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ORIGINAL RESEARCH

Scanning electron microscopy evaluation of the hard tissue barrier after pulp capping with calcium hydroxide, mineral trioxide aggregate (MTA) or ProRoot MTA

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Abstract

The aim of this study was to investigate the morphology and localisation of calcium hydroxide- and mineral trioxide aggregate (MTA)-induced hard tissue barriers after pulpotomy in dogs' teeth. Pulpotomies were performed on maxillary and mandibular premolars of five dogs. The teeth were assigned into three groups according to the pulp-capping agent used. The pulpal wounds were capped with calcium hydroxide ($Ca(OH)_2$ – control), MTA or ProRoot MTA, and the cavities were restored with amalgam. After a 90-day follow-up period, the dogs were euthanised and the teeth were examined under scanning electron microscopy (SEM). An image-processing and analysis software was used to delimit the perimeters of the root canal area and the hard tissue barrier to determine the percentage of root canal obliteration. SEM data were used to assess the morphology, localisation and extension of the reparative hard tissue barriers. ProRoot MTA was statistically different from MTA and $Ca(OH)_2$ ($P < 0.05$) regarding tissue barrier morphology. Localisation data showed that ProRoot MTA was significantly different from $Ca(OH)_2$ ($P < 0.05$) and similar to MTA ($P > 0.01$; $P > 0.05$). No statistically significant difference ($P > 0.01$; $P > 0.05$) was observed between MTA and $Ca(OH)_2$. A larger number of complete (centroperipheral) hard tissue barriers with predominance of dentinal tubules was observed to the ProRoot MTA when compared with the $Ca(OH)_2$ group.

Introduction

Several different therapies and pulp-capping materials have been proposed previously. The most frequently used materials for capping exposed or amputated pulp tissue are calcium hydroxide-based paste/cement, zinc oxide and eugenol cement, adhesive systems and, most recently, mineral trioxide aggregate (MTA) (1–13).

Over nearly one century, the biological properties of calcium hydroxide have been extensively investigated. Its capacity of inducing the formation of calcified tissue barriers as well as the bactericidal and bacteriostatic properties resulting from its high pH are well demonstrated (1,8,14–21). For more than 50 years, calcium hydroxide was the material of choice for capping of pulp remnant in teeth undergoing pulpotomy. However, current investigations have demonstrated that pulps capped with calcium hydroxide or MTA present similar mechanism of healing (9).

MTA was introduced to dentistry in the early 1990's (22,23) and was initially indicated for repair of root perforations. The findings of the first studies were promising and very stimulating with respect to the sealing and repairing properties of this material. Similar to calcium hydroxide, MTA also has a high pH, which suggests that this is one of the main characteristics that pulp-capping agents must have in order to induce, stimulate or at least to provide pulpal healing. It might appear contradictory hence, it was believed that ProRoot MTA and MTA had absolutely the same formulation. It may be

suggested that small differences in the composition, as demonstrated by Estrela *et al.* (2000) (8) and Camilleri *et al.* (2005) (24), would determine the variation in tissue response to these materials.

In clinical situations of pulp exposure or amputation, complete pulp healing is characterised by the formation of a complete calcified tissue barrier and maintenance of the normal histological characteristics of the remaining pulp tissue. In view of this, the purpose of this study was to investigate the morphology and localisation of calcium hydroxide- and MTA-induced hard tissue barriers after pulpotomy in dogs' teeth.

Materials and methods

The research protocol was approved by the local Ethics in Research Committee (Lutheran University of Brazil, Canoas, Brazil) in compliance with the applicable ethical guidelines and regulations of the International Guiding Principles for Biomedical Research Involving Animals (Geneva, 1985).

Five 1-year-old Beagle dogs were used in this study. Until the beginning of the experiment, the health status of the animals was monitored daily to avoid any painful or distressful situations. The dogs were maintained anesthetised and hydrated with saline as the operative procedures were performed on the maxillary and mandibular second, third and fourth premolars of both sides. A total of 53 teeth (94 roots) were included in the study.

After prophylaxis with pumice/water, the teeth were radiographed to evaluate root conditions. Cuspal reduction was performed using diamond burs at high-speed and under copious air/water cooling, and the teeth were isolated with a rubber dam and No. 00 rubber dam clamps (Ivory Company, Philadelphia, PA, USA). Crown access was made with a size 2 round carbide bur (SS White, Rio de Janeiro, Brazil) at high-speed and under copious air/water cooling. The pulp chamber roof was completely removed and the coronal pulp tissue was amputated using sharp sterile curettes adapted to pulp chamber dimensions. Hemostasis was achieved by gently pressing against the remaining pulp tissue with autoclaved cotton pellets. The teeth were assigned into three groups in a randomised manner according to the pulp-capping agent to be used after pulpotomy. The root pulp wounds were capped with one of the following pulp-capping agents: calcium hydroxide (control – pro-analysis $Ca(OH)_2$; J. T. Baker, São Paulo, Brazil), mineral trioxide aggregate – MTA (Loma Linda University, Loma Linda, CA, USA) and ProRoot MTA (Tulsa/Dentsply, Tulsa, OK, USA). The capping agents were protected with a layer of a hard-setting calcium hydroxide cement (Dycal®, Dentsply Ind. e Com. Ltda, Rio de Janeiro, Brazil) and the cavities were restored with a high-copper spherical amalgam alloy (Tytin®; Sybron-Kerr, Romulus, MI, USA). New periapical radiographs were taken in a buccolingual direction to evaluate the quality of pulp-capping procedure.

The animals were housed under strict surveillance by a skilled staff during a follow-up period of 90 days and were then euthanised by sodium thiopental overdose (Thionembutal, Abbot Laboratories, Rio de Janeiro, Brazil).

The bone pieces containing the teeth were removed and reduced in size to fragments measuring approximately 3 mm of coronal height and 3 mm of root height. The specimens were stored in buffered 10% formalin at 4°C for 72 h. Afterwards, the pieces were gently ground with water-cooled fine-grained sandpapers until a final thickness of 2 mm on average was reached and root canal entrances were visualised.

The specimens were immersed in 2.5% glutaraldehyde (Merck KGaA, Frankfurter Darmstadt, Germany) in a 0.1-mol/L sodium cacodylate buffer at pH 7.4 (Merck KGaA), for 12 h at 4°C. After fixation, the specimens were rinsed with 0.2 mol/L sodium cacodylate buffer at pH 7.4 for 1 h with three changes followed by 1 min in distilled water. Right after, they were dehydrated in an ascending ethanol series (25% for 20 min; 50% for 20 min; 75% for 20 min; 90% for 30 min; and 100% for 60 min). The specimens were mounted on stubs, sputter-coated with gold and examined with a SEM (XL20, Phillips, Eindhoven, the Netherlands).

The coronal surface of the specimen was evaluated at ×250, ×300 and ×3000 magnifications. The images were saved as tagged image file format files and were analysed using an image processing and analysis software (ImageLab, Softium Informática, São Paulo, Brazil). The perimeter of the root canal area and the perimeter hard tissue barrier were delimited in order to determine the percentage of obliteration and the extension of the barrier.

Regarding the morphology, the hard tissue barriers were classified as amorphous, when the presence of tubules was inexistent or insignificant; mixed, when the tubules occupied an area proportional to that occupied by amorphous dentin; and tubular, when the presence of tubules was predominant or absolute. The morphological analysis of the hard tissue barriers was performed by SEM micrographs taken at ×3000 magnification. The scores of each tested material were recorded and further interpreted.

Regarding the localisation, the hard tissue barriers were classified as central (at the center of the exposure); peripheral (around the walls of the canal); and centroperipheral (when the formation of a hard tissue barrier was complete).

Chi-squared test and Bonferroni correction were used for statistical analysis of the morphology and localisation data at 5% significance level.

Results

The difference in the number of specimens in each group was due to variables found in every clinical study, such as missing teeth and variations in response from one root to another in the same tooth. These differences were recorded and compensated for in the statistical analysis. The findings regarding the morphology and localisation of the hard tissue barriers are show in Tables 1 and 2.

Calcium hydroxide

Out of 24 specimens, one presented a tubular dentin barrier, corresponding to 4.2% of the total. Mixed dentin barriers were observed in 10 specimens (41.7%). Thirteen specimens presented hard tissue barriers with characteristics of amorphous dentin, corresponding to 54.2% of the cases (Fig. 1).

Regarding the localisation of the hard tissue barriers, no central barrier was observed. In seven specimens (29.2%), the deposition of mineralised tissue occurred in the centroperipheral area, characterising the complete hard tissue barrier formation. In the remaining 17 specimens (70.8%), the hard tissue barrier was formed only in the peripheral area (Fig. 2).

Five specimens did not present formation of a hard tissue repair barrier and were therefore not included in the statistical analysis because they did not produce any morphology or localisation data.

MTA

A total of 29 specimens were considered for evaluation of this pulp-capping agent. Mineralised tissue barriers with characteristics of tubular dentin were observed in seven specimens, corresponding to 24.1% of the total. Twelve specimens (41.4%) presented hard tissues barriers with an amorphous pattern whereas in 10 specimens (34.5%) mixed hard tissue barriers were formed (Fig. 3).

Regarding the localisation of the MTA-induced hard tissue barriers, there was a predominance of centroperipheral barriers, corresponding to 16 cases (55.2%) treated with this capping agent. Central dentin barriers were found in only three specimens (10.3%). In the

Table 1 Morphology of the hard tissue barriers formed with each material

Morphology	$Ca(OH)_2$	MTA	ProRoot MTA	Total*
Amorphous	13 (54.2%)	12 (41.4%)	1 (4.2%)	26 (33.8%)
Mixed	10 (41.7%)	10 (34.5%)	8 (33.3%)	28 (36.4%)
Tubular	1 (4.2%)	7 (24.1%)	15 (62.5 %)	23 (29.9%)
Total	24 (100%)	29 (100%)	24 (100%)	77 (100%)

*Specimens presenting inaccurate reading were rejected. MTA, mineral trioxide aggregate.

Table 2 Localisation of the hard tissue barrier formed with each material

Location	$Ca(OH)_2$	MTA	ProRoot MTA	Total*
Central	0 (0)	3 (10.3%)	2 (7.1%)	5 (6.2)
Peripheral	17 (70.8%)	10 (34.5%)	5 (17.9%)	32 (39.5)
Centroperipheral	7 (29.2%)	16 (55.2%)	21 (75.0%)	44 (54.3)
Total	24 (100%)	29 (100%)	28 (100%)	81 (100%)

*Specimens presenting inaccurate reading were rejected. MTA, mineral trioxide aggregate.

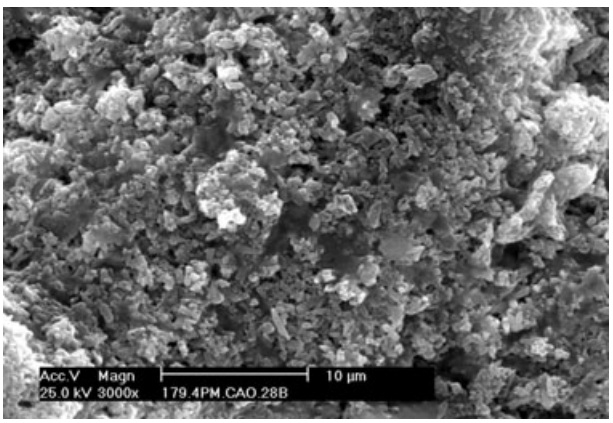

Figure 1 Morphology. Example of an amorphous $Ca(OH)_2$-induced hard tissue barrier.

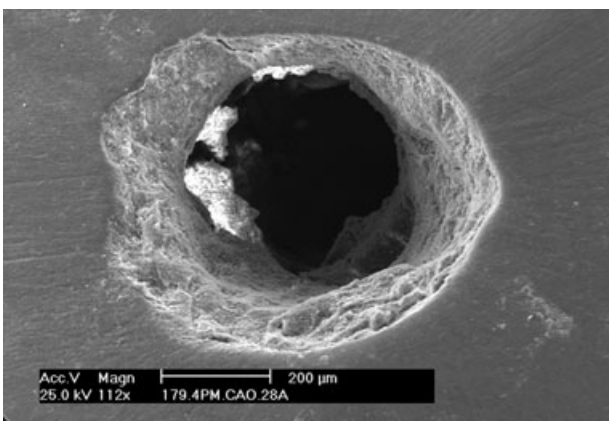

Figure 2 Localisation. Example of a peripheral $Ca(OH)_2$-induced hard tissue barrier.

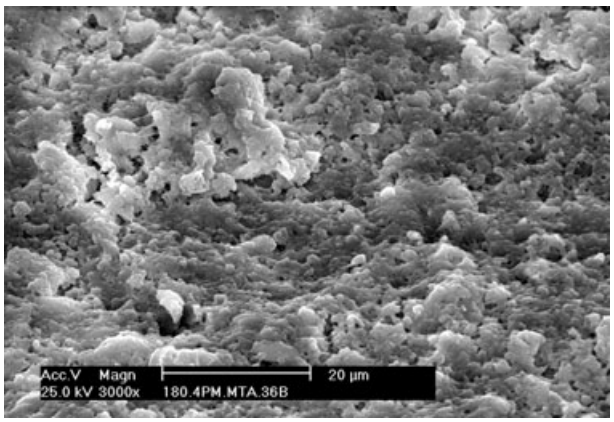

Figure 3 Morphology. Example of a mixed mineral trioxide aggregate-induced hard tissue barrier.

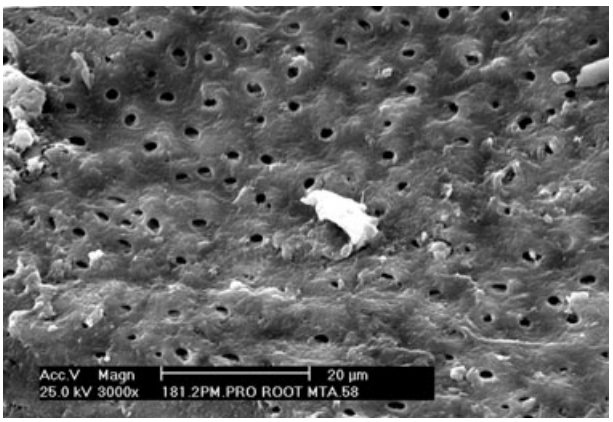

Figure 5 Morphology. Example of a tubular ProRoot mineral trioxide aggregate-induced hard tissue barrier.

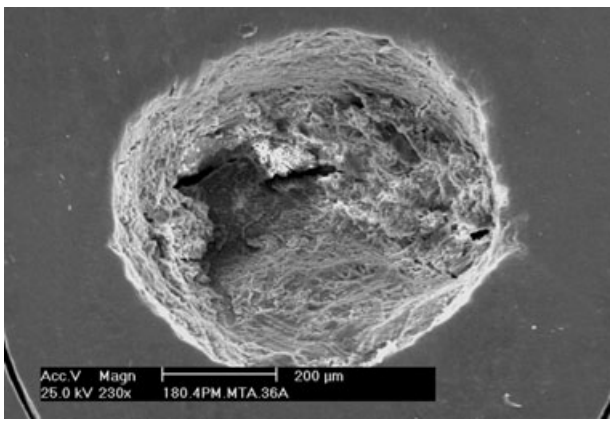

Figure 4 Localisation. Example of a centroperipheral mineral trioxide aggregate-induced hard tissue barrier.

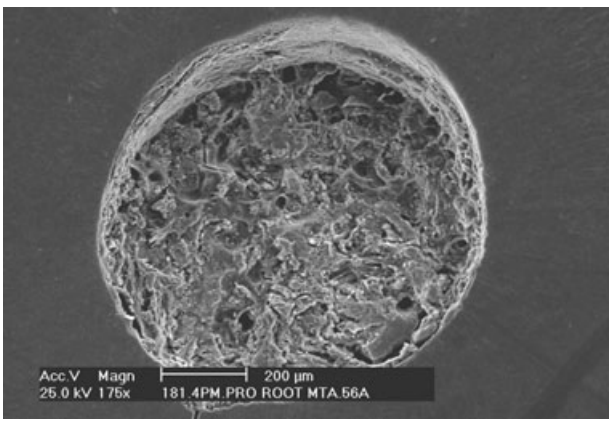

Figure 6 Localisation. Example of a centroperipheral ProRoot mineral trioxide aggregate-induced hard tissue barrier.

remaining 10 specimens (34.5%), the hard tissue barriers had a peripheral localisation (Fig. 4).

Four specimens did not present formation of mineralised tissue barriers and were not included in the statistical analysis.

ProRoot MTA

Fifteen specimens (62.5%) treated with ProRoot MTA presented hard tissue barriers with predominance of dentinal tubules. Mixed hard tissue barriers were found in eight specimens (33.3%). Only one specimen exhibited hard barrier with amorphous characteristics, which corresponds to 4.2% of the cases (Fig. 5).

Regarding the localisation of the barriers in this group, in most specimens (21 cases corresponding to 75%), the formation of a hard tissue barrier occurred in the centro-peripheral area. The incidence of hard barriers formed in the peripheral area was 17.9% (five specimens) and in the central area was 7.1% (two specimens) (Fig. 6).

Three specimens did not present formation of mineralised tissue barriers and were thus not included in the statistical analysis.

Statistical analysis

The statistical analysis of the hard tissue barrier morphology data showed that ProRoot MTA was significantly different from MTA and calcium hydroxide ($P < 0.05$). However, no statistically significant difference ($P > 0.05$) was observed between MTA and calcium hydroxide. The statistical analysis of the hard tissue barrier localisation data showed that ProRoot MTA was significantly different from calcium hydroxide ($P < 0.05$) and was similar to

MTA ($P > 0.01$; $P > 0.05$). No statistically significant difference ($P > 0.01$; $P > 0.05$) was observed between MTA and calcium hydroxide.

Discussion

Pure, pro-analysis calcium hydroxide powder was used as a control in this *in vivo* study because it is well documented that the use of this material as a pulp-capping agent stimulates pulp repair by a specific mechanism (25).

In the present study, most specimens that had the pulpal wound capped with calcium hydroxide after pulpotomy showed the formation of hard tissue barriers with an amorphous pattern. Likewise, in a histochemical analysis of the mechanism of dentin formation in dogs' pulp, Eda (1961) (26) observed predominance in the synthesis of reparative dentin with amorphous characteristics (non-tubular) when pulp was capped with calcium hydroxide. The authors reported that the amorphous areas were characterised as precipitations of coarse calcium granulations, constituting the so-called superficial granular zone, which is associated with the initial synthesis and deposition of disorganised dentin matrix. The second most predominant morphological type of hard tissue barrier observed in the calcium hydroxide group was the mixed type. It has been reported that the layers of the hard tissue barrier have distinct characteristics. Although the external layer is irregular and atubular, the internal layer exhibits the typical dentinal characteristics with pre-dentin and irregular, tortuous, randomly distributed tubules, similar to those present in the reparative dentin (5,25).

It has been widely reported that when directly applied to the exposed pulp tissue, both MTA and its commercial formulation (ProRoot MTA) induce the formation of a hard tissue barrier without a significant local inflammatory response (6,10,12,19,21). The findings of this *in vivo* study showed that, together, tubular and mixed hard tissue barriers were formed in more than 50% of the specimens capped with MTA and ProRoot MTA, with a significant increment in the formation of tubular barriers in the ProRoot MTA group.

It has been reported that, although MTA and calcium hydroxide have a similar mechanism of action on the exposed pulp tissue (9), the inflammatory pulp response to MTA seems to be less intense. This might be attributed to the fact that, immediately after preparation for use, MTA has a significantly lower pH (approximately 10.2) than calcium hydroxide (nearly 13.0). During the setting period, which takes about 4 h, the pH of MTA can increase to 12.5 (23). The difference between the initial pH of calcium hydroxide and MTA, when the materials are placed in contact with the exposed pulp tissue, would justify the occurrence of a more severe pulp injury when calcium hydroxide is used as a pulp-capping agent.

Torabinejad *et al.* (1995) (18) showed a more significant and less organised tissue response in pulps capped with calcium hydroxide compared with pulps capped with MTA. Therefore, the data from the morphology and localisation of the hard tissue barriers formed in the present study suggest that there is a relationship between the influence of materials' pH and the pulp response pattern. The three materials evaluated in this study stimulated pulp repair, which was characterised by the deposition of a hard tissue barrier in the amputated pulp tissue. However, it may be speculated that the main difference in the formation of the mineralised barrier, which is directly associated with pulp cell activity and extracellular matrix organisation, is due to variations in pulp injury, probably more severe in the calcium hydroxide group. This is attributed to the fact that, in addition to its high pH, calcium hydroxide also releases ions calcium and hydroxyl intensively and continuously when applied in moist environments (27).

On the other hand, MTA and ProRoot MTA have a relatively low pH immediately after preparation, thus causing a discrete initial caustic effect when placed in direct contact with the pulp wound. Moreover, the setting reaction of these materials may limit the continuous release of toxic ions to the subjacent pulp tissue. It should also be emphasised that, after final stetting, both MTA and ProRoot MTA have a significant mechanical strength unlike the calcium hydroxide paste, which maintains a puttylike consistency even after cavity restoration. In view of this, it may be speculated that up to the end of the follow-up period after pulpotomy (90 days), the tested MTA cements presented a limited diffusion of their components from the pulp-capping site to the interior of the pulp tissue. Perhaps, it might have been an important factor that contributed to presence of a complete (centroperipheral) hard tissue barrier in several specimens in MTA and ProRoot MTA groups and to the small number of this type of barriers in the roots in which pulp remnant was capped with calcium hydroxide. The few centroperipheral hard tissue barriers formed in this group presented a convex shape and were distant from the capping agent, suggesting the existence of a direct relationship between the capping material, localisation and direction of the barrier and the morphology of the formed tissue (4,27,28).

It is not possible to unequivocally state that external interferences did not occur in the different phases of the present *in vivo* study because distinct responses might be observed in individuals from the same species and even in the same individual. However, to minimise these

interferences, care was taken in the standardisation of pre- and postoperative conditions, storage of specimens, preparation of anatomical pieces and randomisation of specimen distribution for each material. Particular attention to these issues was determinant not to allow that one of the materials had favorable or unfavorable results compared with the others in such a way that it could effectively interfere with the study outcomes.

According to the proposed methodology and based on the experimental conditions of this *in vivo* study, it may be concluded that: (i) all materials used as pulp-capping agents induced the deposition of a hard tissue barrier at 90 days after the pulp therapy (pulpotomy); (ii) most hard tissue barriers formed in those root pulps capped with ProRoot MTA exhibited dentinal tubules; and (iii) there was a predominance of centroperipheral hard tissue barriers in the specimens treated with ProRoot MTA compared to the other pulp-capping agents evaluated in this *in vivo* study.

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