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Histological and histomorphometrical evaluation of furcation perforations filled with MTA, CPM and ZOE

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Abstract

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Aim To evaluate the biological response of the periodontium adjacent to furcation perforations in rat molars filled with Endo-CPM-Sealer (CPM), MTA-Angelus (MTA) or zinc oxide–eugenol cement (ZOE).

Methodology The pulp chamber floors of maxillary right first molar teeth were perforated and sealed with CPM, mineral trioxide aggregate (MTA) or ZOE; the left first molars, without any treatment, were used as controls (CG). After 7, 15, 30 and 60 days, fragments of maxilla were fixed, decalcified and embedded in paraffin. Sections were stained with H&E, Masson's trichrome and submitted to tartrate-resistant acid phosphatase (TRAP) reaction, used as an osteoclast marker. The width of the periodontal space, the numerical density of inflammatory cells and the number of TRAP-positive osteoclasts in the bone surface were measured, and statistical analyses were performed using analysis of variance and Tukey test ($P \leq 0.05$).

Results In all experimental groups, the greatest number of inflammatory cells was observed at 7 days, especially in the ZOE group. In this group, the intense inflammatory process was related to a significant increase ($P \leq 0.05$) in the number of osteoclasts and, thereby, in an increase in the width of the periodontal space. At 60 days, no significant differences in osteoclast numbers amongst CPM, MTA and CG groups occurred; the periodontal space was also significantly reduced in the experimental groups in comparison with the initial periods. However, in the ZOE group, the periodontal space was significantly larger ($P \leq 0.05$) in comparison with MTA-based materials.

Conclusions The periodontium adjacent to perforations filled with MTA and CPM exhibited clear evidence of re-establishment and thus better biocompatibility than ZOE.

Keywords: biocompatibility, furcal perforations, mineral trioxide aggregate.

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Introduction

Perforation is a mechanical or pathological communication between the root canal system and the outer tooth surface caused by caries, resorption or iatrogenic factors (Sinai 1977). The prognosis of the tooth depends on the location and size of the perforation, the presence or not of bacterial contamination and of the immediate sealing of these perforations (Seltzer *et al.* 1970, Sinai 1977, Hamamoto *et al.* 1989).

Studies have reported that the outcome of treating perforations depends in part on the sealing material, which should have good physicochemical properties and be biocompatible (Sinai *et al.* 1989, Pitt-Ford *et al.*

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1995, Yildirim *et al.* 2005). The use of mineral trioxide aggregate (MTA) has been suggested in these cases because of its good sealing ability, marginal adaptation (Lee *et al.* 1993, Torabinejad *et al.* 1995) and biocompatibility (Pitt-Ford *et al.* 1995, Yildirim *et al.* 2005). In addition, it has been reported that MTA can be used in the presence of moisture without affecting its physicochemical and biological properties (Torabinejad *et al.* 1994). However, there are disadvantages when using MTA, such as its extended setting time and difficult handling (Camilleri *et al.* 2005). It has been shown that the addition of substances to MTA can improve its physicochemical properties (Bortoluzzi *et al.* 2006a,b, Camilleri 2008). Considering that MTA is composed basically of Portland Cement (Estrela *et al.* 2000), additives used in civil engineering may be considered in an attempt to improve its clinical deficiencies. Studies have demonstrated that the addition of calcium chloride ($CaCl_2$) to MTA reduces its setting time (Bortoluzzi *et al.* 2006b), increases the release of calcium ions (Bortoluzzi *et al.* 2006b), improves its sealing ability (Bortoluzzi *et al.* 2006a) and facilitates its insertion in cavities (Bortoluzzi *et al.* 2006b), without interfering with its biocompatibility (Abdullah *et al.* 2002, Bortoluzzi *et al.* 2008).

The MTA-based sealer, Endo-CPM-Sealer (CPM Sealer; EGEO S.R.L., Buenos Aires, Argentina), was created in an attempt to combine the physicochemical properties of a root canal sealer with the biological properties of MTA. According to the manufacturer, CPM-Sealer exhibits similar chemical composition and, therefore, has same clinical indications as MTA. This cement, besides $CaCl_2$, contains calcium carbonate as a component that reduces its pH, restricting the surface necrosis in contact with the material, which allows the action of the alkaline phosphatase and, consequently, the deposition of mineralized tissue (Gomes-Filho *et al.* 2009a). It was demonstrated that an Endo-CPM-Sealer has good antimicrobial activity (Tanomaru *et al.* 2008) and a satisfactory radiopacity (Guerreiro-Tanomaru *et al.* 2009). It has also been suggested that this cement releases hydroxyl and calcium ions similar to MTA and, therefore, may be an alternative root-end filling material (Tanomaru-Filho *et al.* 2009). Moreover, culture with fibroblasts revealed that Endo-CPM-Sealer is not cytotoxic (Gomes-Filho *et al.* 2009b).

Although the CPM-Sealer, according the manufacturer, can be used to seal root perforations, there is no study that evaluated the reaction of the periodontium in these conditions. Thus, in this study, the biological response of the periodontium adjacent to furcation perforations of rat molars filled with three different sealing materials: Endo-CPM-Sealer, MTA-Angelus and zinc oxide–eugenol cement was evaluated.

Materials and methods

Experimental model

Principles of laboratory animal care and national laws on animal use were observed in this study, which was authorized by the Ethical Committee for Animal Research of the São Paulo State University, Brazil (Araraquara Dental School-UNESP).

Sixty male Holtzman rats (*Rattus norvegicus albinus*) weighing 200 g obtained from São Paulo State University animal house were used. The rats were divided into groups: MTA (white MTA; Angelus, Londrina, Brazil), CPM (Endo-CPM-Sealer, Lab. EGEO S.R.L, Buenos Aires, Argentina) and ZOE (zinc oxide eugenol, S.S.White, Rio de Janeiro, Brazil) according to the sealing material. Twenty rats were used in each group. The healthy left maxillary first molars, without perforations in the pulp chamber floors, was used as controls (CG).

The animals were anaesthetized with an intraperitoneal injection of ketamine (80 mg kg^{-1} of body weight) combined with xylazine (4 mg kg^{-1} of body weight), and the pulp chamber of the maxillary right first molar was opened with the help of a stereoscopic microscope at ×60 (Wild M7; Wild Heerbrugg, Switzerland) for better visualization of the operative field. A Class I cavity was prepared on the occlusal surface of maxillar molar using a number ¼ sterile round bur (KG Sorensen, Barueri, São Paulo, Brazil) at low speed under saline irrigation. After access, the pulp chamber was washed with physiological saline solution and dried with sterile cotton pellets. Using a sterile size ¼ round bur, the pulp chamber floor in the furcation region was perforated; the round bur was inserted to a depth of 2 mm, measured from the occlusal surface using a rubber stop as reference. This distance required to create the perforation on the floor of the pulp chamber was standardized from a previous evaluation of the dental and periodontal anatomy of the rats. Therefore, the diameter of the perforation achieved, using this technique, was approximately 0.25 mm. Haemorrhage was arrested using physiological saline irrigation and sterile cotton pellets.

The cavities were dried with sterile paper points, and the perforations were filled with white MTA®, Endo-CPM.-Sealer® or Zinc oxide and Eugenol. MTA-based materials were prepared according to the

manufacturer's instructions; zinc oxide–eugenol cement was used in the proportion of 1.0 g of zinc oxide for 0.2 mL of eugenol (Bernabé *et al.* 2005). The materials were placed into the perforations with a small excavator and gently compacted with size 15 paper points. The access cavity was cleaned and restored with light-cure glass–ionomer cement (Vitremer®, 3M; Sumaré, São Paulo, Brazil). All procedures were carried out under a stereoscopic microscope at ×160 (Wild M7; Wild Heerbrugg).

On the 7th, 15th, 30th and 60th days after filling of the perforations, the rats were sacrificed with chloral hydrate (600 mg kg^{-1} of body weight), and fragments of the maxilla (right and left) containing the first molar teeth with surrounding periodontal tissues were removed and immediately immersed in fixative solution.

Histological preparation and examination

The fragments of maxilla were fixed in 4% formaldehyde (prepared from paraformaldehyde) buffered at pH 7.2 with 0.1 mol L^{-1} sodium phosphate, for 48 h. After decalcification for 45 days in 7% EDTA solution (disodium ethylene-diaminetetracetic acid) containing 0.5% formaldehyde, in sodium phosphate buffer 0.1 mol L^{-1}, at pH 7.2, the specimens were dehydrated and embedded in paraffin. Sagittal sections, 6 μm thick, were stained with haematoxylin and eosin (H&E) and Masson's trichrome; two sections per animal were submitted to the tartrate-resistant acid phosphatase (TRAP) histochemical method.

TRAP procedure

The TRAP method was used as an osteoclast marker (Minkin 1982, Cerri *et al.* 2003, Faloni *et al.* 2007). Deparaffinized sections were immersed in an incubation solution prepared by dissolving 20 mg of naphthol AS-BI (Sigma Chemical Company, St Louis, MO, USA) in 500 μL of N-N-dimethylformamide (Sigma Chemical Company) followed by the addition of 50 mL of 0.2 mol L^{-1} sodium acetate buffer (pH 5.0) containing 70 mg of Fast Red Salt (Sigma Chemical Company) as the coupling agent; 50 mmol L^{-1} sodium tartrate dehydrate was added to the solution. After incubation at 37 °C, the sections were washed in distilled water and counterstained with haematoxylin.

Morphometrical analysis

The morphometrical analyses described below were undertaken using a light microscope (BX51; Olympus, Tokyo, Japan) and an image analysis system (Image Pro-Express 6.0, Olympus).

Width of the periodontal space

The width of the periodontal space in the furcation region was obtained from three measurements made between the alveolar bone and the root surfaces of each section (Fujiyama *et al.* 2004). In each animal, three H&E-stained sagittal sections of the first molar with surrounding periodontal tissues were used, totalling 15 sections per group. The shortest distance between the sections was approximately 100 μm. Thus, the mean value of width of the periodontal space/animal and per group was obtained.

Numerical density of inflammatory cells

Three H&E-stained sections per animal were selected at intervals of least 100 μm; in each section, a standardized field of 0.8 mm^2 of the periodontal ligament in the furcation region was analysed, totalling 2.4 mm^2 per animal. In each area, the total number of inflammatory cells was scored, and the number of inflammatory cells per mm^2 was calculated.

Number of TRAP-positive osteoclasts per mm of bone surface

Sections of maxilla containing first molars with surrounding periodontal tissues were used at intervals of least 100 μm. The surface of the alveolar bone of the furcation region was measured by using the image analysis system at ×4. Subsequently, the multinucleated TRAP-positive osteoclasts on the alveolar bone surface measured were counted using the light microscope, at ×400; in each animal, the values were divided by the total length of the bone surface.

Statistical analysis

The differences between the groups were statistically analysed by the SigmaStat 2.0 software (Jandel Scientific, Sausalito, CA, USA); the data were submitted to ANOVA and Tukey test, and the significance level accepted was $P \leq 0.05$.

Results

Morphological and morphometrical findings

Control group

In all experimental periods, the width of the periodontal space in the furcation region was approximately

0.05 mm (Table 1). The periodontal ligament contained several fibroblasts and collagen fibres; usually, the bone surface of the alveolar process was covered by numerous osteoblasts (Fig. 1a,b). In the furcation region, approximately 1.45 TRAP-positive osteoclasts were present on the surface of the alveolar process (Fig. 1c and Table 3).

MTA group

At 7 days, the width of the periodontal space was 0.22 mm (Table 1) and the numerical density of inflammatory cells was approximately 129.41 (Table 2 and Fig. 2a); approximately 5.61 TRAP-positive osteoclasts per mm of bone surface were found (Fig. 2b and Table 3). After 15 days, a significant reduction in the number of inflammatory cells was seen in comparison with 7 days (Table 2); however, significant differences were not observed in the width of the periodontal ligament and in the number of osteoclasts (Tables 1 and 3). Moreover, in two specimens, a layer of elongated cells, resembling epithelial cells, was observed in contact with the MTA that filled the perforations (Fig. 2c). At 30 and 60 days, a significant reduction in the inflammatory process was seen (Fig. 2d–f and Table 2); one specimen, in the period of 30 days, contained an acidophilic and homogeneous material, similar to the bone tissue, which filled partially the dentine perforation; osteoblast-like cells were apposed to the surface of this neoformed tissue (Fig. 2d,e). In addition, in the period of 60 days, a significant reduction in the number of osteoclasts (Table 3) was accompanied by a significant reduction in the width of the periodontal space (Table 2 and Fig. 2f).

CPM group

In the furcation region, a significant increase in the width of the periodontal space (0.18 mm) in comparison with the control group (0.05 mm) was seen at 7 days (Table 1). The numerical density of inflammatory cells was 114.83 (Table 2), and 3.5 osteoclasts per mm of bone surface were found (Table 3 and Fig. 3a). At 15 days, although a reduction in the inflammatory process was observed, the width of the periodontal space remained similar to 7 days (Tables 1 and 2). In these initial periods, portions of CPM were often found in the periodontal ligament; this material was surrounded by inflammatory cells and multinucleated giant cells (Fig. 3a,b). At 30 days, a significant reduction in the numerical density of inflammatory cells (around 26%) was seen in the periodontal ligament in comparison with the initial period (Table 2); the periodontal ligament exhibited bundles of collagen fibres, and several osteoblasts were present on the alveolar bone surface (Fig. 3c). At 60 days, the number of inflammatory cells had reduced significantly (about 27%) when compared to 30 days (Table 2). Moreover, the significant reduction in the osteoclasts number (Table 3) was accompanied by the presence of a continuous layer of osteoblasts on the bone surface. However, in the furcation region of the periodontal ligament of some specimens, a profuse epithelial proliferation was observed in close contact with the root surface (Fig. 3d).

ZOE group

At 7 days, 308 inflammatory cells per mm^2 were seen in the wide periodontal space (average 0.35 mm) of the furcation region (Fig. 4a and Tables 1 and 2). On the alveolar process, 12.65 osteoclasts per mm of bone surface were found (Table 3). At 15 days, although significant reduction was observed in these morphometrical parameters (Tables 1, 2 and 3), an intense inflammatory process was present in the large periodontal space (Fig. 4b). Significant reduction in the inflammatory process was seen at 30 and 60 days (Fig. 4c and Table 2). The number of osteoclasts per mm of bone surface was 8.45 and 4.96 at 30 and 60 days, respectively (Table 3).

Table 1 Width of the periodontal space (in mm) of the furcation region in the control, MTA, CPM and ZOE groups at the different periods

Days	Control	MTA	CPM	ZOE
07	$0.05 \pm 0.005^{a,1}$	$0.22 \pm 0.011^{b,1}$	$0.18 \pm 0.007^{c,1}$	$0.35 \pm 0.006^{d,1}$
15	$0.06 \pm 0.003^{a,1}$	$0.24 \pm 0.010^{b,1}$	$0.18 \pm 0.002^{c,1}$	$0.28 \pm 0.002^{d,2}$
30	$0.05 \pm 0.007^{a,1}$	$0.24 \pm 0.020^{b,1}$	$0.19 \pm 0.012^{c,2}$	$0.29 \pm 0.044^{d,2}$
60	$0.05 \pm 0.004^{a,1}$	$0.16 \pm 0.002^{b,2}$	$0.16 \pm 0.003^{b,1}$	$0.21 \pm 0.013^{c,3}$

The comparison amongst groups ($P \leq 0.05$) is indicated by different superscripts (a, b, c and d) in the various lines.
The comparison amongst periods ($P \leq 0.05$) is indicated by different superscripts (1, 2 and 3) in the various columns.
CPM, Endo CPM-Sealer; MTA, mineral trioxide aggregate; ZOE, zinc oxide eugenol.

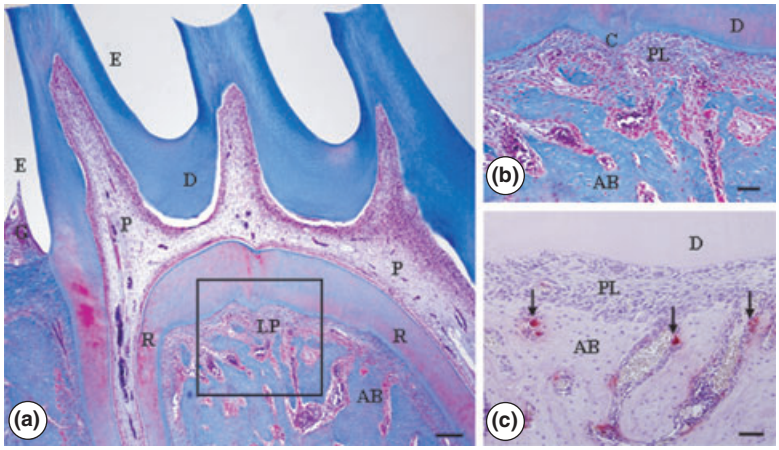

Figure 1 Light micrographs show portions of molar teeth of rats from the control group. (a) A sagittal section of a molar containing periodontal tissues, the periodontal ligament (PL) is between the root surface (R) and alveolar bone (AB). D, dentine; P, dental pulp; E, enamel space; G, gingiva. Masson's trichrome (bar, 200 μm). In (b), outlined area of a, the periodontal ligament (PL) is situated in a narrow space between the surfaces of the cementum (C) and alveolar bone (AB). D, dentine. Masson's trichrome (bar, 100 μm). In (c), tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts (arrows), stained in red, are next to the alveolar bone surface (AB). D, dentine; PL, periodontal ligament. TRAP reaction counterstained with haematoxylin (bar, 100 μm).

Table 2 Number of inflammatory cells per mm^2 of periodontal ligament in the furcation region from the MTA, CPM and ZOE groups at the different periods

Days	MTA	CPM	ZOE
07	129.41 ± 0.86^{a,1}	114.83 ± 0.19^{b,1}	308.89 ± 28.80^{c,1}
15	113.49 ± 3.06^{a,2}	105.32 ± 0.99^{b,2}	265.08 ± 1.87^{c,2}
30	83.49 ± 5.10^{a,3}	84.83 ± 0.28^{a,3}	235.66 ± 3.80^{b,3}
60	75.24 ± 2.29^{a,4}	61.58 ± 1.59^{b,4}	114.99 ± 1.58^{c,4}

The comparison amongst groups ($P \leq 0.05$) is indicated by different superscripts (a, b and c) in the various lines.
The comparison amongst periods ($P \leq 0.05$) is indicated by different superscripts (1, 2, 3 and 4) in the various columns.
CPM, Endo CPM-Sealer; MTA, mineral trioxide aggregate; ZOE, zinc oxide eugenol.

Statistical analyses amongst groups

Width of the periodontal space

According to Table 1, a significant increase in the periodontal space was seen in the different experimental groups in comparison with the control group. In all analysed periods, the width of the periodontal space in the ZOE group was significantly larger in comparison with the groups with MTA and CPM. Although a significant reduction in the periodontal space of the ZOE group was seen at 60 days, the mean value of the periodontal space was similar to the MTA group at 7 days. In the initial periods (7, 15 and 30 days), the width of the periodontal space was significantly higher in the MTA group in comparison with CPM group. However, differences between these groups were not statistically significant in the period of 60 days.

Numerical density of inflammatory cells

At 7 days, the number of inflammatory cells per mm^2 in the periodontal ligament was significantly higher in the ZOE group in comparison with MTA and CPM groups. In all groups, a high cellular density was observed in the initial period (at 7 days) and decreased gradually in the subsequent periods. In addition, the number of inflammatory cells, in all periods analysed, was significantly higher in the ZOE group than in the other experimental groups. Otherwise, a significant reduction in the numerical density of inflammatory cells was observed in the CPM group, in comparison with MTA, except at 30 days; in this period, no statistically significant difference was observed between the CPM and MTA groups (Table 2).

Number of TRAP-positive osteoclasts/bone surface

As shown in Table 3, the number of TRAP-positive osteoclasts per mm of bone surface, in all experimental groups, was significantly higher than the control group at 7, 15 and 30 days. The number of osteoclasts, in the MTA and CPM groups, decreased significantly in comparison with ZOE group in all periods analysed. At 7 days, the number of osteoclasts decreased significantly in the CPM group in relation to MTA group. However, the differences amongst the CPM, MTA and

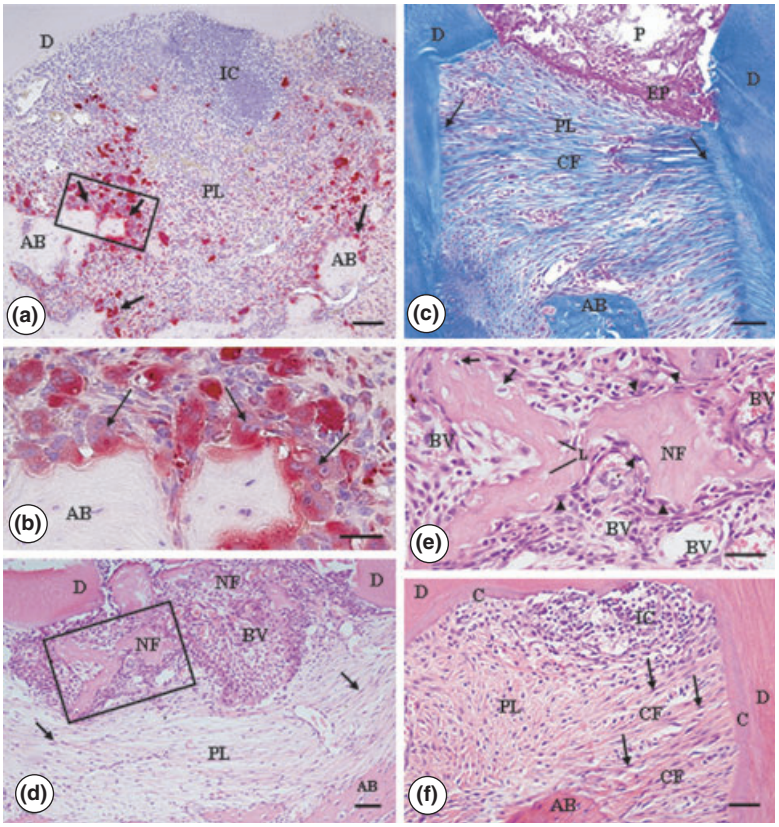

Figure 2 Light micrographs of portions of furcation regions of molar teeth perforated and filled with MTA and examined after 7- (a and b), 15- (c), 30- (d and e) and 60-days (f). In a, numerous inflammatory cells (IC) are present in the wide periodontal space. Tartrate-resistant acid phosphatase (TRAP)-positive osteoclasts (arrows) are next to the alveolar bone (AB) surface. TRAP reaction counterstained with haematoxylin. D, dentine; PL, periodontal ligament (bar, 100 μm). In (b), outlined area of (a), TRAP-positive osteoclasts (arrows) are adjacent to the bone surface. AB, alveolar process (bar, 50 μm). (c) Shows a layer of 2–3 elongated cells (EP) between the perforation in the floor of the pulp chamber (P) and the periodontal ligament (PL). In the PL, some bundles of collagen fibres (CF) penetrate into the root surfaces (arrows). D, dentine; AB, alveolar process. Masson's trichrome (bar, 100 μm). In (d), acidophilic and irregular material (NF) fills partially the perforation of the floor of the pulp chamber and is surrounded by numerous mononuclear cells and blood vessels (BV). Collagen fibres and fibroblasts (arrows) seem to be surrounding the material 'NF'. PL, peridontal ligament; D, dentine; AB, alveolar process. H&E (bar, 100 μm). In (e), outlined area of (d), homogeneous material 'NF' contains lacunae (L) and, sometimes, cells inside them (arrows). Elongated and rounded cells (arrowheads) are in close juxtaposition to the irregular surface of 'NF'. BV, blood vessels. H&E (bar, 50 μm). In (f), some inflammatory cells (IC) are adjacent to the root surface. In the furcation region, fibroblasts (arrows) and bundles of collagen fibres (CF); some of them penetrate into the alveolar bone (AB) and cementum (C). D, dentine. H&E (bar, 100 μm).

control groups were not statistically significant at 60 days.

Discussion

The results demonstrated that MTA-based materials, used for filling of perforations in the pulp chamber floor, exhibited better biological behaviour in comparison with ZOE. This interpretation was based on the analysis of different morphometrical parameters: numerical density of inflammatory cells in the periodontal ligament, width of the periodontal space and the number of TRAP-positive osteoclasts in the alveolar bone surface.

In the present study, the molars used as control group had not the floor of the pulp chamber perforated because the untreated perforations, in contact to saliva, promote intense inflammatory reaction (Pitt-Ford *et al.* 1995, Noetzel *et al.* 2006). Although it is not possible to measure the tissue reaction promoted by traumatic injury as a result of the experimental perforations, the

Days	Control	MTA	CPM	ZOE
07	1.46 ± 0.09^{a,1}	5.61 ± 0.97^{b,1}	3.52 ± 0.46^{c,1}	12.65 ± 0.81^{d,1}
15	1.41 ± 0.20^{a,1}	6.64 ± 1.52^{b,1}	6.51 ± 0.87^{b,2}	10.64 ± 0.54^{c,2}
30	1.50 ± 0.13^{a,1}	5.57 ± 0.52^{b,1}	4.37 ± 0.25^{c,1}	8.45 ± 1.18^{b,3}
60	1.40 ± 0.09^{a,1}	1.89 ± 0.63^{a,2}	1.78 ± 0.23^{a,3}	4.96 ± 0.98^{b,4}

The comparison amongst groups ($P \leq 0.05$) is indicated by different superscripts (a, b, c and d) in the various lines.
The comparison amongst periods ($P \leq 0.05$) is indicated by different superscripts (1, 2, 3 and 4) in the various columns.
TRAP, tartrate-resistant acid phosphatase; MTA, mineral trioxide aggregate; CPM, Endo CPM-Sealer; ZOE, zinc oxide eugenol.

Table 3 Number of TRAP-positive osteoclasts per mm of alveolar bone surface in the control, MTA, CPM and ZOE groups at the different periods

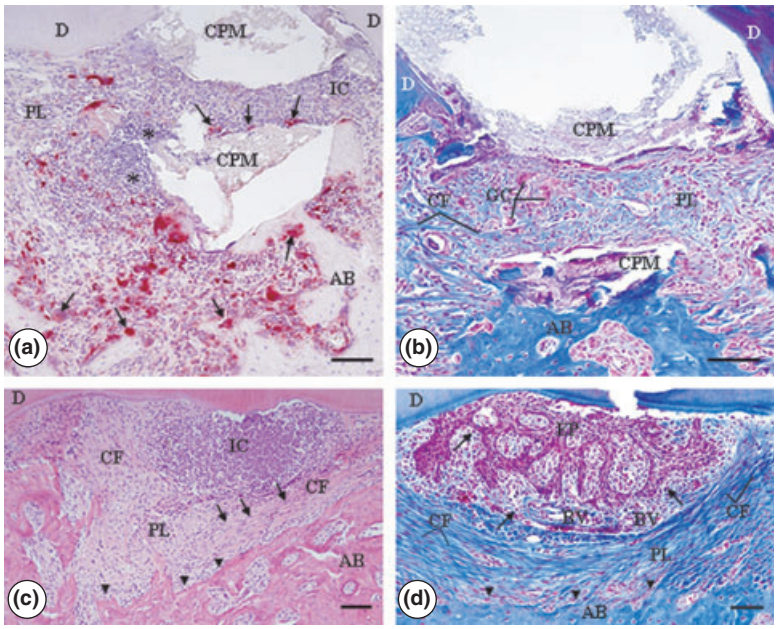

Figure 3 Light micrographs of portions of furcation regions of molar teeth perforated and filled with CPM, and examined after 7- (a), 15- (b), 30- (c) and 60-days (d). In (a), remainders of CPM filling the perforation in the floor of the pulp chamber. In the periodontal ligament (PL), numerous inflammatory cells (IC) are in close contact with CPM. CPM in the periodontal space is involved by inflammatory cells (asterisks) and tartrate-resistant acid phosphatase (TRAP)-positive cells (arrows). The alveolar bone (AB) exhibits TRAP-positive osteoclasts (arrows). D, dentine. TRAP reaction counterstained with haematoxylin (bar, 100 μm). In (b), remainders of CPM are observed inside perforation in the floor of the pulp chamber. Periodontal ligament (PL) exhibits some inflammatory cells and multinucleated giant cells (GC) amongst fibroblasts and collagen fibres (CF). CPM overflowed in the periodontal space is apposed to surface of the alveolar bone (AB). D, dentine. Masson's trichrome (bar, 100 μm). In 3C, an aggregate of inflammatory cells (IC) is next to root surface. Fibroblasts (arrows) between the collagen fibres (CF) are observed. A layer of osteoblasts (arrowheads) is seen on the alveolar bone (AB) surface; note the absence of osteoclasts. D, dentine. H&E (bar, 100 μm). In (d), in the furcation region, a profuse epithelial proliferation (EP) forming an intricate network is adjacent to root surface. In the subjacent tissue, inflammatory cells (arrows) and blood vessels (BV) are observed. Bundles of collagen fibres (CF) seem to be surrounding partially this epithelial proliferation (EP). On the alveolar bone surface (AB), typical osteoblasts (arrowheads) and Sharpey's fibres are observed. D, dentine; PL, periodontal ligament. Masson's trichrome (bar, 100 μm).

high number of inflammatory cells observed in the 7-day period could be, at least in part, caused by this injury. However, the variation in the numerical density of inflammatory cells found in the different experimental groups indicates that the materials released irritant substances.

The morphometrical findings revealed that the number of inflammatory cells was significantly higher

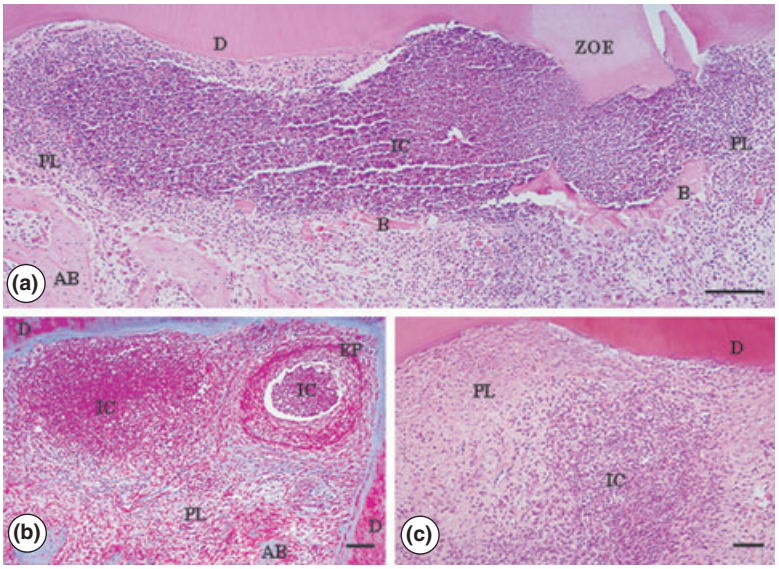

Figure 4 Light micrographs show the portions of furcation regions of molar teeth which were perforated and filled with zinc oxide eugenol (ZOE), and examined after 7- (a), 15- (b) and 60-days (c). In (a), numerous inflammatory cells (IC) are observed through periodontal ligament (PL) in the furcation region. ZOE, filling the perforation in the floor of the pulp chamber; D, dentine; B, bone trabeculae; AB, alveolar process. H&E (bar, 100 μm). In (b), numerous inflammatory cells (IC) are observed in the periodontal ligament (PL); a dense and round aggregate of inflammatory cells (IC) is adjacent to root surface. Another structure appears to be surrounded by epithelial cells (EP). D, dentine; AB, alveolar bone. Masson's trichrome (bar, 100 μm). The figure (c) shows massive presence of inflammatory cells (IC) in the large periodontal space. D, dentine; PL, periodontal ligament. H&E (bar, 100 μm).

in the periodontal ligament of rats in the ZOE group in comparison with MTA and CPM groups indicating, therefore, that the zinc oxide eugenol induced the formation of an intense inflammatory process. This intense inflammatory process is likely associated with slow and prolonged release of eugenol (Molnar 1967, Becker *et al.* 1983). There is evidence that eugenol causes protein denaturation, promoting structural and functional cellular changes and, thereby, cell death (Kozam & Mantell 1978). In addition, the zinc ions released may be responsible for deleterious effects on cell survival (Economides *et al.* 1995).

The inflammatory cells produce and release several factors and interleukins such as interleukin-1 (IL-1), IL-6, tumour necrosis factor α and prostaglandins (PGE_2). These cytokines stimulate the osteoclast formation and activity (Phan *et al.* 2004, Michael *et al.* 2005). Therefore, the intense inflammatory process may be responsible for significant increase in the number of TRAP-positive osteoclasts in the alveolar bone surface observed in the ZOE group in comparison with MTA and CPM groups. The massive presence of osteoclasts exhibiting evident positivity to the histochemical reaction for the detection of TRAP indicated intense resorptive activity and, therefore, explains the significant increase in the periodontal ligament space observed in the ZOE group.

In addition, at 7 days, the MTA promoted greater changes than the Endo-CPM-Sealer. It has been described that MTA stimulates initially the formation of inflammatory process in the periodontal ligament promoting the disorganization of the periodontium (Noetzel *et al.* 2006). It is known that MTA contains, amongst other components, calcium oxide that reacts with water giving rise to calcium hydroxide (Holland *et al.* 2002, Camilleri *et al.* 2005, Camilleri 2007). The release of calcium hydroxide may be responsible for the biocompatibility of the MTA-based cements (Sarkar *et al.* 2005). Therefore, it has been suggested that the action mechanism of MTA is similar to the calcium hydroxide, i.e. initially promotes a surface necrosis of the tissue juxtaposed to material (Holland *et al.* 1999, Shahi *et al.* 2006). Although the CPM Sealer has a similar composition to MTA, it has been described that the reduction in pH by the addition of calcium carbonate allows the action of the alkaline phosphatase and, thereby, provokes smaller areas of surface necrosis in comparison with MTA-Angelus (Gomes-Filho *et al.*

2009a). Considering that the number of inflammatory cells decreased between 7 and 15 days, it is possible to suggest that the irritant effect of these materials decreased after a few days.

The chemical composition of the materials interferes with their biocompatibility. The analysis of the results revealed that MTA induced a more intense disorganization and enlargement of the periodontal space than Endo-CPM- Sealer® up to 30 days. Although the periodontal space, at 30 days, was larger in the MTA group in comparison with the CPM group, a significant reduction in the number of inflammatory cells was observed in the MTA group from 15 to 30 days. This reduction suggests that the irritant effect of MTA decreased 15 days after sealing allowing repair of the periodontium; this hypothesis is reinforced by a significant reduction in the width of the periodontal space at 60 days, indicating bone formation in the alveolar process.

The significant reduction in the number of inflammatory cells verified in all groups, at 60 days, may be responsible for a decrease in the number of TRAP-positive osteoclasts. The reduction of osteoclasts and the presence of active osteoblasts on the bone surface allowed, at least in part, the re-establishment of the alveolar process and, thereby, the reduction in the width of the periodontal space. Although a significant reduction in the number of inflammatory cells was observed in the ZOE group, the mean values at 60 days were similar to those observed in the MTA-based materials in the initial periods (7 and 15 days) indicating the intense damage caused by ZOE. Therefore, these findings may explain the significant increase in the number of osteoclasts in the alveolar surface found in the ZOE group in comparison with other groups, at 60 days. On the other hand, the number of TRAP-positive osteoclasts in the alveolar bone surface in the control, MTA and CPM groups, at 60 days, was not significantly different. These findings associated with neoformation observed in alveolar bone reinforce the concept that these materials are biocompatible. In addition, bone-like material formation was observed in a specimen filled with MTA, after 30 days. The formation of hard tissue in contact with MTA used for filling the perforations in the furcation regions has been reported. However, the formation of structures, similar to mineralized tissue, is often found after 90–180 days of the experiment (Pitt-Ford *et al.* 1995, Yildirim *et al.* 2005). In the present study, the presence of bone-like tissue may be because of MTA releasing calcium into the surrounding tissues, favouring calcium carbonate precipitation (Holland *et al.* 1999, 2002). There is evidence that fibronectin deposited in the calcium carbonate surface allows the cellular proliferation and adhesion and, thereafter, the formation of mineralized matrix (Seux *et al.* 1991). Moreover, calcium ions seem to stimulate the production of osteopontin and bone morphogenetic protein-2 and, by this way, to control the process of formation of mineralized tissue (Rashid *et al.* 2003).

Although CPM promoted less bone destruction in comparison with MTA, this material was occasionally found in the periodontal ligament indicating that extravasation occurred during the sealing of some perforations. The pasty consistency exhibited by CPM used in the present study may explain the overflow of this cement to the periodontal space. It has been reported that the placement of biocompatible matrices such as calcium sulfate, hidroxiapatite or collagen in perforations between the periodontal ligament and the sealing material may avoid the extrusion of cements into the periodontal ligament. However, these matrices caused unfavourable inflammatory reaction and did not aid bone regeneration thus interfering in the repair of the adjacent periodontium (Salman *et al.* 1999, Al-Daafas & Al-Nazhan 2007).

Conclusion

A significant reduction in the number of osteoclasts and in the periodontal space of MTA and CPM groups occurred, indicating that these materials induce bone repair. These findings suggest that MTA-based materials are more biocompatible than ZOE. In this animal model, Endo-CPM-Sealer can be considered as an alternative sealing material in the treatment of root perforations.

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