Histological evaluation of MTA as a root-end filling material

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Abstract

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Aim To assess the histological response associated with grey mineral trioxide aggregate (GMTA) and zinc oxide eugenol (ZOE) as root-end filling materials in teeth where the root canals were not filled and the coronal access cavities were not restored.

Methodology Periapical lesions were developed in 24 premolar teeth in three dogs. The root canals were prepared and half of them were dried, filled and the coronal access restored (closed). The remaining teeth were not root filled and no coronal restoration was placed (open). Apical root-end resections were performed 3 mm from the apex, and root-end cavities were prepared with ultrasonic tips. These were randomly filled with either ZOE or GMTA in the same number of specimens using MAPSYSTEM device. After 180 days the animals were killed and blocks of tissues removed and processed for histological examination. Periradicu-

lar tissue reaction was evaluated, including severity of inflammation and cementum formation. Statistical analysis was performed using ANOVA analysis and Tukey's test.

Results A significant difference was found between the levels of inflammation in the periradicular tissues of the GMTA/closed group, compared with the ZOE/open and ZOE/closed groups (P < 0.05) but not between GMTA/closed and GMTA/open groups. Cementum formation was not found over any ZOE specimens but over MTA in all specimens. No microorganisms were found in the interface between the material and the dentinal walls.

Conclusions GMTA was associated with less periapical inflammation and tissue response when used as a root-end filling material, even when no root filling or coronal restoration was present.

Keywords: apicectomy, MTA, sealability, tissue response.

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Introduction

The main goal of a root-end filling material is to provide an apical seal to prevent the movement of bacteria and the diffusion of bacterial products from the root canal system into the periapical tissues.

The quality of the apical seal obtained by root-end filling materials has been assessed in many ways including the use of dye penetration (Starkey *et al.* 1993, Torabinejad *et al.* 1994, Holt & Dumsha 2000), bacterial penetration (Fischer *et al.* 1998, Siqueira *et al.* 2001, Mangin *et al.* 2003), electromechanical technique (Martell & Chandler 2002), fluid filtration (Wu *et al.* 1998, Fogel & Peikoff 2001) and *in vivo* studies (Torabinejad *et al.* 1995a, 1997, Economiades *et al.* 2003). Scanning electron microscopy (SEM) has also been used to assess the adaptation and the sealing capacity of commonly used root-end filling materials (Abdal & Retief 1982, Stabholz *et al.* 1985, Gondim *et al.* 2003).

Gartner & Dorn (1992) proposed that an ideal root-end filling material should be easy to mani-

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pulate, as well as be radiopaque, dimensionally stable, nonabsorbable, insensitive to moisture, adhesive to dentine, nontoxic and biocompatible. Many materials have been used for root-end fillings in endodontic surgery; however, there is no universally accepted material.

Mineral trioxide aggregate (MTA) was developed with the specific goal of sealing communications between the tooth and external surfaces (Lee et al. 1993). Studies evaluating MTA as a root-end filling material have shown less periapical inflammation, presence of a fibrous capsule and formation of new cementum in contact with the material surface in many cases (Torabinejad et al. 1995a, Bernabé et al. 2005). Similar findings were reported by Holland et al. (2001), in a study in dogs. When MTA was used to repair furcation perforations in dog premolars teeth, deposition of new cementum in contact with the material was also observed (Pitt Ford et al. 1995). A dentine bridge was additionally found covering the pulp tissue that remained after pulp capping with MTA (Faraco & Holland 2001, Holland et al. 2001).

Previous *in vivo* studies utilized MTA only in noninfected or disinfected root canals (Torabinejad *et al.* 1995a, 1997, Economiades *et al.* 2003). These conditions aided the ability of the material to prevent periapical inflammation by eliminating the microorganisms, which create the inflammatory response. It is important to evaluate the behaviour of the materials in more challenging situations, i.e. when the canals are not disinfected and the coronal access cavities are not restored. Thus, the ability of the material to prevent periapical inflammation should be evaluated when contamination from the mouth and canal system may impair the healing process if not prevented by a rootend filling.

The purpose of this study was to evaluate the association of grey mineral trioxide aggregate (GMTA) and zinc oxide eugenol (ZOE) as root-end filling materials with periapical inflammation in dog's teeth, in which the root canals were not filled and the coronal access cavities were not restored.

Materials and methods

Twenty-four maxillary and mandibular premolar teeth from three male adult mongrel dogs (eight teeth/ animal) aged 2–3 years old were used. Procedures in this experiment were conducted according to the guidelines approved by the Research Committee of Paulista State University, Brazil. The animals were anaesthetized with 2 mL of a mixture of xylazine (Rompum; Bayer do Brasil S/A, São Paulo, SP, Brazil) and ketamine hydrochloride (Ketalar; Park Davis-Aché Laboratórios Farmacêuticos S/A, São Paulo, SP, Brazil), in a 1 : 1 ratio, administered intramuscularly and maintained with subsequent anaesthetic injections. The animals were intubated with a cuffed endotracheal tube before beginning the experimental procedures. All procedures were in accordance and approved by the Ethical Committee of Animal Experimentation of the Araçatuba School of Dentistry and by the Brazilian Committee of Animal Experimentation.

At the first intervention, coronal access and pulpal removal were performed without using rubber dam isolation. The root canals were left open to the oral environment for 180 days, to induce the formation of apical pathosis, which was confirmed radiographically (Holland *et al.* 1979).

Afterwards, the animals were anaesthetized, the root canals cleaned and instrumented to the apical delta with a step-back technique to a size 30 master apical file. The canals were irrigated with 1% sodium hypochlorite before the use of each instrument. After instrumentation, half of the canals were dried with paper points and filled with laterally compacted Guttapercha and Sealapex root canal sealer (Syborn Endo, Glendora, CA, USA), and the coronal cavities sealed with amalgam (closed). The remaining specimens were left with no filling of the root canal and no sealing of the coronal cavities (open).

Surgery began with the use of topical antiseptic solutions (iodine solution and 0.12% chlorhexidine gluconate). Flap design consisted of two releasing incisions connected by a sulcular incision. After tissue elevation, ostectomy was performed with chisel and complemented with a Zekrya bur (Dentsply Maillefer, Ballaigues, Switzerland). After removal of the apical lesion with size 85 Lucas surgical curettes (Hu-Friedy, Chicago, IL, USA) and size 35 and 36 curettes (Dentsply Maillefer), 3 mm of apical root was sectioned with a Zekrva bur (Dentsply Maillefer) at high speed, in such a way that the cut surface was perpendicular to the long axis of the root. Root-end cavities of 3 mm depth were prepared with S15 RD, S15 LD and S12 D/90 ultrasonic tips (Gnatus-Satelec, Ribeirão Preto, SP, Brazil) using an ultrasonic device (Gnatus-Satelec). Saline was used for irrigation during the entire surgical procedure.

The root-end cavities were irrigated with saline, aspirated and dried with absorbent paper points. Cavities were filled with either ProRoot grey MTA (Dentsply Caulk, Milford, DE, USA), mixed according to the manufacturers' instructions or ZOE (SS White Artigos Dentários Ltda., Rio de Janeiro, RJ, Brazil) mixed to a firmer consistency (1.0 g of zinc oxide and 0.2 g of eugenol) than that when used a sealer. Twenty-four roots were used for each material, only half were root filled and restored. The materials were inserted into the cavities with the Map-System device (Produits Dentaires SA, Vevey, Switzerland). The bone cavities were allowed to fill with blood and the flaps were sutured with absorbable gut sutures, and the animals maintained on a regular diet.

After 180 days, the animals were killed by anaesthetic overdose. Maxillas and mandibles were removed, fixed in 10% buffered formalin solution, and demineralized in formic acid and sodium citrate solution. Blocks, including one root and surrounding alveolar bone each, were produced and the specimens embedded in paraffin wax, serially sectioned at 6 μ m intervals, and stained with haematoxylin–eosin (H&E) and Brown and Bren stains.

Severity, extent of inflammation and predominant inflammatory cell type in the periradicular tissues adjacent to the root-end filling materials were recorded. Severity of the inflammation was recorded as: none, no inflammatory cells; mild, few inflammatory cells; moderate, inflammatory cells did not obscure the normal tissues and severe, inflammatory cells replaced the normal tissues (Torabinejad *et al.* 1995a). Extent of inflammation from the surfaces of root-end filling materials was recorded as $\leq 0.1, \leq 0.2$, ≤ 0.5 or ≥ 1 mm. The presence or absence of cementum deposition on resected surface, root-end filling materials and bone formation were also recorded. In

addition, the presence or absence of bacteria in the interface between the material and dentine was recorded. Histological examination of specimens was performed by two investigators in a double-blind manner. Statistical analysis was carried out using one-way ANOVA analysis. To determine differences between groups, Tukey's multiple comparison test was performed.

Results

One specimen for each group was excluded from the study because of technical problems that occurred during histological preparation. Consequently, a total of 11 specimens in each group were available for histological examination.

The histological findings in the experimental groups are summarized in Table 1. The periradicular tissues of the roots with ZOE (open or closed) had more severe inflammation than those with MTA in the same situations (Figs 1, 4, 7 and 10). A significant difference was found between the levels of inflammation present in the periradicular tissues of MTA/closed group, compared with ZOE/open and ZOE/closed (P < 0.05), but not with MTA/open. When the widths of inflammation in the periradicular tissues were compared, significant differences were only found between MTA/closed and ZOE/open (P < 0.05). All ZOE and MTA specimens had mononuclear cells, such as macrophages, mast cells, lymphocytes in the periradicular tissues, but there were no statistically significant differences amongst the groups.

Cementum formation was not found over ZOE specimens in any situation (open or closed). However,

Group	ZOE/opened	MTA/opened	ZOE/closed	MTA/closed
No. roots	11	11	11	11
Severity	3 S	0 S	4 S	0 S
	1 MO	0 MO	0 MO	0 MO
	1 F	5 F	3 F	1 F
	5 N	6 N	4 N	10 N
Extent of inflammation	3 × 0.1	7 × 0.1	5 × 0.1	10 × 0.1
	4 imes 0.2	4 imes 0.2	2 imes 0.2	1 × 0.2
	0 imes 0.5	0 imes 0.5	2 imes 0.5	0 imes 0.5
	4 × 1.0	0 × 1.0	2 × 1.0	0 × 1.0
Predominant cells	Mononuclear cells	Mononuclear cells	Mononuclear cells	Mononuclear cells
Cementum/over material (yes/no)	0/11	11/0	0/11	11/0
Cementum/over root-end (yes/no)	11/0	11/0	11/0	11/0
New bone formation (yes/no)	11/0	11/0	11/0	11/0
Presence of bacteria (yes/no)	0/11	0/11	0/11	0/11

 Table 1 Scores of histomorphological events

S, severe; MO, moderate; F, few; N, none.



Figure 1 Photomicrograph showing severe periapical tissue response to root-end cavity filled with ZOE (opened) showing mainly chronic cells, such as macrophages and lymphocytes (H&E, original magnification $\times 100$).



Figure 3 Photomicrograph showing mild inflammatory response to root-end cavity filled with ZOE (closed) (H&E, original magnification $\times 100$).



Figure 2 Photomicrograph showing no inflammation in the periapical tissue and cementum in close contact with rootend cavity filled with MTA (opened) (H&E, original magnification $\times 100$).

cementum was present over MTA in all specimens in both situations (Figs 4 and 10). Cementum formation over the resected surface was not affected by the materials tested (Figs 1, 4, 7 and 10). Bone formation occurred after surgery in all experimental groups. Negative staining was observed in all groups stained for the presence of bacteria. Bacteria were not noted in the interface between the material and the dentinal walls (Figs 2, 5, 8 and 11), but they were observed in



Figure 4 Photomicrograph showing no inflammation in the periapical tissue and cementum in close contact with rootend cavity filled with MTA (closed) (H&E, original magnification $\times 100$).

all groups without filling and sealing in the middle third of the canals (Figs 3, 6, 9 and 12).

Discussion

The experimental model of lesion induction used in this study was based on previously established criteria (Holland *et al.* 1979); the pulp canal systems were exposed to the oral environment for 180 days to induce lesions.



Figure 5 Photomicrograph showing no bacteria in the interface ZOE/dentinal wall (opened) (Brown and Bren, original magnification $\times 400$).



Figure 7 Photomicrograph showing no bacteria in the interface ZOE/dentinal wall (closed) (Brown and Bren, original magnification $\times 400$).



Figure 6 Photomicrograph showing no bacteria in the interface MTA/dentinal wall (opened) (Brown and Bren, original magnification $\times 400$).

This study was performed to examine the association between periapical inflammation and root-end filling materials in an *in vivo* experimental model. Many studies have been used to evaluate the properties of such materials in different ways. In the present study, the materials were tested under challenging conditions.

The present data indicate that the materials had similar behaviour, but the use of GMTA resulted in a more effective healing process. Indeed, GMTA, in both situations (with or without root filling and coronal access restoration), prevented periapical inflammation and was able to evoke the wound healing observed. The



Figure 8 Photomicrograph showing no bacteria in the interface MTA/dentinal wall (closed) (Brown and Bren, original magnification ×400).

3 mm thick layer of MTA was effective in preventing periapical inflammation, in accordance to a previous study (Valois & Costa 2004).

Zinc oxide eugenol also allowed a satisfactory healing process, and it blocked the bacteria from the coronal access when observed with the Brown and Bren staining technique, even though the inflammatory reaction was more intense than that observed with MTA, probably due to its composition (Economiades *et al.* 2003).

The reaction of the tissue to ZOE might have been due to the high concentration of eugenol in the tissue adjacent to the material in that was sufficient to inhibit

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Figure 9 Photomicrograph showing presence of bacteria in the dentinal tubules of the middle third (ZOE/opened) (Brown and Bren, original magnification $\times 400$).



Figure 11 Photomicrograph showing no bacteria in the dentinal tubules of the middle third (ZOE/closed) (Brown and Bren, original magnification ×400).



Figure 10 Photomicrograph showing presence of bacteria in the dentinal tubules of the middle third (MTA/opened) (Brown and Bren, original magnification ×400).

respiration and thus kill cells (Hume 1984). Eugenol could also have initiated, or worsened, the acute immunological reaction (Grossman & Lally 1982). When eugenol is mixed with zinc oxide powder, zinc eugenolate is formed. The release of eugenol from the mixture might have occurred due to hydrolysis (Wilson & Batchelor 1970), which could have maintained tissue inflammation over time. The increased powder/ liquid ratio used in the present study would have decreased the amount of eugenol release (Camps *et al.* 2004) and produced a less irritant response within the tissues (Valle *et al.* 1980).



Figure 12 Photomicrograph showing no bacteria in the dentinal tubules of the middle third (MTA/closed) (Brown and Bren, original magnification $\times 400$).

Thus, ZOE used in a harder consistency than that used as a sealer improved its biological properties as observed in the present results. The improvement of ZOE with the increasing of powder/liquid ratio was not enough to evoke the same tissue response as observed with GMTA. However, no bacteria were found in the interface between the material and the dentinal walls with any material tested.

MTA has been shown to be a more favourable material in studies in which it was compared to amalgam used as root-end filling material in infected

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dog's teeth (Torabinejad *et al.* 1995a). These data are consistent with previous findings, when less inflammatory response with the use of MTA was observed than with intermediate restorative material (IRM) in terms of a short-term exposure (Economiades *et al.* 2003). Similar results were also observed when MTA was shown to be superior to Super EBA, ZOE and IRM in root-end cavities created with burs.

The ability of MTA to reduce periapical inflammation is probably due to its hydrophilic nature and expansion when placed in humid environments (Torabinejad *et al.* 1995b), which potentiality prevents microbial and endotoxin leakage (Adamo *et al.* 1999, Tang *et al.* 2002), even when it is used in root-end cavities contaminated by blood (Torabinejad *et al.* 1994). In the present study, it was seen that, even when used in root canals with no filling material and unrestored coronal access cavities, MTA maintained its ability to reduce periapical inflammation and helped the healing process.

MTA was the only material tested that induced formation of hard tissue, even when used in root-end cavities when the canal system was not filled and the coronal access was not restored. These data are in accordance with previous reports (Torabinejad et al. 1995a). Deposition of a continuous layer of new cementum upon MTA surfaces has been described previously (Pitt Ford et al. 1995). Dentinal bridge formation after direct pulp capping (Faraco & Holland 2001), pulpotomy (Holland et al. 2001) and treatment of perforations (Pitt Ford et al. 1995, Holland et al. 1999a) also support the results of the present study. It was reported that cementum deposition against MTA may be due to several factors, such as sealing ability, biocompatibility or alkaline pH on setting (Torabinejad et al. 1997).

Calcium oxide in MTA reacts with water or tissue fluids, forming calcium hydroxide that may stimulate hard tissue deposition (Holland *et al.* 1999b). This assumption is supported by the presence of granulations birefringent to polarized light, found when MTA is used, which are similar to the calcite crystals observed with the use of calcium hydroxide (Holland *et al.* 1999b).

Conclusions

Based on the results of the present research, MTA had the ability to reduce periapical inflammation and adverse tissue response when used as a root-end filling material, even without root filling and coronal restoration and allowed healing of periradicular tissues with newly formed hard tissue.

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