Mineral trioxide aggregate as a root canal filling material in reimplanted teeth. Microscopic analysis in monkeys

Panzarini SR, Holland R, de Souza V, Poi WR, Sonoda CK, Pedrini D. Mineral trioxide aggregate as a root canal filling material in reimplanted teeth. Microscopic analysis in monkeys.

Abstract – This study analyzed mineral trioxide aggregate (MTA) as a root canal filling material for the immediate reimplantation of monkey teeth. Four adult capuchin monkeys *Cebus apella* were used, which had their maxillary and mandibular lateral incisors on both sides extracted and reimplanted after 15 min. During the extra-alveolar period, the teeth were kept in saline solution and after reimplantation retention was performed with a stainless steel wire and composite resin for 14 days. After 7 days, the reimplanted teeth were submitted to endodontic treatment with biomechanics up to file n. 30 and irrigation with a saturated solution of calcium hydroxide [$Ca(OH)_2$], and then divided into two study groups: group I – root canal filled with a $Ca(OH)_2$ paste, and group II – root canal filled with MTA. Radiographic follow up was performed at 30, 60 and 90 days postoperatively, and after 180 days the animals were killed and specimens were processed for histomorphological analysis. The results revealed that most specimens of both groups presented organized periodontal ligament with no inflammation. The resorptions observed were surface resorptions and were repaired by cementum. Both MTA and $Ca(OH)_2$ were good root canal filling materials for immediately reimplanted teeth, providing good repair and also allowing biological sealing of some lateral canals. There was no significant difference between the study groups ($\alpha = 29.60\%$).

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Key words: tooth reimplantation; calcium hydroxide; MTA

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Accepted 28 September, 2005

Reimplantation of the avulsed tooth with complete reestablishment of the vitality of periodontal fibers is the objective. Nevertheless, the percentage of success observed in clinical follow-up investigations of tooth reimplantation has been low, ranging from 4% to 50% (1).

However, it should be highlighted that the consideration of success of tooth reimplantation should take into account the clinical needs and patient expectations. The period of permanence of the tooth in the dental arch may be enough to meet some of the needs, such as prevent atrophy of the alveolar ridge, allow the patient to better assimilate the tooth loss, and even delay the need of prosthesis (2). The success of tooth reimplantation depends on the maintenance of vitality of the periodontal ligament, allowing its remnants adhered to the avulsed tooth to survive and recover their functions (1).

Resorption is the main cause of failure of reimplantations, and the prognosis of a reimplanted tooth is related to the type of resorption that may lead to complete destruction of the root (3). Root resorption is basically classified as follows: surface resorption which may occur even in the absence of a significant inflammatory process; inflammatory resorption, which occurs in the presence of an inflammatory connective tissue; and replacement resorption, in which periodontal ligament is resorbed or removed and replaced by bone tissue (3, 4).

Degeneration of the periodontal ligament depends on several factors, such as trauma, management of the root, extra-alveolar period and storage medium (3, 5, 6). The need to perform endodontic treatment has been demonstrated by several authors, as the pulp tissue may present necrosis and its toxins may reach the periodontal ligament through the dentinal tubules or root canal, definitely contributing to an increase in the resorption process (7–9).

The $Ca(OH)_2$-distilled water paste is the temporary root canal filling material most often employed in reimplanted teeth, because of its antimicrobial and healing process properties, which may delay or interrupt the process of root resorption (8, 9). However, the importance of performing periodical changes of the dressing to maintain its action has been highlighted (10).

In the last decade, a new cement called mineral trioxide aggregate (MTA) was developed at Loma Linda University (USA) with the objective of sealing communications between the interior of the tooth and its external surfaces. In addition to the treatment of root perforations, MTA has demonstrated good outcomes when employed as a retrograde filling material in endodontic surgery, endodontic treatment of teeth with incompletely formed roots, capping material in conservative pulp therapies (11) and as a root canal filling material (12, 13). Considering that its properties are similar to those of $Ca(OH)_2$ (14, 15) and because of the physical characteristics of the material (16), this study compared the healing of reimplanted teeth with root canals filled with either of these two materials.

Materials and methods

Experimental procedures

Four adult male monkeys (*Cebus apella*; University of the State of São Paulo 'Júlio de Mesquita Filho' – UNESP, Dental School of Araçatuba, Tufted Capuchin Monkey Procreation Center) in good health were used in this study. The research protocol was approved by the UNESP – Dental School of Araçatuba Animal Care and Use Committee. All guidelines regarding the care of animal research subjects were strictly followed. The animals were initially sedated by inhalation of sulphuric ether and anesthetized by an intraperitoneal injection of sodium thionembutal (Abbott Laboratórios do Brasil Ltda, São Paulo, Brazil), at a dosage of 30 mg kg^{-1} of body weight.

After anesthesia of the animals, the maxillary and mandibular lateral incisors at the right and left side were extracted and reimplanted after 15 min. During this extra-alveolar period, the teeth were kept in saline solution. After reimplantation, the teeth received retention with 0.7 stainless steel wire (Morelli, Sorocaba, Brazil) and composite resin (TPH, Dentsply, Petrópolis, Brazil), kept for 14 days. Following treatment, the animals were submitted to a dosage of benzathine penicillin – 320 000 UI (Wyeth, São Paulo, Brazil).

Seven days later, the teeth were submitted to coronal opening, pulpectomy, biomechanical preparation and the root canals were irrigated with a saturated solution of $Ca(OH)_2$ with the purpose of making the dentin alkaline (17). Following this, the canals were filled with aid of a lentulo spiral (Dentsply, Petrópolis, Brazil), with the following materials: group I – $Ca(OH)_2$ paste and propylene glycol; group II – MTA cement (Ângelus, Londrina, Brazil). Finally, the coronal cavities were sealed with chemically cured glass ionomer cement (Vidrion, SS White, Rio de Janeiro, Brazil), and specimens were radiographically followed up at 30, 60 and 90 days postoperatively.

After 180 days, the animals were killed by an overdose of anesthetic and perfused with 10% buffered formalin solution at pH 7. The specimens were processed in the laboratory, serially sectioned in a longitudinal direction of 6 μm thickness and the sections attained were stained with hematoxylin and eosin for histomorphological analysis and by the Brown and Brenn method for histomicrobiological analysis. Analysis of the outcomes was performed by one of the authors, according to the 17 events listed below, which were quantified with scores from one to four, one being the best outcome and four being the worst. Data were submitted for statistical analysis by the Mann–Whitney test.

Events considered in analysis of the outcomes:

1 Area of epithelial insertion
- cementoenamel junction;
- ligament below the cementoenamel junction;
- much below the cementoenamel junction (near medium third);
- absence of epithelial insertion.

2 Acute and chronic inflammatory process close to the area of epithelial insertion.

2.1 Intensity of inflammatory process based on the criteria described by Wolfson and Seltzer (18).
- absence or occasional presence of inflammatory cells;
- small number of inflammatory cells. Up to 10 inflammatory cells per field with 400× magnification;
- moderate number of inflammatory cells. From 11 to 50 inflammatory cells per field with 400× magnification;
- large number of inflammatory cells. More than 50 inflammatory cells per field with 400× magnification.

2.2 Extension of the inflammatory process
- absence or occasional presence of inflammatory cells;
- inflammatory process restricted to the lamina propria of the internal aspect of the epithelium;
- inflammatory process extending apically toward the small portion of connective tissue underlying the lamina propria of the internal aspect of the gingival epithelium;
- inflammatory process reaching the area of the alveolar bone crest.

3 Periodontal ligament

3.1 Organization of the periodontal ligament
- periodontal fibers inserted in bone and cementum throughout the extension of the ligament;
- periodontal fibers inserted in bone and cementum at two-third of the extension of the ligament;
- periodontal fibers inserted in the bone and cementum at one-third of the extension of the ligament.
- absence of periodontal fibers with insertion in bone and cementum.

3.2 Intensity and extension of acute and chronic inflammatory process in the periodontal ligament.

3.2.1 Intensity – same criteria employed close to the area of epithelial insertion.

3.2.2 Extension of the inflammatory process
- absence or occasional presence of inflammatory cells;
- inflammatory process present only in the apical or coronal periodontal ligament, or small lateral area;
- inflammatory process reaching more than half of the lateral periodontal ligament on the tooth root;
- inflammatory process throughout the periodontal ligament.

4 Tooth root

4.1 Active and inactive root resorption
- absence of root resorptions or repaired resorptions;
- areas of inactive resorptions (absence of clastic cells);
- small areas of active resorptions;
- extensive areas of active resorptions.

4.2 Extension of root resorption – the diameter of resorbed areas was measured (in micrometers) in representative sections. The mean of the values achieved on the buccal and lingual aspects allowed assignment of the following scores:
- absence of resorption;
- mean extension of 1–1000 μm;
- mean extension of 1001–5000 μm;
- mean extension larger than 5001 μm.

4.3 Depth of root resorption – the depth of resorption areas was measured (in micrometers) in representative sections. The mean of the values achieved on the buccal and lingual aspects allowed assignment of the following scores:
- absence of resorption;
- mean depth of 1–100 μm;
- mean depth of 101–200 μm;
- mean depth larger than 201 μm;

4.4 Repair of root resorption areas.
- absence of resorption or deposition of newly formed cementum throughout the extension of resorption areas;
- deposition of newly formed cementum in half or more of the extension of resorption areas;
- deposition of newly formed cementum in less than half of the resorption areas;
- absence of deposition of newly formed cementum at the resorption areas.

5 Bone tissue – area of active and inactive resorption
- absence of resorption areas;
- presence of inactive resorption areas (absence of clastic cells);
- presence of small areas of active resorption;
- presence of extensive areas of active resorption.

6 Ankylosis
- absence of ankylosis;
- small points of ankylosis;
- one-third of the root with ankylosis;
- more than one-third of the root with ankylosis.

7 Microorganisms
- absence of microorganisms;
- presence of microorganisms not related with the root resorption or inflammation areas;
- presence of small areas with microorganisms related to the inflammation or resorption areas;
- many or extensive areas with microorganisms related to inflammation or root resorption areas.

Results

Group I: $Ca(OH)_2$

In five of six treated cases, epithelial insertion occurred at the cementoenamel junction, with no presence of inflammatory cells in the adjacent connective tissue (Fig. 1). One specimen exhibited epithelial insertion below the cementoenamel junction, besides intense chronic inflammatory process close to the connective tissue adjacent to the lamina propria of the internal aspect of the epithelium, extending to the underlying connective tissue.

The periodontal ligament was well organized in five of six treated cases, both in the apical portion of the tooth root and on the lateral areas. Periodontal fibers were observed inserted in the newly formed bone and cementum in all these cases, and also on

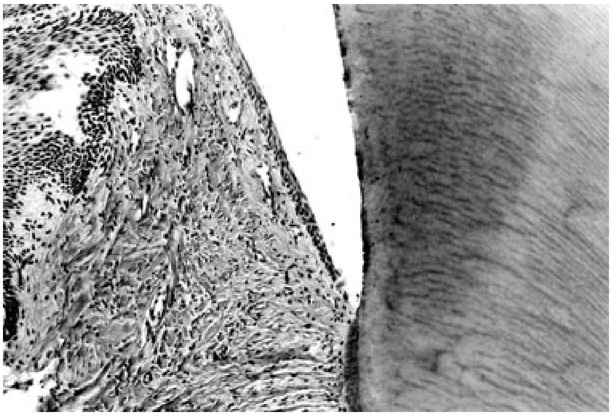

Fig. 1. Group I – $Ca(OH)_2$ – epithelial insertion at the cementoenamel junction. Absence of inflammatory infiltrate. Hematoxylin and eosin (HE) 100×.

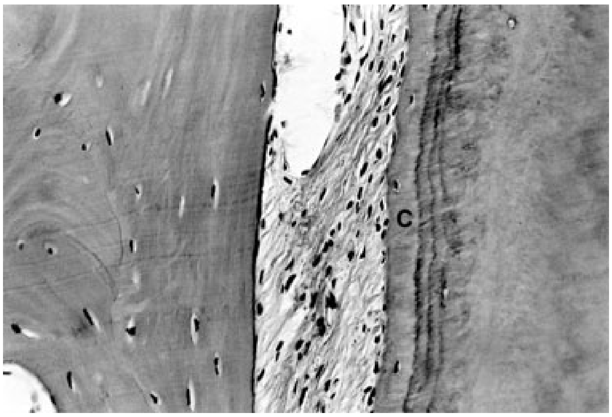

Fig. 2. Group I – $Ca(OH)_2$ – well-organized lateral periodontal ligament with fibers inserted in the bone tissue and newly formed cementum (C). HE 200×.

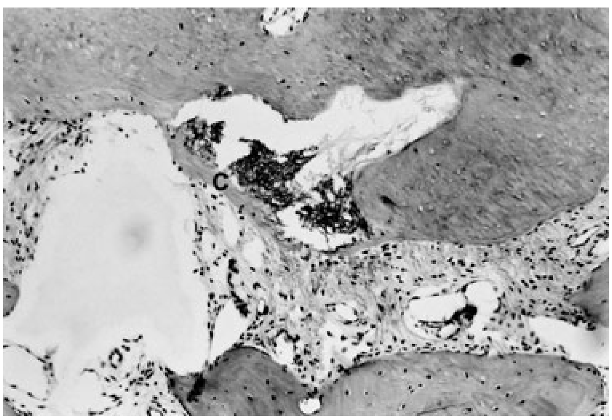

Fig. 3. Group I – $Ca(OH)_2$ – biological sealing (C – newly formed cementum) of the apical foramen of an apical lateral canal. HE 100×.

the lateral aspect of the tooth root (Fig. 2). In one specimen, the periodontal fibers were inserted in bone and cementum at only one-third of the periodontal ligament. Moreover, this case exhibited chronic inflammatory infiltrate close to the apical portion of the tooth root. Half of the specimens displayed biological sealing of some apical lateral canals with newly formed cementum (Fig. 3).

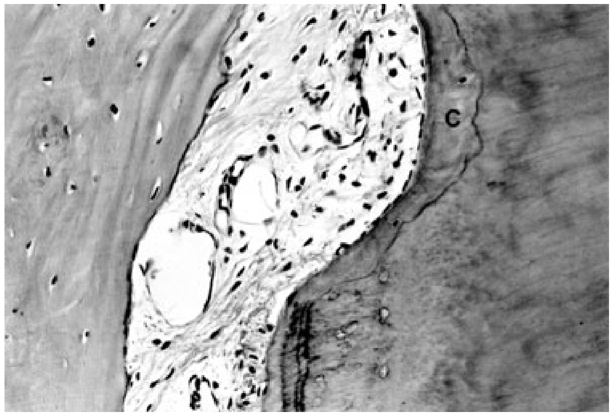

Fig. 4. Group I – $Ca(OH)_2$ – area of lateral resorption at the apical third of the root, with dentinal exposure, covered with newly formed cementum (C) and well-organized periodontal ligament. HE 200×.

Root resorptions were observed in all specimens. In five of six cases investigated, their extension was smaller than 1000 μm and the depth ranged from 100 to 200 μm. However, all cases were covered by newly formed cementum at the previously resorbed area (Fig. 4). There were no findings of bone resorption, except for one case with resorption area without clastic cells. There were no areas of ankylosis. The Brown and Brenn staining revealed presence of Gram-negative microorganisms inside

Table 1. Weighted means achieved for the study groups, based on scores assigned to the different histomorphological events considered

Histomorphological details	$Ca(OH)_2$	MTA
Epithelial insertion		
Area	1.16	1.0
Intensity of acute inflammatory infiltrate	1.0	1.0
Extension of acute inflammatory infiltrate	1.0	1.0
Intensity of chronic inflammatory infiltrate	1.50	1.16
Extension of chronic inflammatory infiltrate	1.33	1.16
Periodontal ligament		
Organization	1.33	1.16
Intensity of acute inflammatory infiltrate	1.0	1.0
Extension of acute inflammatory infiltrate	1.0	1.0
Intensity of chronic inflammatory infiltrate	1.16	1.50
Extension of chronic inflammatory infiltrate	1.16	1.16
Root resorption		
Active and inactive	1.0	1.16
Extension	2.16	2.33
Depth	2.50	2.16
Repair	1.0	1.0
Bone tissue		
Resorption	1.16	1.66
Ankylosis	1.0	1.0
Presence of bacteria	1.50	1.50
Global score – weighted mean	1.29	1.30

MTA, mineral trioxide aggregate.

some dentinal tubules. The scores assigned to the different histopathologic events are presented in Table 1.

Group II: MTA

Epithelial reinsertion occurred at the cementoenamel junction in all cases. A small number of chronic inflammatory cells were observed in only one case, at the lamina propria of the internal aspect of the epithelium (Fig. 5).

Except for one case in which the periodontal fibers were partly organized with the presence of intense chronic inflammatory infiltrate at the apical portion, the others presented complete organization with the absence of inflammatory cells (Fig. 6). The periodontal fibers were inserted in newly formed cementum and bone, both in the lateral (Fig. 6) and apical ligament (Fig. 7).

Half of the specimens displayed the presence of biological sealing of some apical lateral canals with newly formed cementum (Fig. 8). Some apical lateral canals were not penetrated by MTA, reaching or not the periodontal ligament. In one specimen, the MTA present in the periodontal ligament was being covered by newly formed cementum (Fig. 9).

Root resorptions were observed in four of six cases investigated. From these four cases, only one area exhibited inactive resorption with partial deposition of cementum. These specimens displayed root resorptions with extension ranging from 1200 to 3000 μm. The depth of resorptions ranged from 80 to 170 μm. Except for one specimen, the others presented resorption areas covered by newly formed cementum (Fig. 6). Concerning the bone tissue, two cases exhibited small areas of active resorption. There were no cases of ankylosis.

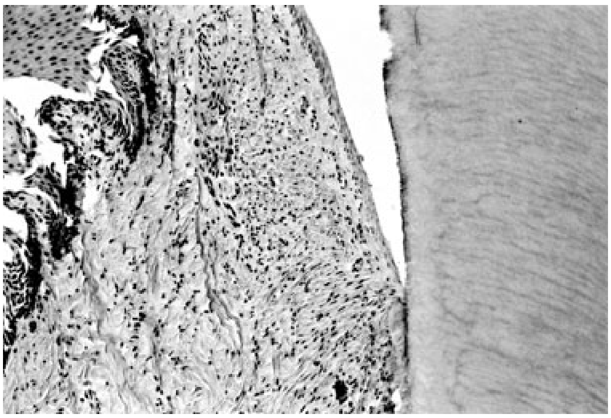

Fig. 5. Group II – MTA – epithelial insertion at the cementoenamel junction. Little chronic inflammatory infiltrate. HE 100×.

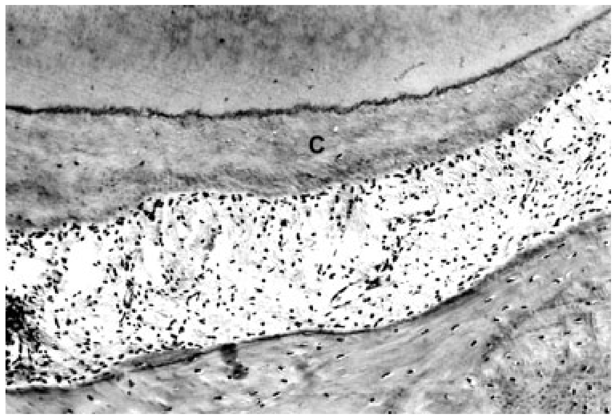

Fig. 7. Group II – MTA – well-organized apical periodontal ligament, absence of inflammatory cells and presence of newly formed cementum (C) and bone tissue. HE 100×.

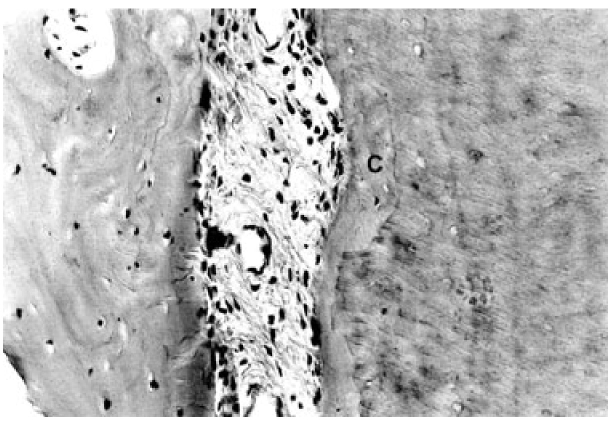

Fig. 6. Group II – MTA – fibers of the lateral periodontal ligament inserted in newly formed cementum (C) and bone tissue. Absence of inflammatory infiltrate. HE 200×.

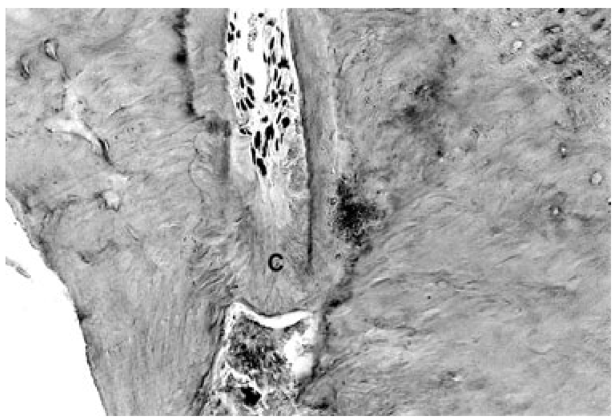

Fig. 8. Group II – MTA – biological sealing (C – newly formed cementum) of an apical lateral canal by a newly formed cementum bridge and vital remaining pulp. HE 200×.

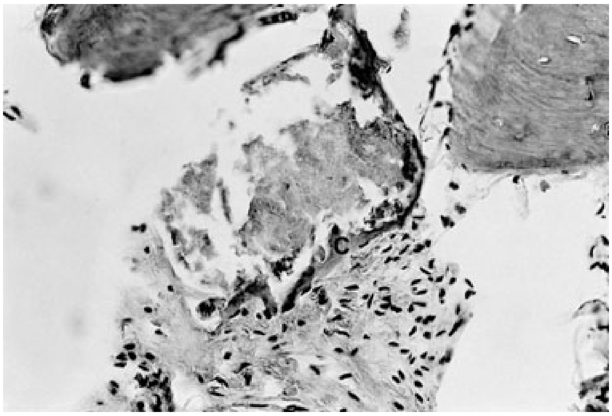

Fig. 9. Group II – MTA inside the apical periodontal ligament being surrounded by newly formed cementum (C). HE 200×.

The Brown and Brenn staining revealed the presence of Gram-negative microorganisms in only one specimen, which exhibited chronic inflammatory infiltrate in the periodontal ligament. The means of scores of the different histological events assigned to all specimens in both experimental groups are presented in Table 1. Statistical analysis by the Mann–Whitney test revealed similar outcomes between both study groups ($\alpha = 29.60\%$).

Discussion

Healing of a reimplanted tooth is a complex process that depends on the potential for reestablishment of different types of cells such as osteoblasts, cementoblasts and fibroblasts, and proportion of involvement of each (5). There is consensus in the literature that the success of tooth reimplantation depends on maintenance of vitality of the cementum periodontal ligament (1). This was evident in the present study, in which periodontal ligament cells were preserved, reattached and well organized, and inflammatory root resorption, replacement resorption or ankylosis were not observed. Two critical aspects for maintenance of this vitality are the extra-alveolar period and storage medium (1). According to the literature, the immediate replantation has to be performed up to 30 min after the occurrence of trauma and the tooth may be kept in a dry environment (1, 5). However, the extra-alveolar period may be extended if a humid environment is employed (6, 19).

From a practical standpoint, pasteurized milk and saline solution are considered satisfactory media (19). In this study, in which the teeth were kept in saline solution for 15 min before reimplantation, there was complete repair of the periodontal ligament, with absence of external root resorption.

Every time one tooth is avulsed, there is rupture of the vascular-nervous bundle and the pulp tissue is impaired. Although studies have assumed the possibility of pulp revascularization (20), this is not likely to occur in teeth with completely formed roots. Thus, following the protocol proposed by the International Association of Dental Traumatology, the tooth should be submitted to endodontic treatment and receive temporary filling with $Ca(OH)_2$ at 10 days after reimplantation (21).

Endodontic treatment is important and necessary, as there is a direct relationship between pulp necrosis and inflammatory root resorption in reimplanted teeth. Several studies have demonstrated that this process may be prevented or controlled by endodontic therapy with utilization of $Ca(OH)_2$ (7, 9).

Studies have been conducted on reimplanted monkey teeth using $Ca(OH)_2$ as a root canal dressing. Some authors reported that this material, due to its high pH, would cause damage to the cells in the periodontal ligament at the apical region, leading to replacement resorption. Thus, its application is recommended after 7–10 days, when repair of the ligament is more advanced (8, 22–24).

When endodontic treatment is initiated 7 days after reimplantation, the $Ca(OH)_2$ dressing may be replaced by definitive filling after 1 month (21); however, if treatment is initiated after establishment of inflammatory root resorption, it should be maintained for longer periods (9).

The success of $Ca(OH)_2$ as a root canal dressing is related to its anti-bacterial and calcifying action provided by hydroxyl and calcium ions, inactivating enzymes that act in the mechanism of nutrition of bacteria or directly on the plasmatic membrane and activating tissue enzymes as alkaline phosphatase, which influences mineralization (25).

The speed of ionic dissociation of $Ca(OH)_2$ may be related to the vehicle employed. As the hydrosoluble vehicles allow fast dissociation (10), they should be selected in cases of dental trauma. For that reason, in the present study, propylene glycol was employed as the $Ca(OH)_2$ vehicle in one study group.

The mechanism of action of $Ca(OH)_2$ is well known and has been described *in vitro* by Seux et al. (26) and *in vivo* by Holland et al. (27). When $Ca(OH)_2$ has direct contact with the tissues, there is dissociation into calcium and hydroxyl ions. The hydroxyl ions penetrate into the tissue, producing surface protein denaturation because of its high pH (14, 27). There is also penetration of calcium ions, which is combined with carbonic gas at the area between the denatured and alive tissue giving rise to calcium carbonate granules as calcite, birefringent to polarized light. Below these granulations and between them, there is also precipitation of small amorphous granules of calcium salts, characterizing an area of dystrophic calcification (26, 27).

Another material with similar properties as $Ca(OH)_2$ is MTA. The excellent outcomes of studies primarily related to tissue response, when employed for pulp capping, internal root resorption, endodontic treatment of teeth with incompletely formed roots and for root canal filling (11, 28, 29), served as a stimulus to investigate it in one of the present study groups, because of its biological properties.

The mechanism of action of MTA is similar to that of $Ca(OH)_2$, with formation of Von Kossa-positive granules, birefringent to polarized light, under which calcified tissue is deposited. In the case of MTA, the calcium ions originate from the $Ca(OH)_2$ formed after reaction of calcium oxide with the water present in the vehicle and connective tissue (14, 15, 29).

The results of the MTA group were similar to those of the $Ca(OH)_2$ group, histologically revealing the biological sealing of some apical lateral canals with newly formed cementum and absence of root resorption. Moreover, in the cases in which the material overflowed to the periapical region, it was partly covered with newly formed cementum. These findings corroborate studies that report the ability of MTA to stimulate the differentiation of new cementoblasts that will deposit matrix for cementogenesis (16, 30, 31). The specimens radiographed at 30, 60 and 90 days also revealed normality of the tooth supporting structures and absence of root resorption in both study groups.

Conclusion

The data obtained in the present study demonstrated that MTA provided similar results compared to the $Ca(OH)_2$ paste, which suggests that it is a viable option for root canal filling in cases of immediate reimplantation.

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