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Periapical tissue reactions to calcium hydroxide and MTA after external root resorption as a sequela of delayed tooth replantation

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Abstract – Clinical experience has shown that most avulsed teeth are replanted after a long extra-alveolar time and dry or inadequate wet storage, causing necrosis of periodontal ligament cells. This condition invariably leads to development of external root resorption, leaving the filling material in contact with the periapical connective tissues. In this study, the periapical tissue reactions to calcium hydroxide (CH) and mineral trioxide aggregate (MTA) were evaluated after occurrence of external root resorption as an expected sequela of delayed tooth replantation. Twenty male Wistar rats (*Rattus norvegicus, albinus*) had their right upper incisor extracted and maintained in dry storage for 60 min. Then, the dental papilla, enamel organ, pulp tissue, and periodontal ligament were removed, and the teeth were immersed in a 2% acidulated phosphate sodium fluoride solution, pH 5.5, for 10 min. The teeth were randomly assigned into two groups ($n = 10$), in which the canals were filled with either a CH and saline paste (CH group) or MTA (MTA group). The sockets were irrigated with saline, and the teeth were replanted. After 80 days, it was possible to observe large areas of replacement root resorption and some areas of inflammatory root resorption in both groups. More severe inflammatory tissue reaction was observed in contact with calcium hydroxide compared with the mineral trioxide aggregate. New bone formation was more intense at the bottom of the socket in the MTA group. In conclusion, as far as periapical tissue compatibility is concerned, intracanal MTA can be considered as a viable option for root canal filling in delayed tooth replantation, in which external root resorption is an expected sequela.

Avulsion of permanent teeth, while not very frequent, is the most serious of all dental injuries because of its esthetic and functional impacts to the patient. Despite its limitations, replantation should be the treatment of choice, because the period of permanence of the tooth in the dental arch may be sufficient to fulfill needs like preventing atrophy of the alveolar ridge, allowing the patient to have better acceptance of tooth loss, and even delaying the need of prosthesis (1–4).

When replantation is performed under conditions where the viability of the periodontal ligament (PDL) cells is maintained, repair may occur with PDL fiber reattachment. In most situations, however, the conditions are not so favorable, and the avulsed teeth are exposed to a dry medium for long periods, causing the death of the PDL cells, which may start pathological resorption processes that lead to tooth loss (5). Immediate replantation occurs in very few cases mainly because patients as well as health professionals do not know that avulsed teeth can be replanted or are not familiar with replantation procedures (6, 7).

The contamination of late replanted teeth can be controlled by the treatment of root surface and administration of systemic antibiotics. Root canal therapy is also recommended because there is a direct relationship between the occurrence of pulp necrosis and inflammatory root resorption in replanted teeth. Calcium hydroxide (CH) is by far the most widely used filling material in these cases (3, 8). The benefits of CH as a root filling material are derived from its ionic dissociation. The action of calcium and hydroxyl ions released from CH on the tissues and bacteria explain its biological and antimicrobial properties (9) as demonstrated both *in vitro* (10) and *in vivo* (11).

Mineral trioxide aggregate (MTA) has the same mechanism of action of CH. Although originally indicated for the repair of root and furcal perforations and retrofilling of root-end cavity preparations (12), MTA has also been used as a capping material in conservative pulp therapies and a root canal filing material (13–15). In addition to having antimicrobial activity, MTA can also induce bone regeneration and accelerate the repair of bone defects by stimulation of osteoblast differentiation (16, 17). This action can be interesting for cases of external root resorption, as the filing material could contribute to the repair of areas of bone resorption, creating more favorable local conditions for future rehabilitation with osseointegrated implants.

Clinical experience has shown that most avulsed teeth are replanted after a long extra-alveolar time and dry or inadequate wet storage, causing necrosis of periodontal ligament cells. This condition invariably leads to the development of external root resorption, leaving the filling material in contact with the periapical connective tissues. In this study using a rat tooth model, the periapical tissue reactions to CH and MTA were evaluated after occurrence of external root resorption as an expected sequela of delayed tooth replantation.

Material and methods

The research protocol was approved by the Animal Care and Use Committee of the Araçatuba Dental School, UNESP (protocol number 2008-003266). All guidelines regarding the care of animal research subjects were strictly followed.

Twenty adult male Wistar rats (*Rattus norvegicus, albinus*) weighing between 150 and 250 g were selected for the study. The animals were housed in plastic cages under climate-controlled conditions (12 h light/12 h dark; thermostatically regulated room temperature) and were fed a standard solid chow (Ração Ativada Produtor; Anderson & Clayton S.A. Indústria e Comércio, São Paulo, SP, Brazil) and water *ad libitum*, except for the 12 preoperative hours.

The surgical procedures were performed under general anesthesia. The animals received an intramuscular injection of xylazine hydrochloride (Dopaser; Laboratório Calier do Brasil Ltda., Osasco, SP, Brazil; 0.03 ml per 100 g body weight) to attain muscular relaxation and were anesthetized with ketamine hydrochloride (Dopalen; AgriBrands do Brasil Ltda., Paulínea, SP, Brazil; 0.07 ml per 100 g body weight). Asepsis of the anterior maxilla was performed with 1% iodine polyvinylpyrrolidone (Riodeine; Indústria Farmacêutica Rioquímica Ltda., São José do Rio Preto, SP, Brazil) followed by non-traumatic extraction of the maxillary right incisor of all animals. The teeth were held by their crowns, fixed on a red wax plate, and kept dry at room temperature for 60 min. Thereafter, the dental papilla and the enamel organ of each tooth were removed with a no. 15 scalpel blade (Embramac Exp. e Imp., Campinas, SP, Brazil), and the pulp tissue was extirpated through a retrograde via with a slightly curved no. 35 Hedström file (25 mm; Sybron Kerr Corporation, Orange, CA, USA). Root canals were irrigated with saline (Ariston Ind. Quím. e Farm. Ltda., São Paulo, SP, Brazil) followed by aspiration with a 25×6 needle couple to a disposable syringe.

The root surface-adhered PDL was removed mechanically with a no. 15 scalpel blade (Embramac Exp. E Imp. São Paulo, SP, Brazil), and the teeth were immersed in 20 ml of a 2% acidulated-phosphate sodium fluoride solution, pH 5.5 (0.1 M phosphoric acid, pH 2.0, diluted in 2% sodium fluoride solution, pH 8.0; Apothicário Farmácia de Manipulação, Araçatuba, SP, Brazil) for 10 min. After root surface treatment, fluid aspiration was performed, and the root canals were dried with absorbent paper points (Dentsply Ind. e Com. Ltda., Petrópolis, RJ, Brazil). The teeth were randomly assigned into two groups: in the CH group ($n = 10$), canals were filled with CH and saline paste using a no. 40 lentulo spiral (Dentsply Ind. e Com. Ltda.) at low speed; in the MTA group ($n = 10$), canals were filled with white MTA (Angelus, Londrina, PR, Brazil) using a no. 40 lentulo spiral (Dentsply Ind. e Com. Ltda) at low speed.

The sockets were gently irrigated with saline, and the teeth were replanted. All animals received a single intramuscular dose of benzathine G penicillin 20 000 IU (Fort Dodge; Animal Health Ltda., Campinas, SP, Brazil).

Eighty days after replantation, the rats were sacrificed by anesthetic overdose. The anatomic specimens containing the replanted teeth were removed, fixed in 10% formalin for 24 h, and decalcified in a 4.13% EDTA solution, pH 7.0. After decalcification, the specimens were embedded in paraffin and longitudinal 6-μm-thick sections were obtained and stained with hematoxylin and eosin for histomorphometric analysis under optical microscopy.

Analysis of the outcomes was performed by one of the authors in a blind fashion, according to the 16 histomorphometric parameters listed later, which received scores 1–4, 1 being the best result and 4 the worst, with intermediate outcomes for scores 2 and 3 (14, 18). This list of histomorphometric parameters can be used to immediate and delayed tooth replantation studies, and therefore some of the attributed scores apply only to one or another condition.

Area of epithelial attachment.

1 Cemento-enamel junction.
2 Ligament below the cemento-enamel junction.
3 Much below the cemento-enamel junction (near the middle third).
4 Absence of epithelial attachment.

Acute and chronic inflammatory processes close to the area of epithelial attachment.

Intensity of inflammatory process based on the criteria described by Wolfson and Seltzer (19).

1 Absence or occasional presence of inflammatory cells.
2 Small number of inflammatory cells (≤10 cells per field with 400× magnification).
3 Moderate number of inflammatory cells (11–50 cells per field with 400×·magnification).

4 Large number of inflammatory cells (> 50 cells per field with 400× magnification).

Extension of the inflammatory process.

1 Absence or occasional presence of inflammatory cells.
2 Inflammatory process restricted to the lamina propria of the internal aspect of the epithelium.
3 Inflammatory process extending apically toward the small portion of connective tissue underlying the lamina propria of the internal aspect of the gingival epithelium.
4 Inflammatory process reaching the area near the alveolar bone crest.

PDL.

PDL organization.

1 PDL fibers inserted in bone and cementum throughout the ligament extension.
2 PDL fibers inserted in bone and cementum at two-thirds of the ligament extension.
3 PDL fibers inserted in the bone and cementum at one-third of the ligament extension.
4 Absence of PDL fibers with insertion in bone and cementum.

Intensity and extension of acute and chronic inflammatory processses in the PDL.

Intensity – same criteria used for the area of epithelial insertion.

Extension of the inflammatory process.

1 Absence or occasional presence of inflammatory cells.
2 Inflammatory process present only in the apical or coronal PDL, or in a small area of the lateral PDL.
3 Inflammatory process reaching more than half of the lateral PDL.
4 Inflammatory process in the entire PDL extension.

Tooth root.

Active and inactive root resorption.

1 Absence of root resorption or repaired resorptions.
2 Areas of inactive resorption (absence of clastic cells).
3 Small areas of active resorption.
4 Extensive areas of active resorption.

Extension of root resorption – the diameter of the resorbed areas was measured (in micrometers) in representative sections. The mean of the values obtained on the buccal and lingual aspects allowed assignment of the following scores:

1 Absence of resorption.
2 Mean extension of 1–1000 μm.
3 Mean extension of 1001–5000 μm.
4 Mean extension larger than 5001 μm.

Depth of root resorption – the depth of the resorption areas was measured (in micrometers) in representative sections. The mean of the values obtained on the buccal and lingual aspects allowed assignment of the following scores:

1 Absence of resorption.
2 Mean depth of 1–100 μm.
3 Mean depth of 101–200 μm.
4 Mean depth larger than 201 μm.

Repair of root resorption areas.

1 Absence of resorption or deposition of newly formed cementum throughout the extension of the resorbed areas.
2 Deposition of newly formed cementum in half or more of the extension of the resorbed areas.
3 Deposition of newly formed cementum in less than half of the resorbed areas.
4 Absence of deposition of newly formed cementum in the resorbed areas.

Bone tissue – areas of active and inactive resorption.

1 Absence of resorption areas.
2 Presence of inactive resorption areas (absence of clastic cells).
3 Presence of small areas of active resorption.
4 Presence of extensive areas of active resorption.

Ankylosis.

1 Absence of ankylosis.
2 Small areas of ankylosis.
3 One-third of the root with ankylosis.
4 More than one-third of the root with ankylosis.

Data were submitted to statistical analysis by the Mann–Whitney *U*-test to analyze the differences found in each parameter and the characteristics in each group. The significance level was set at 5%.

Results

CH group

In all specimens, epithelial attachment occurred at the cemento-enamel junction, with presence of fibroblasts and few inflammatory cells in the adjacent connective tissue. The PDL space was filled by fibrous connective tissue, with fibers arranged parallel to the root surface and some areas filled by bone tissue (Fig. 1). In all specimens, areas of active cemental and dentin resorption were replaced by bone, and few areas of ankylosis were also observed. Two specimens exhibited areas of inflammatory root resorption in the cervical and apical thirds. All specimens presented an acute inflammatory infiltrate in the apical region in contact with the filling material (Fig. 2). A less intense inflammatory infiltrate was observed in the connective tissue close to areas of calcification (Fig. 3). Only few areas of new bone formation were observed at the bottom of the socket (Fig. 4). The scores assigned to the different histopathologic events are presented in Table 1.

MTA group

Epithelial attachment occurred at the cemento-enamel junction in all cases. Only few inflammatory cells were observed in the adjacent connective tissue. In some specimens, the PDL space was diminished and filled by connective tissue, with the fibers arranged parallel to the root surface and absence of inflammatory cells. In the other specimens, the PDL space was completely filled by bone tissue. In all cases, areas of active cemental and dentin resorption were seen along the three root thirds. In some areas, the cementum was in direct contact with the bone tissue, characterizing the occurrence of ankylosis (Fig. 5). Dentin was replaced by bone tissue in several areas of the root. In the apical region, most specimens exhibited connective tissue with few inflammatory cells close to the MTA particles (Fig. 6). Con-

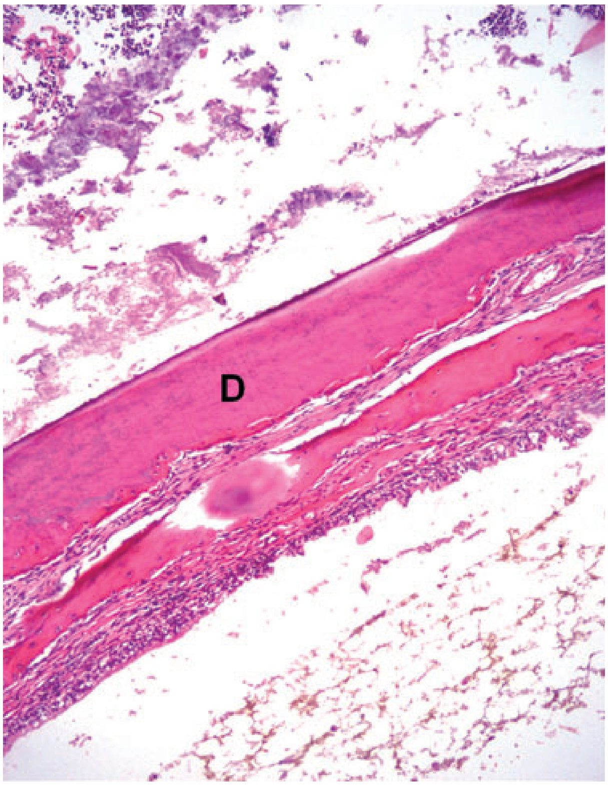

Fig. 1. CH group. Fibrous connective tissue with the fibers arranged parallel to the root surface. Dentin (D). HE. Original magnification 160×.

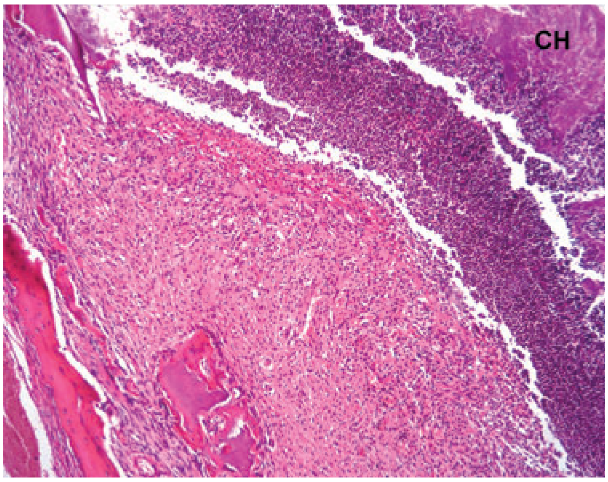

Fig. 2. CH group. Presence of an acute inflammatory infiltrate in the apical foramen region in contact with the filling material. Calcium hydroxide (CH). HE. Original magnification 100×.

nective tissue with chronic inflammatory infiltrate in contact with the filling material was observed in only few cases. The PDL space was narrowed or completely occupied by bone tissue (Fig. 7). The scores of the different histologic events assigned to all specimens in both experimental groups are presented in Table 1. Statistical analysis using the nonparametric Mann–

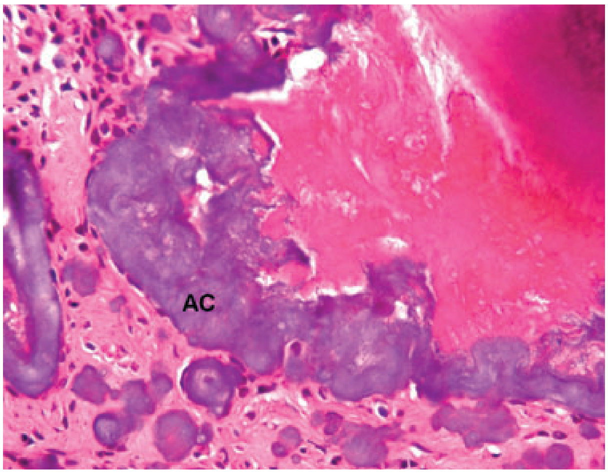

Fig. 3. CH group. Areas of calcification (AC) induced by the calcium hydroxide. HE. Original magnification 400×.

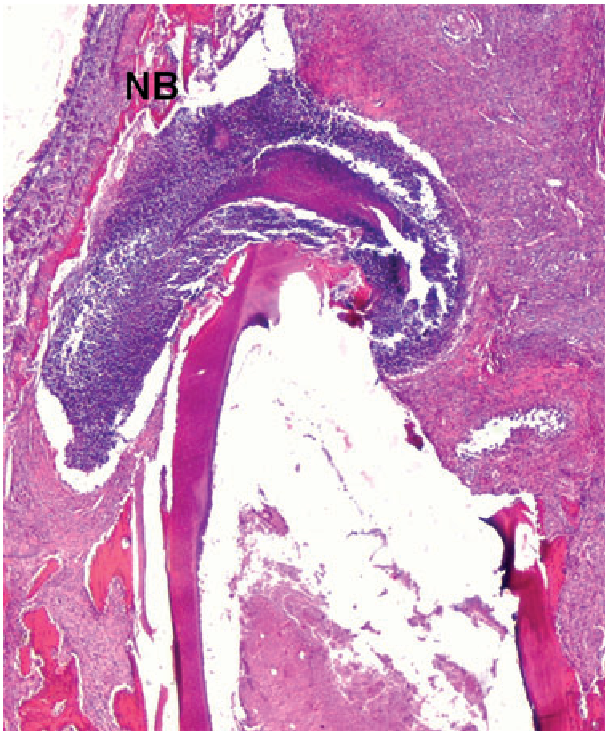

Fig. 4. CH group. Discrete new bone formation (NB) at the bottom of the socket. HE. Original magnification 25×.

Whitney test showed no statistically significant difference ($P > 0.05$) between the two groups.

Discussion

In cases of dental avulsion, the pulp and the PDL suffer extensive damage, and the repair of replanted teeth depends on a combination of factors including the extra-alveolar time and the storage conditions (3, 20, 21). Development of ankylosis and replacement root resorption are expected when the PDL is necrotic (1–3, 5, 21).

Table 1. Histomorphological details scores related to the groups

Histomorphological details	Ca(OH)$_2$				MTA			
Score	1	2	3	4	1	2	3	4
Epithelial insertion								
Area	10/10	0/10	0/10	0/10	10/10	0/10	0/10	0/10
Intensity of acute inflammatory infiltrate	1/10	9/10	0/10	0/10	7/10	0/10	3/10	0/10
Extension of acute inflammatory infiltrate	4/10	5/10	1/10	0/10	7/10	0/10	3/10	0/10
Intensity of chronic inflammatory infiltrate	1/10	9/10	0/10	0/10	0/10	10/10	0/10	0/10
Extension of chronic inflammatory infiltrate	0/10	10/10	0/10	0/10	0/10	10/10	0/10	0/10
Periodontal ligament								
Organization	0/10	0/10	0/10	10/10	0/10	0/10	0/10	10/10
Intensity of acute inflammatory infiltrate	0/10	1/10	9/10	0/10	0/10	4/10	6/10	0/10
Extension of acute inflammatory infiltrate	0/10	0/10	10/10	0/10	0/10	1/10	9/10	0/10
Intensity of chronic inflammatory infiltrate	0/10	4/10	3/10	3/10	0/10	3/10	7/10	0/10
Extension of chronic inflammatory infiltrate	0/10	0/10	10/10	0/10	1/10	9/10	0/10	0/10
Active root resorption	0/10	0/10	0/10	10/10	0/10	0/10	0/10	10/10
Extension of root resorption	0/10	0/10	0/10	10/10	0/10	0/10	0/10	10/10
Depth of root resorption	0/10	0/10	0/10	10/10	0/10	0/10	0/10	10/10
Repair of root resorption	0/10	0/10	0/10	10/10	0/10	0/10	0/10	10/10
Bone tissue	0/10	0/10	10/10	0/10	0/10	0/10	10/10	0/10

MTA, mineral trioxide aggregate.

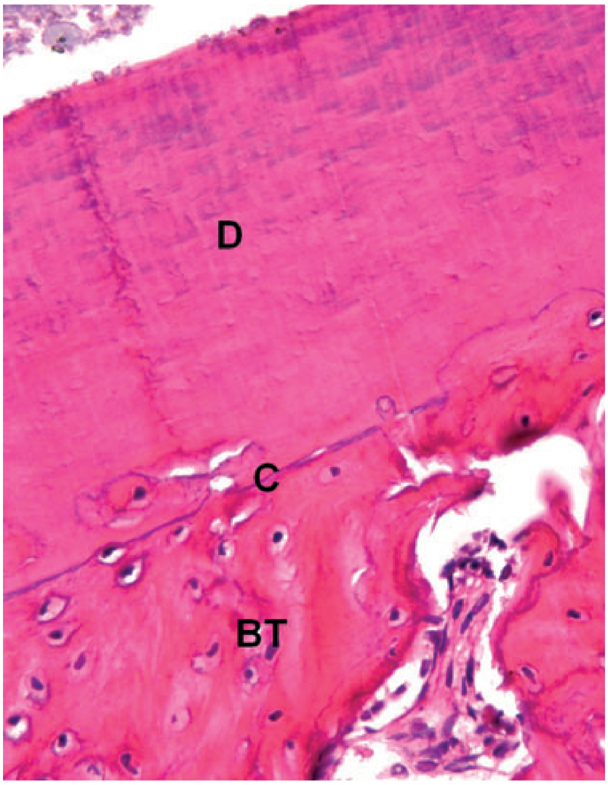

Fig. 5. MTA group. Ankylosed area. Dentin (D); bone tissue (BT); cementum (C). HE. Original magnification 400×.

It is, thus, important to use biocompatible root canal filling materials, as they will be in contact with the periapical tissues as a result of the resorption.

According to the International Association of Dental Traumatology's guidelines for the management of avulsed permanent teeth with extraoral dry time longer than 60 min, root canal treatment can be performed

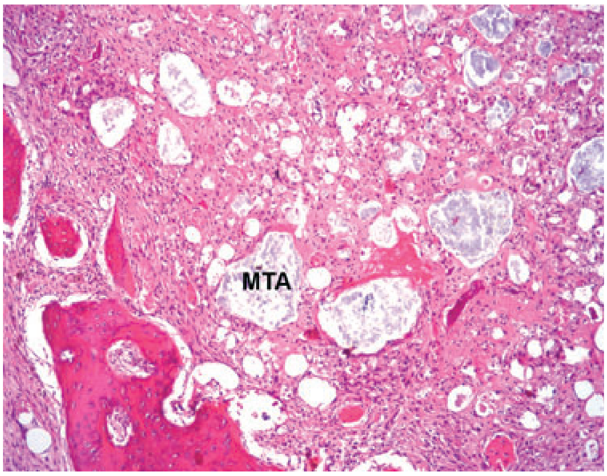

Fig. 6. MTA group. Connective tissue with few inflammatory cells surrounding the MTA (MTA). HE. Original magnification 100×.

extraorally prior to replantation, or it can be performed 7–10 days after replantation (1). The root canal should be filled with a CH dressing (1, 8, 22).

The mechanism of action of CH is well known and has been described *in vitro* (10) and *in vivo* (11). It has been demonstrated that CH activates tissue enzymes, such as alkaline phosphatase, which act on the mineralization of bone and cementum, the dentin tubules, and root resorption (8, 9). A previous study has tested an oily CH suspension as an adjunct to guided bone regeneration and found that this material is a poor candidate for such purpose, as it induced less new bone formation than the control and may hamper bone healing (23). In the present study, the amount of newly formed bone at the bottom of the socket was smaller in the CH group.

The specimens filled with CH paste exhibited an intense inflammatory infiltrate in the regions of the tissue

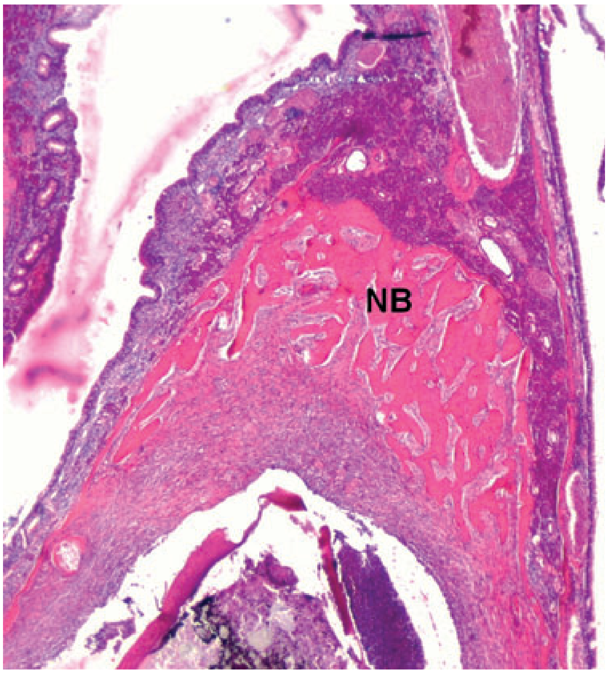

Fig. 7. MTA group. New bone formation (NB) at the bottom of the socket. HE. Original magnification 25×.

that remained in direct contact with the material after root resorption. This can be explained by the alkalinity of CH, as the high pH of this material causes protein denaturation, producing a local inflammatory reaction. On the other hand, mild or no inflammation was observed around the areas of mineralization induced by the CH paste, showing that calcite deposition promotes a favorable condition for mineralized tissue deposition (8, 9, 11).

Another probable explanation for the presence of an intense inflammatory infiltrate in the tissues in contact with the material is that the efficacy of CH pastes starts lowering 10 days after placement. As no changes in CH paste were carried out, it may be suggested that the decrease in pH on dentin walls after solubilization of the paste in contact with the periapical tissue fluids might have contributed to reduce the efficiency in controlling inflammatory resorption (8, 9).

Despite its excellent biological properties, CH has some disadvantages such as long treatment duration (8), need of changes (8), and weakening of the dental structure after prolonged intracanal therapy (24, 25). This latter effect could be attributed to the strong alkalinity of CH, as it may neutralize, dissolve, or denature dentin organic matrix components that act as coupling agents between the hydroxyapatite crystals and collagen fibers, leading to a collapse of dentin structure (25). In this aspect, MTA can provide better results than CH, because it permits restoring the tooth in the same session, thus reducing the treatment time and preventing tooth fracture and coronal leakage (24–26). This material also prevents changes in the mechanical properties of dentin (24, 26, 27) and presents good biocompatibility (12, 14, 15, 27).

MTA and CH have similar mechanisms of action. Calcium ions released from both materials react with the carbon dioxide present in the connective tissue, originating calcium carbonate. This compound precipitates in the form of calcite crystals that act as calcification nuclei, promoting deposition of mineralized tissue as well as antimicrobial activity (27).

According to Torabinejad et al. (28), most of the MTA molecules are calcium and phosphate ions. These ions are also present in the dental tissues, which gives an excellent biocompatibility to this material. Regarding toxicity, scanning electron microscopic analysis revealed that osteoblasts were adhered and spread over the surface (29) and that the material stimulated the production of osteocalcin and mineralized matrix by cementoblasts (30). In the present study, new bone formation at the bottom of the socket was more intense in the MTA group.

The biocompatibility of MTA has been demonstrated in direct contact with cell cultures. The findings of different studies have shown that MTA does not cause decrease in cell growth (31), increases interleukin-8 concentration, which is a growth factor that stimulates angiogenesis (32), does not directly increase the production and release of prostaglandin E2 (34), and does not cause cell death (36). These events are important for connective tissue repair. Therefore, the success of MTA applications, as reported in several *in vivo* studies, corroborates its biocompatibility and clinical viability (14, 15, 17, 26, 27, 34).

Panzarini et al. (18) investigated the action of MTA as a root canal filling material for the immediate and delayed replantation of monkey teeth. The authors found that MTA presented similar results to CH paste in preventing the occurrence of inflammatory root resorption, thus providing good repair and allowing apical closure by deposition of mineralized tissue in some specimens.

The study showed that MTA in direct contact with the tissues promotes less intense inflammatory reaction and greater new bone formation at the bottom of the socket in comparison with CH. These results are similar to those of other authors who evaluated the periapical tissue response to MTA and observed a small number of inflammatory cells and fibrous capsule formation on the teeth treated with this material (27, 35). The properties of the root canal filling materials used in delayed tooth replantation are very important, as the occurrence of external root resorption in these cases may result in exposure of the intracanal medications to the periapical region. Once in contact with the periapical tissues, these materials may have a direct influence on the repair of resorbed areas, affecting new bone formation and consequently the future rehabilitation, especially when the use of osseointegrated implants is considered. The resorption rate of MTA is slower than that of CH paste. Although it is not yet known how long this process takes, the present study showed that a great amount of MTA had not been resorbed 80 days after placement, but the material still remained biocompatible after this time.

Despite its excellent biological properties, the use of MTA as a filling material may have some disadvantages that should be considered. The physical properties of MTA result in difficult handling and even can make it impossible to remove the material if endodontic retreatment is needed (14, 36). Tooth crown discoloration even with use of white MTA attributed to a possible chemical reaction might also occur (35–37), and the higher cost of MTA compared with CH is another factor to be considered.

Because of its biocompatibility with the periapical tissues, MTA can be considered as a viable clinical option for root canal filling in delayed tooth replantation, in which external root resorption is more likely to occur.

In conclusion, as far as periapical tissue compatibility is concerned, the use of MTA as root canal filling material can be considered as a viable option for late replanted teeth in which external root resorption is expected sequela after replantation.

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