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Effect of MTA-based sealer on the healing of periapical lesions

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ABSTRACT

Some manufacturers have recently added specific components to improve the ease of handling and insertion material properties of MTA in order to create MTA-based sealers. Objective: The aim of this study was to evaluate the healing of periapical lesions in canine teeth after a single session of endodontic treatment with MTA Fillapex® compared with Sealapex® or Endo-CPM-Sealer®. Material and Methods: Sixty-two root canals were performed on two 1-year-old male dogs. After coronal access and pulp extirpation, the canals were exposed to the oral cavity for 6 months in order to induce periapical lesions. The root canals were prepared, irrigated with a solution of 2.5% sodium hypochlorite and filled with gutta-percha and different sealers, according to the following groups: 1) Sealapex®; 2) Endo-CPM-Sealer®; and 3) MTA Fillapex®. Some teeth with periapical lesions were left untreated for use as positive controls. Healthy teeth were used as negative controls. After 6 months, the animals were sacrificed and serial sections from the roots were prepared for histomorphologic analysis and stained with hematoxylin and eosin and the Brown and Brenn technique. The lesions were scored according to pre-established histomorphologic parameters and the scores statistically analyzed using the Kruskal-Wallis test. Results: All 3 materials produced similar patterns of healing ($p>0.05$); in particular, persistent inflammation and absence of complete periapical tissue healing were consistently noted. Conclusions: Preparation of the infected root canals followed by filling with the materials studied was insufficient to provide complete healing of the periapical tissues.

Key words: Endodontics. Dental materials. Material testing.

INTRODUCTION

The biomaterial mineral trioxide aggregate (MTA) was approved for endodontic use by the U.S. Food and Drug Administration in 1998[21]. MTA has been shown to promote favorable tissue reactions characterized by the absence of severe inflammation, the presence of a fibrous capsule, and the induction of mineralized repair tissue[2,9]. MTA is highly biocompatible when used for pulp capping[14], root perforations[17] and retrograde fillings[2]. However, despite its positive characteristics, MTA does not have the appropriate physical properties for use as a sealer[28].

Some manufacturers have recently added specific components to improve the ease of handling and insertion material properties of MTA in order to create MTA-based sealers. Some examples of such materials currently on the market are ProRoot Endo Sealer® (Dentsply, Tulsa, OK, USA), Endo-CPM-Sealer® (EGEO SRL, Buenos Aires, Buenos Aires, Argentina) and MTA Fillapex® (Angelus, Londrina, PR, Brazil).

According to the manufacturer, Endo-CPM-Sealer® has a chemical composition similar to that of MTA but with the addition of calcium carbonate to reduce the post-set pH level from 12.5 to 10.0. This restricts the necrosis of the surface in contact with the material, enabling the action of alkaline phosphatase[9]. Endo-CPM-Sealer® has satisfactory plasticity, adhesion, flow, radiopacity, and antimicrobial activity[11,27]. The

biocompatibility of Endo-CPM-Sealer® is similar to that of Angelus MTA®: both produced a moderate chronic inflammatory response (apparent on the 7th and 15th days post-procedure) that decreased with time[9]. This material has also been shown to stimulate mineralization of the subcutaneous tissue of rats[9].

MTA Fillapex® is composed of salicylate resin, resin diluent, natural resin, bismuth oxide, silica nanoparticles, and mineral trioxide aggregate. According to the manufacturer, it has the following physical properties: working time, 35 min, flow capacity, 27.66 mm, setting time, 130 min, optical density, 77%, and solubility, 0.1%. Moreover, it is easily manipulated. MTA Fillapex® presented solubility values higher than required by the ANSI/ADA[4]. According to a cytotoxicity assay, MTA Fillapex® was more cytotoxic in fibroblast cultures in the beginning but it developed the best behavior after 48 hours[3]. A subcutaneous study showed that MTA Fillapex® produced a moderate chronic inflammatory reaction evident on the 7th day that resolved over a short period of time (15 days), similar to that induced by Angelus MTA® and faster than that induced by Sealapex®[9]. However, the behaviour of MTA Fillapex® has not yet been evaluated in an application model study, such as in the treatment of chronic apical lesions in canine teeth.

Therefore, this study aimed to evaluate the healing of periapical lesions in canine teeth after a single endodontic treatment using Sealapex® (SybronEndo, Glendora, CA, USA), Endo-CPM-Sealer® or MTA Fillapex® as sealers.

MATERIAL AND METHODS

Sixty-four roots of 2 male Beagle dogs were used in this study: eight central incisors, eight intermediate incisors, eight lateral incisors, eight canines, four second superior premolars (2 roots each), four third superior premolars (2 roots each), four third inferior premolars (2 roots each) and four fourth inferior premolars (2 roots each). The care of the animals was in accordance with the guidelines of the Araçatuba School of Dentistry-UNESP Ethical Committee, which approved the project before the beginning of the experiments.

The animals were anaesthetized by intramuscular injection of a combination of xylazine (Cristália Chemicals Pharmaceuticals, Ltd., Itapira, SP, Brazil; 0.05 mL/kg body weight) and a mixture of tiletamine hydrochloride and zolazepam hydrochloride (Zoletil-50; Virbac, São Paulo, SP, Brazil; 0.2 mL/kg body weight). The teeth studied were subjected to coronary opening and pulp extirpation up to the apical barrier. The canals were left open and exposed to the oral environment in order to induce periapical lesions. After 6 months, control radiographs were taken and the teeth were fitted with rubber dams and subjected to endodontic treatment.

The fifty-four experimental root canals were biomechanically prepared up to the apical barrier with a #40 K-file and abundantly irrigated with 2.5% sodium hypochlorite (NaOCl) throughout the instrumentation process. Over-instrumentation with #20 K files was then performed in such a way as to create an artificial main canal foramen. The canals were irrigated, dried with paper points, and filled with 17% EDTA for 3 min. The EDTA was removed by irrigation with 2.5% NaOCl followed by a final irrigation with saline solution[12]. The root canals were filled to 1 mm before the apex with gutta-percha points and sealed by the active lateral condensation technique with Sealapex®, Endo-CPM-Sealer®, or MTA Fillapex®. The sealers were prepared according to the manufacturers' instructions. The pulp chambers were cleaned and the access cavities sealed with intermediate restorative material (Coltosol; Vigodent, Rio de Janeiro, RJ, Brazil) and composite resin (TPH Spectrum; Dentsply, Tulsa, OK, USA). These procedures divided the roots into 3 experimental treatment groups of 18 roots each as follows: Group I: Sealapex®, Group II: Endo-CPM-Sealer®, and Group III: MTA Fillapex®.

As positive controls (Group IV), 4 roots were selected for pulp extirpation and their canals left exposed to the oral environment with no additional treatment for 6 months (after which the animals were sacrificed). As negative controls (Group V), 4 roots remained completely healthy until the end of the experiment once they were not subjected to any treatment.

Six months after treatment, the animals were sacrificed with an overdose of anaesthetic. The maxilla and mandibles containing the roots were removed, fixed in 10% neutral-buffered formalin solution, and decalcified in 17% EDTA solution. Segments of the jaws, each containing 1 root, were prepared for histological examination using standard procedures. Briefly, the specimens were embedded in paraffin, serially sectioned at 6-µm thickness, and stained with hematoxylin and eosin or by the Brown and Brenn technique.

Some roots were discarded due to histological artefacts, and only 12 roots treated with each material were tested and 4 roots from each control group were analyzed. The histological details of the newly formed cementum (thickness, extension, and biological closure of the main and accessory canals), cementum resorption, bone tissue resorption, inflammatory reaction (chronic or acute, number of cells, and extension of the reaction), periodontal ligament (thickness and organisation), root canal filling limit, and presence of debris, giant cells, and microorganisms were noted. All of the above-

listed histomorphological events were scored from 1 to 4 (from the best to the worst) as described in Figure 6 and according to a previous study[12]. The statistical significance of the results was analyzed by the Kruskal Wallis test (p=0.05).

RESULTS

Positive control group

All specimens showed apical cementum resorption areas of different sizes. The areas of resorption were either active or inactive without signs of healing (Figure 1B). The apical periodontal space was intensely and extensively invaded by tissue containing acute and chronic inflammatory infiltrates; the latter was characterized by lymphocytes, plasma cells, and macrophages (Figure 1C). Areas of unrepaired bone tissue resorption were observed in all specimens. Many giant cells were observed in all specimens. Brown and Brenn staining showed gram-negative microorganisms in the main canal, apical accessory canals, and cementum lacunae of all specimens (Figure 1D).

Negative control group

Newly formed cementum greater than 60 µm thick was present in all specimens. No areas of active resorption of cementum or bone were observed in any specimen (Figure 2B). Mild inflammatory infiltrates were observed within the ramifications or adjacent to the foramina in all specimens (Figures 2C,D). The thickness of the periodontal ligament was variable, ranging up to 200 µm, and the ligament was inserted into the cementum and the bone across the apical portion in all specimens. No giant cells or bacteria were observed (Figure 2E).

Sealapex®

Eosinophilic newly formed cementum was observed in 9 specimens analyzed, but the new cementum repaired only 1/3 or fewer of the areas of resorption in 8 specimens. Active resorption in the lateral surfaces of the roots was observed in 6 specimens.

Only 1 specimen showed complete closure of the delta by the newly formed cement, while 2 specimens showed complete closure of the main canal (Figures 3F,G). Ten specimens showed severe chronic inflammatory infiltration of the periodontal ligament (Figure 3C). However, the periapical tissue that grew into the canal showed mild inflammatory infiltrates close to the material and mineralized tissue in close contact with the material in all specimens (Figure 3D).

The root canal filling limit was beyond the intended limit in 9 specimens and short of the intended limit in only 3 specimens. Inactive bone was observed in 4 specimens, resorption with a few active areas in 6 specimens, and resorption with

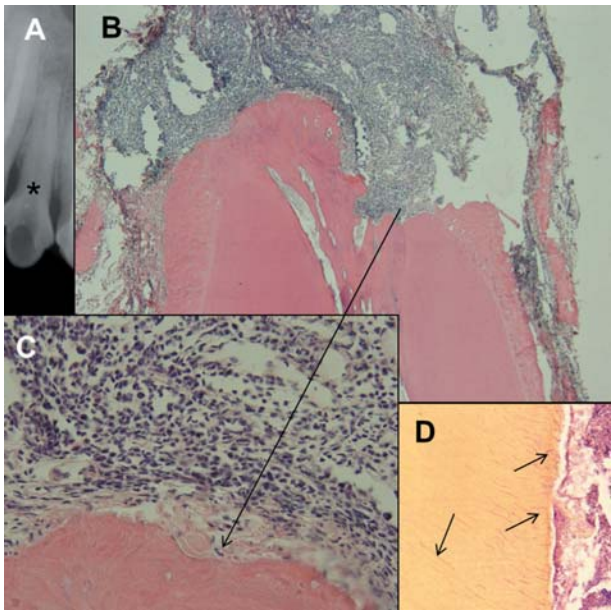

Figure 1- Representative images of the positive control group. (A) Radiographic examination (*) 180 days after pulpectomy [Original; Rx]. (B) Panoramic view [×100; hematoxylin & eosin (H.E.)]. (C) Severe inflammatory infiltrate and extensive areas of resorption (arrow) [×400; H.E.]. (D) Gram-negative microorganisms in the dentinal tubules (arrows) [×400; Brown and Brenn (B.B.)]

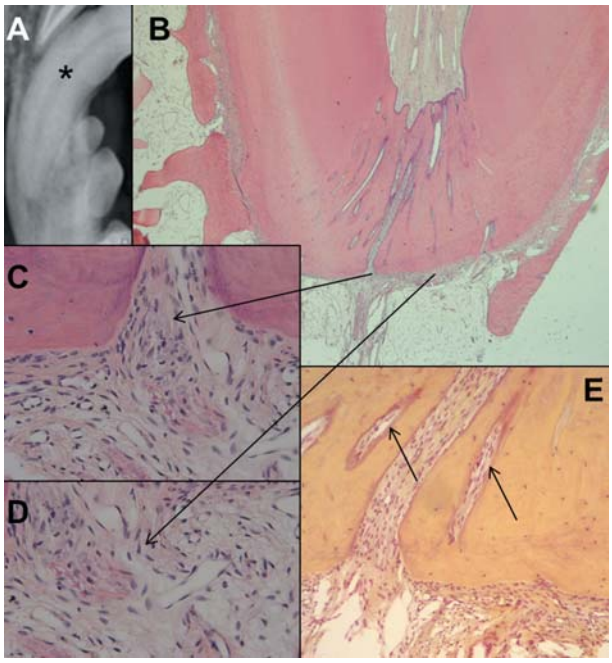

Figure 2- Representative images of the negative control group. (A) Radiographic examination (*) healthy tooth [Original; Rx]. (B) Panoramic view [×100; hematoxylin & eosin (H.E.)]. (C,D) Mild inflammatory infiltrate and tissue organization (arrows) [×400; H.E.]. (E) Absence of microorganisms in cementum lacunae (arrows) [×400 Brown and Brenn (B.B.)]

many active areas in 2 specimens. No debris was observed in 8 specimens, while discrete pockets of debris were present in 4 specimens. Many giant cells were observed in 8 specimens, moderate numbers in 3 specimens, and discrete giant cells in 1 specimen.

Brown and Brenn staining showed gram-positive and gram-negative microorganisms, with gram-negative organisms predominating in the ramifications of the main canal and especially in the cementum lacunae in all specimens (Figure 3E).

Endo-CPM-Sealer®

Eosinophilic newly formed cementum was observed in 5 of the specimens analyzed but repaired only 1/3 or fewer of the areas of resorption in 4 specimens. Areas of active cementum resorption were observed in all specimens.

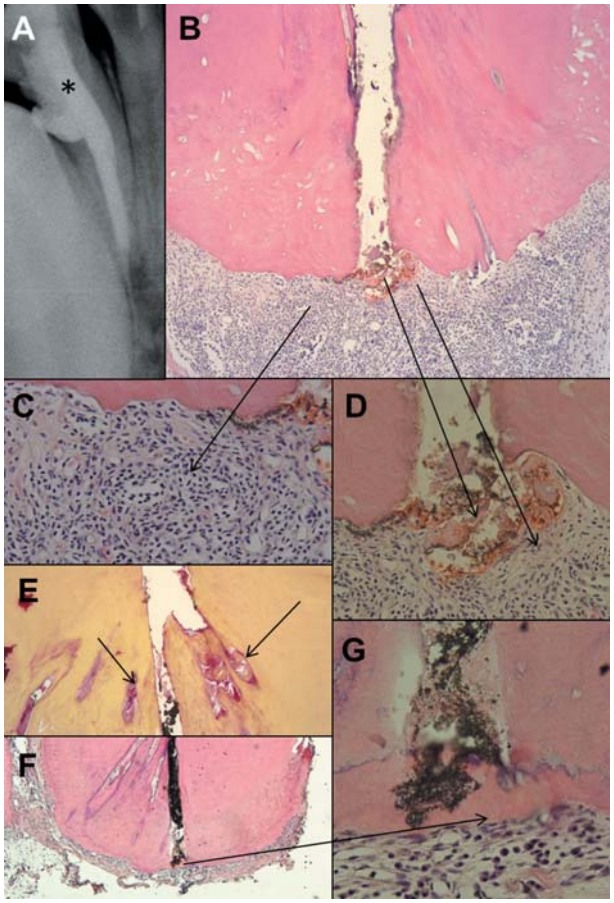

Figure 3- Representative images of the Sealapex® group. (A) Radiographic examination (*) 180 days after filling [Original; Rx]. (B) Panoramic view showing lack of complete closure of the main canal [×100; hematoxylin & eosin (H.E.)]. (C) Severe inflammatory infiltrate (arrow) [×400; H.E.]. (D) Pulp stump with mild inflammatory infiltrate and mineralized tissue (arrows) [×400; H.E.]. (E) Gram-negative microorganisms in cementum lacunae (arrows) [×400; Brown and Brenn (B.B.)]. (F) Panoramic view [×100; H.E.]. (G) Newly formed cementum in the vicinity of the main canal (arrow) [×400; H.E.]

No complete closure of the apical delta and only 1 example of complete closure of the main canal by the newly formed cement was observed (Figure 4E). Severe chronic inflammatory infiltrates were observed in 11 specimens (Figure 4D) and a moderate inflammatory infiltrate in 1 additional specimen. Moderate inflammatory infiltrates were observed in the pulp stump in all specimens, and mineralized tissue was noted in close contact with the material in most specimens (Figure 4F).

The limit of filling was beyond the intended apical limit in 9 specimens and short of the intended limit in only 3 specimens. Resorption of bone tissue with few active areas was observed in 10 specimens and resorption with many active areas in 2 specimens. Debris was present in only 1 specimen. Large numbers of giant cells were observed in all specimens.

Brown and Brenn staining showed gram-negative microorganisms in the ramifications of the main canal and especially in the cementum lacunae of all specimens (Figure 4C).

MTA Fillapex®

Eosinophilic newly formed cementum was observed in 9 specimens analyzed and repaired 1/3

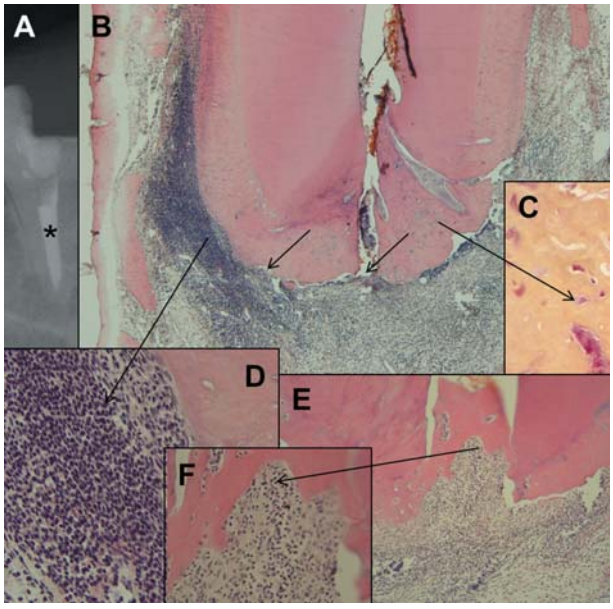

Figure 4- Representative images of the Endo-CPM-Sealer® group. (A) Radiographic examination (*) 180 days after filling [Original; Rx]. (B) Panoramic view showing areas of resorption and the absence of newly formed cementum (arrows) [×100; hematoxylin & eosin (H.E.)]. (C) Gram-negative microorganisms in cementum lacunae (arrows) [×400; Brown and Brenn (B.B.)]. (D) Severe inflammatory infiltrate (arrow) [×400 - H.E.]. (E) Panoramic view [×100 - H.E.]. (F) Pulp stump with moderate inflammatory infiltrate and mineralized tissue (arrow) [×400; H.E.]

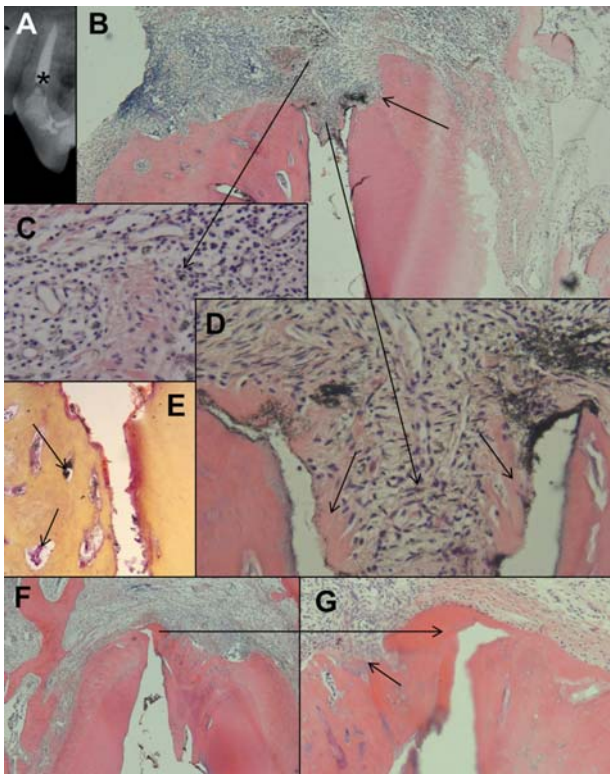

Figure 5- Representative images of the MTA Fillapex® group. (A) Radiographic examination (*) 180 days after filling [Original; Rx]. (B) Panoramic view showing areas of resorption and the absence of newly formed cementum (arrow) [×100; hematoxylin & eosin (H.E.)]. (C) Severe inflammatory infiltrate (arrow) [×400; H.E.]. (D) Pulp stump with mild inflammatory infiltrate and mineralized tissue (arrows) [×400; H.E.]. (E) Gram-positive and gram-negative microorganisms in cementum lacunae (arrows) [×400; Brown and Brenn (B.B.)]. (F) Panoramic view [×100; H.E.]. (G) Newly formed cementum in the vicinity of the main canal, unrepaired areas of resorption, and severe inflammatory infiltrate (arrows) [×400; HE]

or fewer of the areas of resorption in all 9 cases. Areas of active cementum resorption were seen in 7 specimens.

Complete closure of the apical delta in a few ramifications was observed in 9 specimens and complete closure of the main canal in 1 specimen (Figures 5F,G). Moderate chronic inflammatory infiltrates were observed in 2 specimens and severe inflammatory infiltrates in 10 specimens (Figure 5C). Mild inflammatory infiltrates and the presence of mineralized tissue in close contact with the material were seen in the pulp stumps of all specimens (Figure 5D).

The root canal filling limit was beyond the intended apical limit in 11 specimens and short of the intended limit in only one specimen. Resorption of bone tissue with many active areas was observed in only 1 specimen, resorption with few active areas in 9 specimens, and no active areas of resorption in 2 specimens. No debris was observed in 7 specimens and discrete pockets of debris in 5 specimens (Figures 5B,D). Large numbers of giant cells were observed in 10 specimens.

Brown and Brenn staining showed gram-negative microorganisms in the ramifications of the main canal and especially in the cementum lacunae of all specimens (Figure 5E).

Comparison among the Groups

The scores assigned to the various histomorphological events were subjected to the Kruskal-Wallis test. This test ranked the experimental groups from the best to the worst as follows: (a) Sealapex®, (b) MTA Fillapex®, and (c) Endo-CPM-Sealer®. The scores did not differ significantly among the groups ($p>0.05$) (Figures 6 and 7), owing mainly to the absence of periapical tissue healing. However, the pulp stump exhibited mild inflammatory reaction only in the Sealapex® and MTA Fillapex® groups.

DISCUSSION

The experimental model of lesion induction used in this study was based on previously established criteria[12]. Moreover, in the present study, the root canal filling materials were evaluated without the use of an intracanal dressing; this choice was made to reduce the number of variables, not necessarily as an option for clinical use.

The results obtained in this study for the healing of periapical lesions in canine teeth were unsatisfactory regardless of the sealer used. MTA Fillapex® gave results similar to those obtained with Sealapex® and Endo-CPM-Sealer® with regard to the different histopathological criteria investigated. The similarity of the results may be because all contain calcium oxide (CaO) and may therefore have similar dominant mechanisms of biological action. CaO may react with water or tissue fluids to form calcium hydroxide, which then dissociates into calcium and hydroxyl ions and alkalizes the tissue to form calcite crystals[15].

Several factors can influence the results of endodontic treatment of teeth with periapical lesions, including the biomechanical preparation (instrumentation and irrigation), intracanal dressing, and filling[20]. The instruments penetrate almost the entire main root canal but not the ramifications and cementum lacunae, in which microorganisms may lodge and cause apical periodontitis[12,26].

The use of irrigation solutions is intended to reduce the number of microorganisms, remove debris, and neutralize organic compounds; however, due to the risk of leakage through the apical foramen, the irrigation solutions must be biocompatible and non-irritating to the periapical tissues[16]. At high

concentrations, NaOCl has potent antimicrobial activity due to the release of secondary chlorates, which lead to increased tissue dissolution, and it is recommended for the treatment of teeth with periapical lesions[1].

The antimicrobial activity of 2.5% NaOCl against *Enterococcus faecalis*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Streptococcus mutans*, *Streptococcus sanguis*, and *Streptococcus sobrinus* and its effectiveness at disinfecting the root canal have been demonstrated in some *in vitro* studies[24,25]. Furthermore, NaOCl solutions at concentrations of 1%, 2.5%, and 5% have been shown to have similar antimicrobial activities[25]. Although irrigation with 2.5% NaOCl was used in association with instrumentation in this study, bacteria were still observed in most specimens of all 3 experimental groups.

The results of this study showed that the filling materials used did not effectively combat endodontic infection, since the periapical tissue

Figure 6- Distribution of specimens of each group according to scores of histopathologic parameters

Histopathologic parameters	Scores	Groups				
		G I (n=12)	G II (n=12)	G III (n=12)	G IV (n=4)	G V (n=4)
New formed cementum (thickness):	1 - above 60 µm	9	5	9	0	4
	2 - between 20 and 59 µm	0	0	0	0	0
	3 - between 1 and 19 µm	0	0	0	0	0
	4 - absence	3	7	3	4	0
New formed cementum (extension):	1 - repairs all resorption areas or covers pre-existing areas	0	0	0	0	4
	2 - repairs 1/2 to 2/3 areas of resorption	1	0	0	0	0
	3 - repairs 1/3 or less of resorption areas	8	4	9	0	0
	4 - absence of cementum repairing resorption areas	3	8	3	4	0
Biological closure of the apical delta canals:	1 - complete	1	0	0	0	4
	2 - complete in most ramifications	0	4	0	0	0
	3 - complete in a few ramifications	7	8	9	0	0
	4 - absence	4	0	3	4	0
Biological closure of the main canal:	1 - complete	2	1	1	0	4
	2 - partial closure	1	0	0	0	0
	3 - cementum deposition on the sidewalls	0	2	2	0	0
	4 - absence	9	9	9	4	0
Areas of cementum resorptions:	1 - absence	0	0	0	0	4
	2 - partially repaired areas	1	0	1	0	0
	3 - not repaired areas	5	0	4	0	0
	4 - active resorption areas	6	12	7	4	0
Inflammatory infiltrate (intensity):	1 - absence or negligible number	0	0	0	0	0
	2 - small: number of cells less than 10	1	0	0	0	4
	3 - moderate: number of cells between 10 and 25	1	1	2	0	0
	4 - severe: number of cells greater than 25	10	11	10	0	0
Inflammatory infiltrate (extension):	1 - absence	0	0	0	0	0
	2- within the ramifications or next to the foramen	0	0	0	0	4
	3 - just beyond of foramen	1	0	0	0	0
	4 - invade the whole apical periodontal space	11	12	12	4	0

was not completely repaired. Although sealers have antimicrobial activity[5,6,18,23], this was evidently insufficient to control the infection as an intracanal treatment, especially after the setting time[17]. Furthermore, these results were similar to those of previously reported studies that showed that teeth treated in a single appointment did not heal adequately compared with those treated with calcium hydroxide paste as an intracanal dressing[12,22]. Consistent with this fact, the complexity of the root canal system formed by the main canals and ramifications must be considered an important factor in the disinfection strategy that justifies the use of an intracanal dressing[12,19]. Calcium hydroxide dressing is widely used as an intracanal dressing because it inhibits bacterial enzymes and activates tissue enzymes such as alkaline phosphatase resulting in a mineralizing effect[7]. Radiographic study evaluating the healing of periapical lesions treated in one or two visits using calcium hydroxide as an intracanal dressing and found that the additional disinfecting with calcium hydroxide resulted in a 10% increasing on the healing rates[29].

An interesting positive observation was that MTA Fillapex® and Sealapex® produced less inflammatory reaction in the region of the pulp stump in close

Figure 7- Specimens distribution by group according to the scores of histopathologic parameters

Histopathologic parameters	Scores	Groups				
		GI (n=12)	GII (n=12)	GIII (n=12)	GIV (n=4)	GV (n=4)
Bone tissue (resorption):	1 - absence	0	0	0	0	0
	2 - inactive areas	4	0	2	0	4
	3 - few active areas	6	10	9	4	0
	4 - many active areas	2	2	1	0	0
Periodontal ligament (thickness):	1 - up to 200 µm	12	12	12	4	4
	2 - between 201 to 300 µm	0	0	0	0	0
	3 - between 301 to 400 µm	0	0	0	0	0
	4 - above 401 µm	0	0	0	0	0
Periodontal ligament (organization):	1 - insert of the cementum to the bone in all the apical portion	0	0	0	0	4
	2 - insert of the cementum to the bone in part of the apical portion	0	0	0	0	0
	3 - insert parallel to the tooth surface	7	3	7	0	0
	4 - absence of organization	5	9	5	4	0
Root canal filling limit:	1 - before (CDC limit)	3	3	1	-	-
	2 - beyond of the CDC limit	9	9	11	-	-
	3 - just beyond of the foramen	0	0	0	-	-
	4 - beyond of the foramen	0	0	0	-	-
Presence of debris:	1 - absence	8	11	7	-	-
	2 - discreet presence	4	1	5	-	-
	3 - moderate presence	0	0	0	-	-
	4 - intense presence	0	0	0	-	-
Presence of giant cells:	1 - absence	0	0	0	0	4
	2 - discreet: 1 to 3 cells	1	0	0	0	0
	3 - moderate: 4 to 6 cells	3	0	2	0	0
	4 - severe: 7 or more cells	8	12	10	4	0
Presence of microorganisms:	1 - absence	0	0	0	0	4
	4 - presence	12	12	12	4	0
Inflammatory infiltrate (pulp stump):	1 - absence or negligible number	0	0	0	0	4
	2 - small: number of cells less than 10	12	0	12	0	0
	3 - moderate: number of cells between 10 and 25	0	12	0	0	0
	4 - severe: number of cells greater than 25	0	0	0	4	0
Presence of mineralization (pulp stump):	1 - presence	12	8	12	0	0
	4 - absence	0	4	0	4	4

contact with the filling materials, demonstrating the superior biocompatibility of these materials in an application model. These findings are in accordance with results obtained in a rat model that showed that MTA Fillapex® and Sealapex® were biocompatible and stimulated mineralization[8-10,13]. These calcifications are thought to originate from the CaO present in MTA Fillapex® and in Sealapex®. When in contact with water, CaO can be converted into $Ca(OH)_2$ and dissociated into Ca^{2+} and OH^-. The diffusion of hydroxyl ions from the root canal increases the pH level at the surface of the root adjacent to the periodontal tissues, possibly interfering with osteoclastic activity and promoting alkalinization in the adjacent tissues, which favors healing[10].

This study demonstrated that even with the use of biocompatible materials that can stimulate tissue mineralization, the complete repair did not occur without the root canal system disinfection.

CONCLUSIONS

It can be concluded that the endodontic treatment performed in a single session using these materials cannot support complete healing of the periapical tissues of canine teeth.

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