered formalin solution for 72 h. The teeth were decalcified in 50% formic acid-sodium citrate for 6–8 weeks, prepared according to normal histological techniques and embedded in paraffin. Six-micron thick sections were cut with a microtome parallel to the main vertical axis of the tooth. The sections, mounted on glass slides, were stained with haematoxylin and eosin (H/E). Brown and Brenn technique was used to stain bacteria.

The sections were blindly evaluated by an experienced and calibrated pathologist, according to the Mestrener *et al.* (2003) modified criteria: hard tissue bridge; inflammatory response, giant cells and particles of capping materials (Table 2). Each histomorphological observation was evaluated on a 1–4 scoring system, with 1 being the best result and 4 being the worst result. The presence of microorganisms was evaluated, but the response to this criterion is only positive or negative. All teeth with the presence of microorganisms were excluded from statistical analysis. For each tooth, multiple sections were available. The worst score in the sections from the same tooth was recorded.

The overall scores attributed to each group were subjected to nonparametric Kruskal–Wallis statistical analysis. This test was performed for the overall histological examination (hard tissue bridge, inflammatory response, giant cells and particles of capping material). The comparisons between averages were performed by comparing the ranks with appropriately computed critical values ($\alpha = 0.05$) using the Mann–Whitney *U*-test. This test is considered powerful for several independent samples (Conover 1980).

Table 2 Scores used during the histological examination: hard tissue bridge, inflammatory response, giant cells and particles of capping materials

Scores	Hard tissue bridge[a]
1	Complete hard tissue bridge-closure of the exposure area
2	Partial hard tissue bridge-little communication of the capping material with dental pulp.
3	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
Scores	Inflammatory response[b]
1	No inflammatory reaction
2	With inflammatory reaction
3	Abscess
4	Necrosis
Scores	Giant cells
1	Absent
2	Mild number
3	Moderate number
4	Great number
Scores	Particles of the capping materials
1	Absent
2	Mild number
3	Moderate number
4	Great number

[a]Evaluated with a micrometric ocular in three different points of the bridge.
[b]Item evaluated in different areas with a magnification of 400×.

Results

None of the teeth was associated with sensitivity during the period of the study. All restorations were clinically acceptable without fractures or marginal discoloration at the enamel borders by the time of the extraction. The number of specimens classified in each score for all groups is shown in Table 3. Five teeth were excluded on the assumption of the presence of microorganisms (two in the group Angelus 30 days; two in group Dentsply 30 days and; one in group Dentsply 60 days). All groups performed well in terms of hard tissue bridge formation, inflammatory response and other pulpal findings. No significant difference was observed between groups ($P > 0.05$).

Histomorphological features

Group MTA-Angelus 30 days

Five specimens exhibited complete and two partial hard tissue bridge formation (Fig. 1). In Fig. 1 a layer of odontoblast cells can be seen. Only in one case no hard tissue bridge was observed. In five specimens, a chronic inflammatory infiltrate with different intensities and extensions was seen (Fig. 1). Giant cells and particles of the capping materials were found in one case.

Group MTA-Angelus 60 days

Six specimens exhibited complete hard tissue bridges (Fig. 2), whilst in four the bridge was partial, with communications between the capping material and the

Table 3 Number of specimens (%) for each group in each criterion as well as multiple comparisons

Groups	Hard tissue bridge					Inflammatory response					Giant cells					Particles of the capping materials					
Scores	1	2	3	4	a	1	2	3	4	a	1	2	3	4	a	1	2	3	4	a	b
Angelus 30	5	–	2	1	1.5	3	4	1	–	2.0	7	–	1	–	1.0	7	–	1	–	1.0	1.0 a
Angelus 60	6	1	3	–	1.0	7	3	–	–	1.0	9	1	–	–	1.0	9	1	–	–	1.0	1.0 a
Dentsply 30	5	1	1	1	1.0	2	6	–	–	2.0	8	–	–	–	1.0	6	1	1	–	1.0	1.0 a
Dentsply 60	5	–	3	1	1.5	3	5	–	1	2.0	8	–	–	1	1.0	8	–	–	1	1.0	1.0 a

Different letters mean significant differences ($P < 0.05$).
[a]Medians for each group in each sub-item of the criteria.
[b]Overall medians for the criteria.

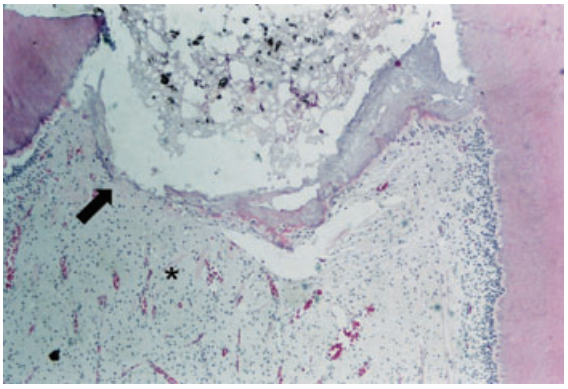

Figure 1 MTA-Angelus, 30 days. There is a partial, irregular and thin hard tissue bridge, and the capping material communicates with the dental pulp (black arrow). A chronic inflammatory infiltrate in this region of the pulp with varying intensities can be observed (black asterisk, HE, 40×).

pulpal tissue. The hard tissue bridge was usually thick and near to the exposure site with the formation of a new layer of odontoblast cells. In three specimens, a chronic inflammatory infiltrate was observed (Fig. 2). Giant cells and particles of the capping material were found in one case. Microorganisms were not found in this group.

Group MTA Dentsply 30 days

Five specimens exhibited complete hard tissue bridges and in two cases the hard tissue bridges were partial (Fig. 3). Near the hard tissue bridge a new layer of odontoblast cells was found. No hard tissue bridge formation was observed in one specimen. In six specimens, a chronic and acute inflammatory infiltrate with varying intensities was observed (Fig. 3). Capping particles or dentine fragments were observed in two specimens. No giant cells were seen in this group.

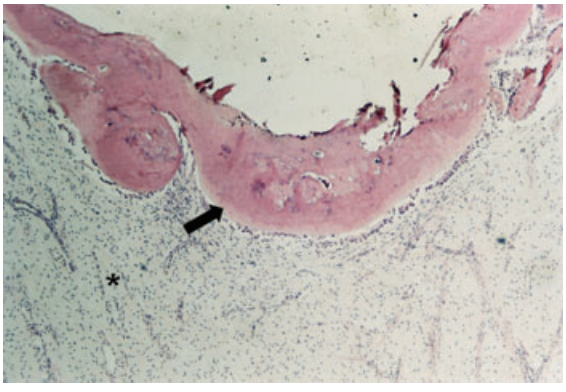

Figure 2 MTA-Angelus, 60 days. There is a complete and thick hard tissue bridge (black arrow). The dental pulp close to the hard tissue bridge shows a chronic inflammatory infiltrate with different intensities and extensions (black asterisk, HE, 40×).

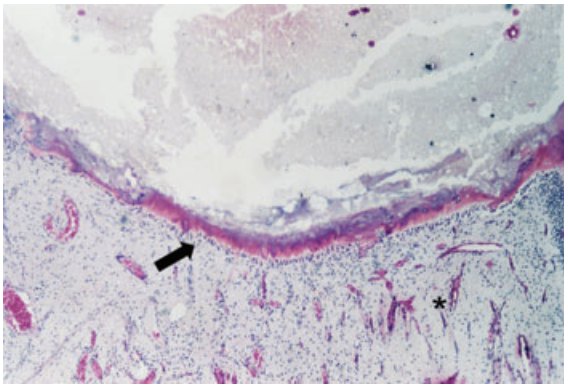

Figure 3 MTA Dentsply, 30 days. There is an irregular and thin hard tissue bridge with no communication between capping material and dental pulp (black arrow). A layer of odontoblast cells can be observed around the black arrow. A chronic inflammatory infiltrate with different intensities and extensions can be observed (black asterisk, HE, 40×).

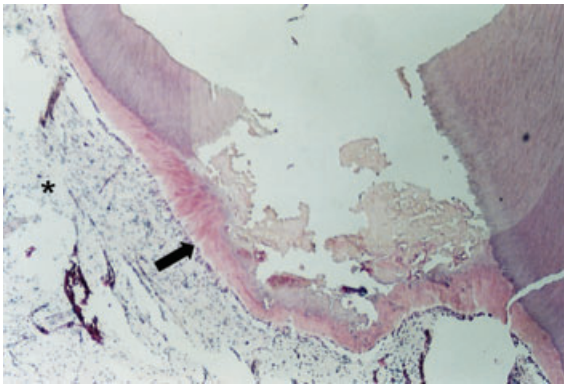

Figure 4 MTA Dentsply, 60 days. There is a complete, irregular and thick hard tissue bridge (black arrow). A chronic inflammatory infiltrate with different intensities and extensions can be observed (black asterisk, HE, 40×).

Group MTA Dentsply 60 days

Five specimens exhibited complete hard tissue bridge formation (Fig. 4) with three having a partial bridge. In the latter, the capping material was in contact with the pulpal tissue. The hard tissue bridge was usually thick, and below the exposure site a new layer of odontoblasts around the hard tissue bridge could be seen (Fig. 4). No hard tissue bridge was observed in one specimen. In six specimens, a chronic inflammatory infiltrate was observed and in three specimens, normal pulpal tissue, near the hard tissue bridge formation was present. Presence of the capping material was associated with macrophages and particles of capping material inside the pulp tissue were found in one specimen.

Discussion

If the formation of complete or partial hard tissue bridge with none or little communication between capping material and dental pulp is considered as clinical success of pulp capping, it can be concluded that all groups achieved pulp healing. In spite of the differences in composition between the white and the grey MTA ProRoot (Camilleri *et al.* 2005, Song *et al.* 2006, Oliveira *et al.* 2007), this seems to have little effect on their performance in terms of pulp healing, as the results of this study, which employed grey MTA, were similar to those of earlier investigations employing white MTA ProRoot (Iwamoto *et al.* 2006, Nair *et al.* 2008).

Iwamoto *et al.* (2006) evaluated white MTA in comparison with calcium hydroxide cement. Forty-five teeth were histologically examined using the HE technique approximately after 4.5 months. Around 91% of MTA-treated and 78% of calcium hydroxide cement-treated teeth 'developed a partial or total bridge'. The results were very similar to that of the present study. Nair *et al.* (2008) compared white MTA with calcium hydroxide cement after three observation times (1 week, 1 month and 3 months). In their study, 33 teeth were histologically evaluated under light microscopy and scanning and transmission electron microscopy (SEM and TEM). After 1 and 3 months, MTA presented 100% of bridge formation against only around 33% of calcium hydroxide. The low rate of success of calcium hydroxide achieved in the study of Nair *et al.* (2008) could be due to the selection of the site and size for pulp exposure amongst others.

In terms of the hard tissue bridge, both materials gave good results after 30 and 60 days. Obviously, as can be seen in Table 3, some differences were observed. Differences in their composition, particle size and shape of these materials could be the reason for these slight differences.

According to Dentsply, ProRoot is composed of 75% Portland cement, 20% bismuth oxide and 5% gypsum in weight (Dentsply Tulsa Dental 1998). On the other hand MTA-Angelus contains only Portland cement (80%) and bismuth oxide (20%). As a result of greater amount of Portland cement in MTA-Angelus (Estrela *et al.* 2000, Santos *et al.* 2005) the pH of MTA-Angelus is higher than that of ProRoot (Duarte *et al.* 2003).

Other studies that evaluated the composition of MTA (Torabinejad et al. 1995a,b, Estrela et al. 2000, Camilleri *et al.* 2005, Song *et al.* 2006, Oliveira *et al.* 2007) found that there are, indeed, slight differences in their chemical compositions when compared with the information provided by the manufacturers of Angelus and Dentsply (Torabinejad *et al.* 1995a, Estrela *et al.* 2000, Camilleri *et al.* 2005, Oliveira *et al.* 2007), as well as physical features, such as particle size and shape (Hashem & Hassanien 2008, Komabayashi & Spångberg 2008).

According to the manufacturers' information, the proportions of bismuth oxide are the same in both materials evaluated. Bismuth oxide normally would be used in excess of 10% by weight for adequate radiopacity in dental acrylic resin (Bloodworth & Render 1992). In these concentrations, properties such as strength and aesthetics were reduced. In addition, it is known that bismuth is toxic (Bloodworth & Render 1992). However, according to Camilleri *et al.* (2004), the lack of biocompatibility of bismuth oxide did not seem to affect the biocompatibility of the

MTA, presumably because of the presence of calcium hydroxide.

Although the exact mechanism by which MTA induces hard tissue bridge formation is not understood completely, the indications show that the initiation of reparative dentinogenesis of MTA and $Ca(OH)_2$ cement are similar (Faraco & Holland 2001, Tziafas *et al.* 2002). Recently, Min *et al.* (2007) evaluated the cellular effects of Portland cement, the basic component of the MTA on cultured human pulp cells. The authors also compared Portland cement with other materials including calcium hydroxide cement. They concluded that Portland cement is biocompatible and allows the expression of mineralization-related genes on cultured human pulp cells, which are responsible for inductive process on hard tissue bridge formation with MTA cement.

Another finding that deserves attention is the fact that in some cases, small particles of the capping materials were observed within the pulpal tissue for both materials. As these particles may induce calcification similar to that which occurs with dentine chips, their presence could have been responsible for retarding the healing process of some specimens, although controversy exists as to whether these particles, which have been accidentally forced into the pulp, promote or retard healing (Stanley 1989).

Microorganisms were only observed in the specimens extracted after 30 days and in only two specimens from each product. Only one specimen had microorganisms after 60 days. The bacteria were found probably due to a faulty overlying filling or possibly poor operative technique. The presence of microorganisms in the pulp indicates contamination and therefore the tissue response was not in response to the material. This is the reason why teeth with microorganisms were excluded from the analysis.

The low incidence of microbial contamination could have been related to the seal provided by the MTA materials and the provisional restorative material as well as the bactericidal properties of MTA (Torabinejad *et al.* 1995b, Estrela *et al.* 2000). However, one cannot rule out the fact that the histochemical staining technique used for the detection of bacteria in the present investigation was of low sensitivity making identification of bacterial difficult, especially when in small numbers (Stanley 1998). In addition, bacteria are lost or removed from dental tissues during histological preparation (Bergenholz *et al.* 1982, Torstenson 1995).

It must be emphasized that this study was performed in sound teeth. In most clinical scenarios, pulp exposure frequently occurs as a result of a carious process in which the level of inflammation is much higher. Had inflamed pulps been capped, the results of this investigation could have been different.

Conclusion

Both commercial MTA products [ProRoot (Dentsply) and MTA-Angelus (Angelus)] produce a good pulp response when placed in healthy teeth on exposed pulp tissue.

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