The effect of mineral trioxide aggregate on the apexification and periapical healing of teeth with incomplete root formation

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Abstract

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Aim To evaluate the influence of mineral trioxide aggregate (MTA) on apexification and periapical healing of teeth in dogs with incomplete root formation and previously contaminated canals and to verify the necessity of employing calcium hydroxide paste before using MTA.

Methodology Twenty premolars from two 6-month old dogs were used. After access to the root canals and complete removal of the pulp, the canal systems remained exposed to the oral environment for 2 weeks. Canal preparation was then carried out using Hedström files, under irrigation with 1% sodium hypochlorite, 1 mm short of the radiographic apex. After drying, the canals of two premolars in each dog were left empty (control group). The other eight teeth in each animal were divided into two experimental groups. The apical thirds of the canals of group 1 were filled with MTA. In the teeth of group 2, the canals were dressed with a calcium hydroxide–propylene glycol paste. After 1 week, the paste was removed and the apical third was filled with MTA. All teeth were restored with reinforced zinc oxide cement (IRM) and amalgam. The animals were killed 5 months later, and blocks of the teeth and surrounding tissues were submitted to histological processing. The sections were studied to evaluate seven parameters: formation of an apical calcified tissue barrier, level of barrier formation, inflammatory reaction, bone and root resorption, MTA extrusion, and microorganisms. Results of experimental groups were analysed by Wilcoxon's nonparametric tests and by the test of proportions. The critical value of statistical significance was 5%.

Results Significant differences ($P < 0.05$) were found in relation to the position of barrier formation and MTA extrusion. The barrier was formed in the interior of the canal in 69.2% of roots from MTA group only. In group 2, it was formed beyond the limits of the canal walls in 75% of the roots. MTA extrusion occurred mainly in roots from group 2. There was similarity between the groups for the other parameters.

Conclusions Mineral trioxide aggregate used after root canal preparation favoured the occurrence of the apexification and periapical healing. The initial use of calcium hydroxide paste was not necessary for apexification to occur, and has shown to be strongly related to the extrusion of MTA and formation of barriers beyond the limits of the root canal walls.

Keywords: apexification, mineral trioxide aggregate, open apices, periapical healing.

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Introduction

When teeth with incomplete root formation suffer pulp necrosis, the formation of dentine stops, and root development ceases. Consequently, the canal remains large, with thin and fragile walls, and the apex remains open. These features make instrumentation of the canal difficult and hinder the formation of an adequate apical stop. In such cases, in order to allow the condensation of the root filling material and to promote an apical seal, it is imperative to create an artificial apical barrier or induce the closure

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of the apical foramen with calcified tissue (apexification).

Calcium hydroxide pastes have become the material of choice to induce apexification (Leonardo *et al.* 1993, Felippe *et al.* 2005). Despite its efficacy, this dressing has several disadvantages, such as variability of treatment time, number of appointments and radiographs, difficulty in patient follow-up, delayed treatment (Shabahang *et al.* 1999) and possibility of increased tooth fracture after calcium hydroxide use for extended periods (Andreasen *et al.* 2002).

Studies have indicated mineral trioxide aggregate (MTA) as an alternative to calcium hydroxide (Tittle *et al.* 1996, Shabahang *et al.* 1999). MTA is a powder aggregate, containing mineral oxides (Lee *et al.* 1993). Besides its noncytotoxicity (Osorio *et al.* 1998), it has good biological action (Torabinejad *et al.* 1995c,d, Torabinejad *et al.* 1998) and stimulates repair (Regan *et al.* 2002, Economides *et al.* 2003), because it allows cellular adhesion, growth and proliferation on its surface (Zhu *et al.* 2000). When used in dogs' teeth with incomplete root formation and contaminated canals, MTA often induced the formation of apical barrier with hard tissue (Tittle *et al.* 1996, Shabahang *et al.* 1999).

From experimental studies described in the literature (Tittle *et al.* 1996, Shabahang *et al.* 1999), clinical protocols (Shabahang & Torabinejad 2000), as well as clinical cases (Torabinejad & Chivian 1999, Witherspoon & Ham 2001, Giuliani *et al.* 2002, Levenstein 2002, Maroto *et al.* 2003), MTA was placed into canals only after the use of calcium hydroxide paste for 1 week. Therefore, the reaction of the apical tissues when this material is applied immediately after canal preparation, in teeth with incomplete root formation, is not known.

The aim of this study was to evaluate the effect of MTA on apexification and periapical healing in dogs' teeth with incomplete root formation and contaminated canals, and to verify whether calcium hydroxide paste was required prior to the use of MTA.

Material and methods

The research project was approved by the Animal Ethics Screening Committee of the Federal University of Santa Catarina (Florianópolis, SC, Brazil).

The procedures were performed as described by Felippe *et al.* (2005). A total of 40 root canals from 20 maxillary and mandibular premolars of two 6-month old Beagle dogs were used. After intramuscular sedation with a mixture of xilasine (Rompum, Abbott, São Paulo, SP, Brazil) and cetamine cloridrate (Ketamin, Aster, São Paulo, SP, Brazil), the dogs were anaesthetized intravenously with 5% sodium thiopental solution (Thionembutal, Abbott) at the dose of 5-mg Kg^{-1} body weight. During the operative procedures, the animals received an infusion of saline solution and intravenous anaesthetics, as required.

Pre-operative radiographs were exposed to confirm the presence of open apices. After endodontic access and determination of canal length, pulp tissue was removed with K-files (Dentsply Maillefer, Ballaigues, Switzerland). Size 60 Hedström files (Dentsply Maillefer, Ballaigues, Switzerland) were introduced up to the radiographic apex and were used in a filing action to remove the remnants of apical pulp tissue totally. The canals were irrigated with distilled water and after haemostasis, a cotton pellet was placed in the entrance of each canal and the teeth were left without a coronal restoration for 2 weeks.

After this period, radiographs were exposed that demonstrated the presence of apical pathosis of variable size associated with all roots. The canals were cleaned under aseptic conditions to the radiographic apex using size 50–60 K-files. Hedström files sizes 70 and 80 were subsequently used 1 mm short of the radiographic apex, using gentle filing movements. During preparation, the canals were irrigated with 1% sodium hypochlorite solution. After drying with sterile paper points, the canals of two premolars of each dog were left unfilled (control group); the coronal restoration consisted of reinforced zinc oxide cement (IRM) and amalgam. For the other eight premolars in each animal, the canals were dried with sterile paper points. The teeth were randomly assigned to two experimental groups.

In group 1, 16 canals were filled with a mixture of MTA (Pro Root MTA, Dentsply Tulsa Dental, Tulsa, OK, USA) with distilled water, in a proportion of 3 : 1. The MTA was placed into the canals with a lentulo-spiral, introduced 3 mm short of the radiographic apex and condensed in the apical third by gentle packing with paper points. The teeth were radiographed to check the MTA apical plug and a sterile cotton pellet was then placed in the access cavity. The coronal restoration consisted of IRM and amalgam.

In group 2, 16 canals received a calcium hydroxide paste (pure calcium hydroxide, 0.612 mg, Reagen, Rio de Janeiro, RJ, Brazil) mixed with propylene glycol (0.4 mL, Quimidrol, Joinville, SC, Brazil). The paste was placed into the canals with a lentulo-spiral, introduced

3 mm short of the radiographic apex. The teeth were radiographed for complete filling of the canals, and sealed with IRM. After 1 week the canals were irrigated with saline solution, and a size 80 K-file was introduced 1 mm short of the radiographic apex and gently manipulated to remove the paste. Irrigation was repeated until the solution was clear of any calcium hydroxide residue. After drying, the apical 3–4 mm of the canals was filled with MTA. All other procedures and the coronal restoration were similar to group 1.

Five months after mechanical canal preparation, the animals were killed and the jaws dissected and fixed in 10% formol solution. After decalcification in formic acid–sodium citrate solution, the specimens were reduced, soaked in paraffin, and trimmed until the apical foramen was exposed. The blocks were cut into 6-μm thick sections and stained with haematoxylin-eosin (HE) and by Brown–Brenn technique (BB).

Typical sections from each root were analysed by three examiners under light microscopy for seven parameters:

- *Apical calcified tissue barrier:* which was classified as (i) absent – there was no new calcified tissue in the apical foramen, (ii) incomplete – apical calcified tissue was interrupted by fibrous or inflammatory tissue, or (iii) complete – apical calcified tissue extending from one root canal wall to the other
- *Level of barrier formation:* (i) within the interior of the canal, (ii) at the limit of the foramen or (iii) beyond the limits of the root canal walls
- *Inflammatory reaction:* which was classified as (i) absent or mild – scattering of inflammatory cells with no structural damage, (ii) moderate – focal accumulations of inflammatory cells, no tissue necrosis with some disruption of structure, or (iii) severe – extensive inflammatory cell infiltrate with replacement of tissues, abscess
- *Bone resorption:* characterized by the presence of clastic cells on the bone surface and enlargement of the periodontal space
- *Root resorption:* characterized by the presence of clastic cells on the root surface or by perceptible exposed dentine surface
- *Extrusion of MTA:* presence of material beyond the limits of the root walls
- *Microorganisms:* presence of microorganisms in the root canal, inside dentinal tubules or in the periodontal tissues.

Wilcoxon's nonparametric tests were used to determine significant differences between the groups for the formation of apical barrier and the intensity of the inflammatory reaction. The other parameters were analysed by the test of proportions. The critical value of statistical significance was 5%.

Results

Before the conclusion of the experiment, four roots from the control group were lost because of severe destruction of the periodontal tissues. Another seven roots were lost during the histological processing. The histological analysis was carried out on 29 specimens; 13 specimens from group 1 (MTA), 12 specimens from group 2 (calcium hydroxide + MTA) and four specimens from the control group.

The histological data obtained are shown in Tables 1–3. In relation to the experimental groups, the statistical analysis revealed significant differences in the histological results in two of the seven parameters studied.

Table 1 Absolute frequency for calcified tissue barrier and level of barrier formation for each group studied

	Calcified tissue barrier			Level of barrier formation		
Groups (*n*)	Absent	Incomplete	Complete	Intracanal	At the foramen	Extracanal
1 (13)	0	6	7	9	3	1
2 (12)	0	10	2	1	2	9

Table 2 Absolute frequency for inflammatory reaction for each group studied

	Inflammatory reaction			
Groups (*n*)	Absent	Mild	Moderate	Severe
1 (13)	9	1	1	2
2 (12)	5	4	2	1

Table 3 Absolute frequency for bone and root resorption, extrusion of mineral trioxide aggregate (MTA), and presence of microorganisms for each group studied

Groups (*n*)	Bone resorption	Root resorption	Extrusion of MTA	Microorganisms
1 (13)	3	3	1	0
2 (12)	3	4	9	0

• *Apical calcified tissue barrier:* this was present in all specimens of groups 1 and 2. The percent of complete barriers was 53.8% for the teeth from group 1 and 16.7% for group 2. The test of proportions showed equivalence between the groups ($Z = 1.93$; $P = 0.0536$).

• *Level of formation of the barrier:* in 69.2% of the roots of group 1, the barrier was formed in the interior of the canal (Fig. 1). However, 75% of the barriers for group 2 occurred beyond the limits of the root canal walls (Fig. 2). The Wilcoxon test revealed significant differences between the groups ($\chi^2 = 191.0$; $P = 0.0218$).

• *Inflammatory reaction:* chronic inflammation was observed in 30.8% and 58.3% of the specimens of the groups 1 and 2, respectively. The test of proportions did not reveal any significant differences between the groups ($Z = 1.39$; $P = 0.1645$). No significant differences were observed for the intensity of the inflammatory reaction ($\chi^2 = 19.50$; $P = 0.2681$).

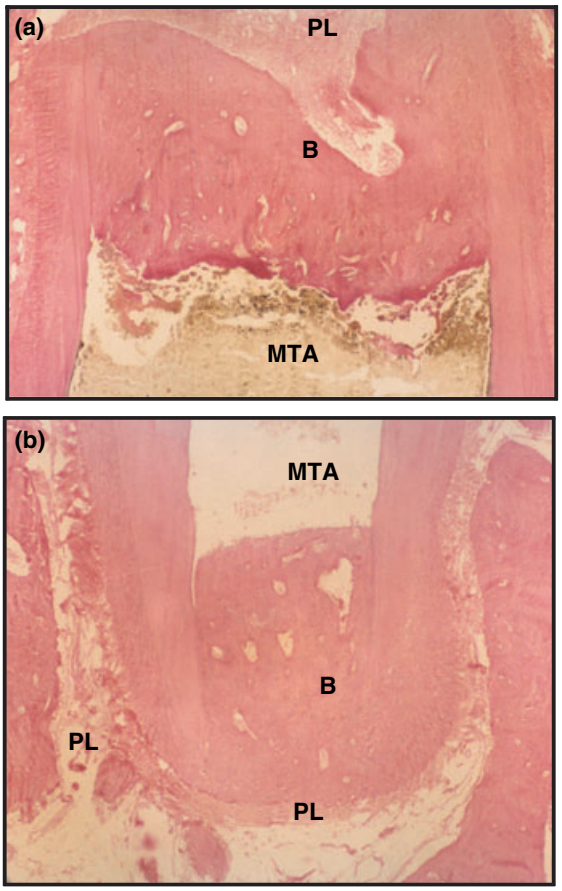

Figure 1 (a) and (b) – Apical area of teeth from group 1 showing complete barrier formation (B) and normal periodontal ligament (PL) (haematoxylin-eosin, ×12.8).

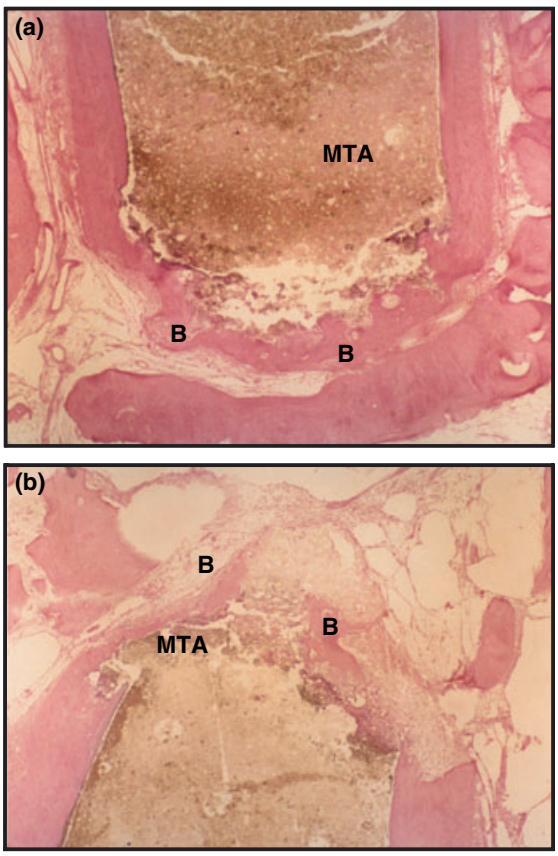

Figure 2 (a) and (b) – Apical area of teeth from group 2 showing incomplete calcified barriers (B). Note the presence of mineral trioxide aggregate beyond the limits of the root canal walls (haematoxylin-eosin, ×12.8).

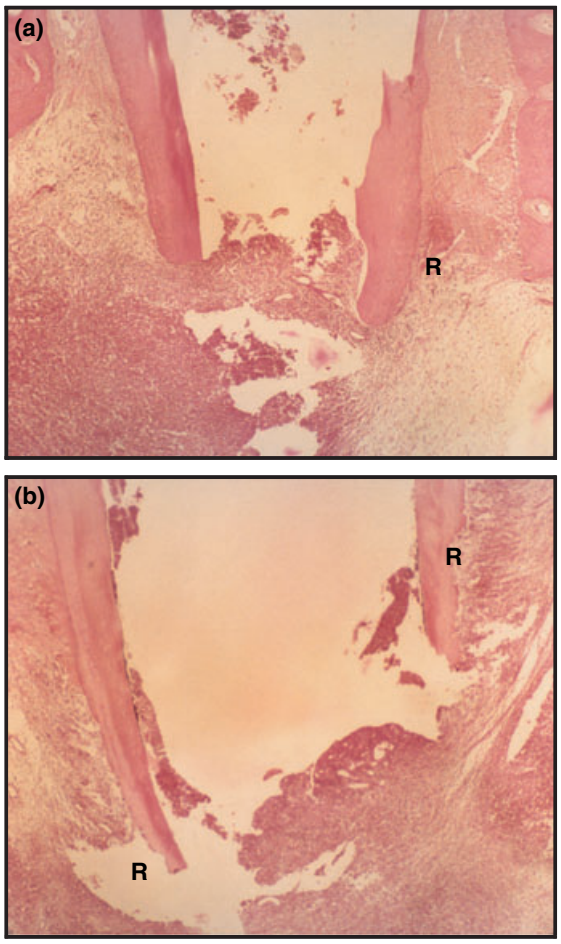

Figure 3 (a) and (b) – Apical area of teeth from control group. Note the absence of apical closure, extensive root resorption (R) and severe chronic inflammatory reaction (haematoxylin-eosin, ×12.8).

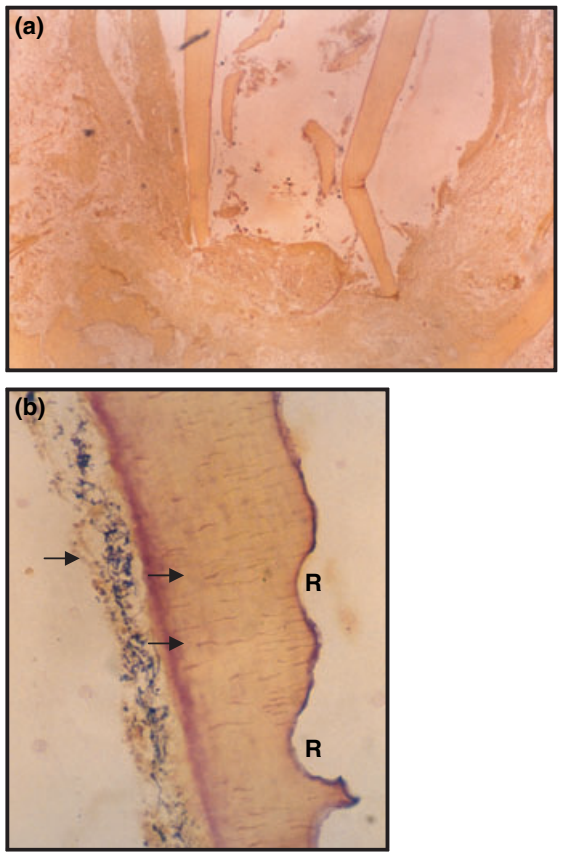

Figure 4 (a) Apical area of a tooth from control group [Brown–Brenn technique (BB), ×12.8]. (b) Higher magnification showing root resorption (R) and microorganisms (arrows) in root canal and dentinal tubules (BB, ×64).

- *Bone resorption:* this was observed in 23.1% of the roots of group 1 and 25% of the roots of group 2. The test of proportions did not show significant differences between the groups ($Z = 0.11$; $P = 0.0912$).
- *Root resorption:* this was observed in 23.1% of the specimens of group 1 and 33.3% of the specimens of group 2. The test of proportions did not show any differences between the groups ($Z = 0.57$; $P = 0.0568$).
- *Extrusion of MTA:* extrusion occurred in 7.7% of the specimens of group 1 and 75% of the specimens of group 2. The test of proportions rejected the hypothesis of similarity between the groups ($Z = 3.43$; $P = 0.0006$).
- *Microorganisms:* none of the specimens of groups 1 and 2, stained by Brown–Brenn technique revealed microorganisms. The histological evaluation of the teeth from control group revealed the absence of apical closure, severe bone and root resorption, severe chronic inflammatory reaction, and connective tissue infiltrated with inflammatory cells (Fig. 3). The Brown–Brenn staining technique revealed the presence of microorganisms in the root canal and inside dentinal tubules (Fig. 4).

Discussion

The absence of an apical calcified tissue barrier, the presence of a severe inflammatory reaction, bone and root resorption and microorganisms, all observed in teeth from the control group, revealed that instrumentation and irrigation with sodium hypochlorite did not disinfect the canals. These findings are similar to other investigators that demonstrated the difficulty of

performing root canal preparation in teeth with incomplete root formation (Leonardo *et al.* 1993, Felippe *et al.* 2005).

Regardless of whether the calcium hydroxide was used or not (group 2 and group 1, respectively), apical repair and the barrier formation occurred in 100% of the sections evaluated. These results are similar to those obtained by Shabahang *et al.* (1999) who observed apical closure with a calcified barrier in 93% of the roots treated with MTA after using calcium hydroxide paste for 1 week.

It was possible to observe deposition of calcified tissue over the MTA for specimens from the experimental groups. This tissue was formed over and was continuous with the previous cementum, similar to that reported by Torabinejad *et al.* (1995a) and Pitt Ford *et al.* (1995).

The cellular response to MTA and mechanism of deposition for barrier formation are unknown and need further investigation. It is believed that the deposition of hard tissue over the material is related to features such as good sealing ability, biocompatibility, and alkaline pH (Torabinejad *et al.* 1995b, 1997); the presence of calcium and phosphate ions in its formulation (Torabinejad *et al.* 1995b); the capacity to attract blastic cells and to promote a favourable environment for cementum formation (Pitt Ford *et al.* 1995, Torabinejad *et al.* 1995a); osseous and cementum-conductive effect (Shabahang *et al.* 1999, Moretton *et al.* 2000); the stimulus to adhesion and cell proliferation (Koh *et al.* 1998), stimulus to expression of alkaline phosphatase by fibroblasts (Bonson *et al.* 2004) and osteocalcin and other interleukins by osteoblasts (Koh *et al.* 1997, Mitchell *et al.* 1999).

The similarity of the results found for both groups can be explained when MTA is compared with calcium hydroxide (Holland *et al.* 2001). Assuming that the MTA when it is hydrated forms, among other chemicals, calcium hydroxide, it is hypothesized that repair and apical barrier formation may occur by the same mechanisms already explained in studies where calcium hydroxide was used (Holland *et al.* 1999a, 2001, Felippe *et al.* 2005).

The presence of necrotic tissue was observed between MTA and the barrier in specimens where the histological technique preserved the aggregate. Soares (1996) and Haglund *et al.* (2003) also demonstrated the presence of denaturated proteins and dead cells adjacent to the MTA. This finding suggests that when MTA contacts connective tissue, it promotes protein denaturation and necrosis by coagulation (Moretton *et al.* 2000, Yaltirik *et al.* 2004), and its behaviour is similar to calcium hydroxide paste (Holland *et al.* 1977).

The barriers identified in this study, presented different thicknesses and shapes. In both groups, the barrier formed presented two distinct, continuous layers, without precise limits between them. The outermost layer, towards the periodontal ligament, was irregular, more compact and with few included cells. The innermost layer, facing the MTA, was irregular and frequently exhibited gaps with cell and tissue inclusions. These features may reflect the conditions and rate of barrier formation and probably are resulting from the irregular arrangement of MTA particles in contact with the apical tissue.

While the statistical analysis did not demonstrate differences between the groups, evaluation of the data related to the features of the barrier indicate that the immediate application of MTA resulted in three times the number of complete barriers. In some specimens from experimental groups the apical tissue barrier was incomplete. It is possible that it would have been complete had the follow-up period been longer.

In the majority of the specimens of group 1, the barrier was formed within the canal. It is possible that flaws in the filling or the presence of granulation tissue inhibited barrier formation at the desired level, as can be observed in some radiographs. In group 2, the majority of barriers were formed beyond the limits of the roots, similar to the cases of extrusion of MTA. It is likely that both the sodium hypochlorite solution and the calcium hydroxide paste caused necrosis and the dissolution of tissue present in the foramen area, leading to extrusion of the MTA that was placed in the canal in the next session. Previous authors have described tissue dissolution following use of these products (Hasselgren *et al.* 1988, Turkun & Cengiz 1997, Wadachi *et al.* 1998). As only one tooth from group 1 showed MTA extrusion, it seems that 1-week calcium hydroxide treatment had an important role to play in tissue dissolution and MTA extrusion.

In the specimens from groups 1 and 2, the inflammation was mainly in the proliferative phase, and it was possible to observe different amounts of mononuclear cells (lymphocytes, plasmocites and macrographs), which permitted subjective classification of the severity of the reaction present. Regardless of the presence and severity of the inflammatory reaction, the apical barrier of hard tissue was formed in all specimens. Other authors also verified apexification in

the presence of apical inflammation with the use of calcium hydroxide (Chosack *et al.* 1997, Felippe *et al.* 2005) or MTA (Tittle *et al.* 1996, Shabahang *et al.* 1999) in teeth with incomplete root formation. The absence of inflammation in the majority of the specimens of the group 1 confirms that the material is well tolerated and corroborates the findings of other authors after using MTA in bone tissue (Torabinejad *et al.* 1998), for root canal filling (Holland *et al.* 1999b), and for the sealing of contaminated root perforations (Pitt Ford *et al.* 1995).

It must be pointed out that root and bone resorption seen in a few specimens was characterized by the presence of very few clastic cells. These observations, along with the presence of reparative cementum over the previously resorbed surfaces, indicate the evolution of the repair process.

While none of the sections of the specimens of the groups 1 and 2, stained by the Brown–Brenn technique, showed microorganisms, their presence cannot be totally disregarded because only two sections of each root were stained by this technique. However, the results suggest that the procedures provided conditions for the achievement of disinfection and its maintenance.

This study demonstrated that the application of MTA immediately after root canal preparation favoured the establishment of a normal periodontal ligament and formation of new bone and cementum. The MTA behaved in a similar manner to the calcium hydroxide paste, even in the presence of exudate and contamination observed at the time of preparation, and promoted the disinfection of the canal and stimulated the formation of an apical barrier of hard tissue.

The histological responses observed in this study indicate that the MTA is a reliable material and should be considered effective in teeth with incomplete root formation. Its application results in predictable apical closure and reduction of the treatment time, number of appointments and radiographs, particularly in young patients. Besides, it seems that treatment of teeth with incomplete root formation using MTA has another advantage compared with calcium hydroxide treatment. Andreasen *et al.* (2002) showed that the fracture strength of immature teeth is markedly decreased following long-term calcium hydroxide treatment. The advantage of a material that promotes the immediate formation of an artificial apical plug and that maintains the capability to induce apexification with time means that the definitive root filling can be placed immediately after the material sets.

Conclusions

When applied as an apical plug, MTA favoured apexification and periapical healing, regardless of the previous use of calcium hydroxide paste. The previous use of the calcium hydroxide paste was associated with MTA extrusion and the formation of barriers beyond the limits of the root canal walls.

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