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Effect of root surface treatment with propolis and fluoride in delayed tooth replantation in rats

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Abstract – Replantation is an acceptable option for treatment of an avulsed permanent tooth. Nevertheless, an extended extraoral period damages the periodontal ligament and results in external root resorption. The purpose of this study was to assess by histologic and histometric analysis, the influence of propolis 15% (natural resinous substance collected by *Apis mellifera* bees from various plants) and the fluoride solution used as root surface treatment on the healing process after delayed tooth replantation. Thirty Wistar (*Rattus norvegicus albinus*) rats were submitted to extraction of their upper right incisor. The teeth were maintained in a dry environment for 60 min. After this, the pulp was extirpated and the papilla, enamel organ and periodontal ligament were removed with scalpel. The teeth were divided into three experimental groups: Group I – teeth immersed in 20 ml of physiologic saline; Group II – teeth immersed in 20 ml of 2% acidulated phosphate sodium fluoride; Group III – teeth immersed in 20 ml of 15% propolis. After 10 min of immersion in the solutions, the root canals were dried and filled with calcium hydroxide paste and the teeth were replanted. The animals were euthanized 60 days after replantation. The results showed that similar external root resorption was seen in the propolis and fluoride groups. Teeth treated with physiologic saline tended to have more inflammatory root resorption compared with those treated with fluoride or propolis. However, the comparative analysis did not reveal statistically significant differences ($P > 0.05$) between the treatment modalities when used for delayed tooth replantation.

An acceptable treatment option in case of dental avulsion is the immediate replantation of the tooth into its socket to preserve the vitality of the structures adhered to the root surface. Periodontal ligament (PDL) healing by reattachment of its fibers to the root surface occurs only when cell viability is maintained (1).

After replantation, the alveolar portion of the PDL is separated from the cemental portion by blood clot. If the avulsed tooth is replanted with vital cemental PDL remnants, the blood clot may organize as a granulation tissue that may regenerate under ideal conditions of immediate replantation (2, 3). In most situations, however, exarticulated teeth are replanted under unfavorable conditions after being exposed to a dry medium for long periods. As a result, the PDL may undergo cell necrosis and, in such cases, the granulation tissue may be replaced by bone tissue and start a resorption process (2).

It is well known that the cementum is a structure resistant to resorption and the loss of cementoblasts secondary to trauma is a predisposing condition to root resorption; this is the most frequent cause of failure of tooth replantation. This justifies the need for root surface treatment, in cases of delayed replantation, to eliminate necrotic tissue remnants so as not to alter the outcomes of the replanted teeth (1).

Different substances have been used for root surface treatment, namely: acetazolamide (4), citric acid (5), hydrochloric acid (6), Emdogain (7), enzymes (8), fluorides (9, 10), calcium hydroxide (11), sodium hypochlorite (9–13), rifocin (14), tetracycline (15), adenosine triphosphate (16), C vitamin (9) and alendronate (17). None of them, however, has proven effective in increasing the survival of avulsed teeth submitted to delayed replantation.

The use of fluoride has been suggested to increase the resistance of root surface to resorption. Fluoride is likely to act on cementum and dentin, converting hydroxyapatite into fluorapatite, which is more resistant to resorption, or even inhibiting clastic cell formation (10).

Propolis is a resinous hive substance produced by honeybees from products collected from plants. It is known to possess valuable antimicrobial, antiviral, fungicidal, local anesthetic, antiulcer, immunostimulating, hypotensive and cytostatic properties (18), and may also be used for root surface treatment in cases of delayed tooth replantation.

In dentistry, the use of propolis has been proposed in different areas including cariology (18, 19), oral surgery (20, 21), endodontics (22, 23), oral pathology (24) and periodontology (25, 26). In dental traumatology, there is only one study that evaluated *in vitro* the use of propolis as a storage medium for avulsed teeth (27). The results of this study were considered favorable because propolis had a significantly higher capacity to preserve PDL cell vitality after tooth avulsion than Hank's balanced solution, milk and saline.

Given the broad-ranging action of this substance and the lack of similar investigations, the purpose of this study was to compare the efficacy of a 15% propolis solution and a 2% acidulated-phosphate sodium fluoride solution for root surface treatment in delayed replantation of rat teeth.

Material and methods

The research proposal was reviewed by the Ethics in Animal Research Committee of the School of Dentistry Araçatuba (São Paulo State University, Brazil) and the study design was approved.

Thirty male Wistar rats (*Rattus norvegicus, albinus*) weighing 250–300 g were used. The animals were fed ground solid ration (Ração Ativada Produtor, Anderson & Clayton S.A., São Paulo, SP, Brazil) and water *ad libitum*, except for the preoperative 12 h. The animals received an intramuscular injection of xilazine chlorhydrate (Anasedan; AgriBrands do Brasil Ltda., Jacareí, SP, Brasil; 0.03 ml per 100 g body weight) for muscular relaxation and were anesthetized with ketamine chlorhydrate (Dopalen; AgriBrands do Brasil Ltda.; 0.07 ml per 100 g body weight). Asepsis of the anterior maxilla was performed 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 #15 scalpel blade (Embramac Exp. e Imp., Campinas, SP, Brazil) and the pulp tissue was extirpated through a retrograde via with a slightly curved #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. The teeth had their root surface gently scraped with a #15 scalpel blade (Embramac Exp. e Imp.) to remove PDL remnants mechanically.

Thereafter, the teeth were randomly assigned to three groups ($n = 10$), according to the root surface treatment protocols accomplished before replantation: Group I = immersion in 20 ml of saline (Ariston Ind. Quím. e Farm. Ltda) for 10 min; Group II = immersion 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; Group III = immersion in 20 ml of a 15% propolis and propyleneglycol solution (Apothicário Farmácia de Manipulação) 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) and filled with a calcium hydroxide-based paste (5 ml propyleneglycol, 5 g calcium hydroxide, 2 g zinc oxide and 0.015 g colophony; Discipline of Endodontics, School of Dentistry of Araçatuba) injected in a retrograde way.

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).

Sixty days after replantation, the rats were euthanized by anesthetic overdose. The anatomic pieces 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 histologic and histometric analysis under optical microscopy. Only the lingual surface of the roots was examined because in rats the PDL fibers attach only to this region of the root.

For histometric analysis, eight sections were analyzed from eight different slides of each experimental group. Root surface extension on the slide was divided into thirds. The middle third was chosen for evaluation because the cervical and apical portions were damaged because of the action of the forceps and scalpel blade for tooth extraction and removal of dental papilla. Images were captured with an optical microscope with a Leitz Aristoplan ×40 objective lens (Leica Microsystems, Bensheim, Germany) coupled to a video camera (JVC TK-1270 Color Video Camera, Tokyo, Japan) connected to microcomputer. Microsoft VidCap video capture software (Microsoft Corp., Redmond, WA, USA) was used. Final images were analyzed using ImageLab® image-analysis software (Diracom Bio Informática, Vargem Grande do Sul, SP, Brazil) for measurement of the resorption areas and ankylosis perimeter.

Absolute numerical values were converted into percentages and classified according to the following inflammatory and replacement resorption scores: 1 = no resorption; 2 = 0.1% to 50% of the area with resorption; 3 = 51% to 99% of the area with resorption; 4 = 100% of the area with resorption. Means and standard deviations were calculated for each type of resorption and each root surface treatment. Data were analyzed statistically by Kruskal–Wallis test at 5% significance level.

Results

During the course of the study, two animals per group died, which reduced the sample size to 24 ($n = 8$).

The results were described after qualitative analysis of the following structures 60 days after tooth replantation: gingival mucosa, PDL, cementum, dentin, alveolar bone wall and bottom of the socket.

Group I (Saline)

The gingival mucosa epithelium was close to the cementoenamel surface, presenting fibroblasts and few lymphocytes. The PDL space was filled by a fibrous connective tissue, with fibers disposed parallel to the root surface (Fig. 1), and by bone tissue in few areas (Fig. 2). Cementum and dentin presented replacement resorption areas along all three root thirds in three specimens (Fig. 2). Inflammatory resorption areas with several lymphocytes were predominantly observed in the specimens of this group (Fig. 3). Areas in which the bone tissue was in direct contact with the cementum were rarely observed (Fig. 4). The alveolar wall exhibited bone apposition, causing narrowing and/or filling of the PDL space. Newly formed bone trabeculae were observed at the bottom of the socket.

Group II (2% acidulated-phosphate sodium fluoride)

The gingival mucosa epithelium was close to the cementum surface, below the cementoenamel junction. The underlying tissue exhibited fibroblasts and few lymphocytes. In some root areas, the PDL space was filled by connective tissue with fibers arranged parallel to the root surface, while in other areas it was filled by newly formed bone (Fig. 5). Cementum and dentin were intact in few areas of the root surface. In most specimens, active replacement resorption was noticed on all alveolar thirds (Fig. 6) and there were a few spots where the cementum was in direct contact with the bone tissue (Fig. 7). In

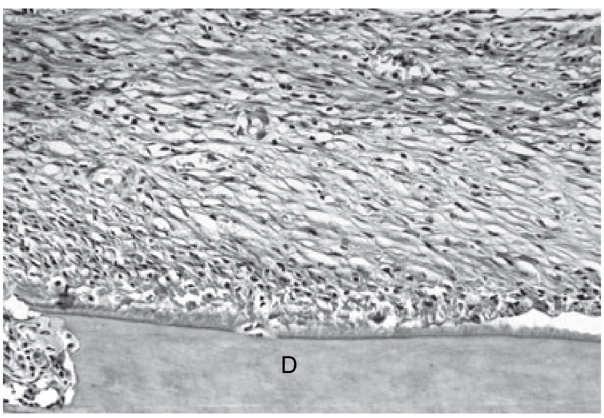

Fig. 1. Group I (saline). Fibrous connective tissue filling the periodontal ligament space, with fibers disposed parallel to the root surface. Dentin (D). H&E. Original magnification: ×160.

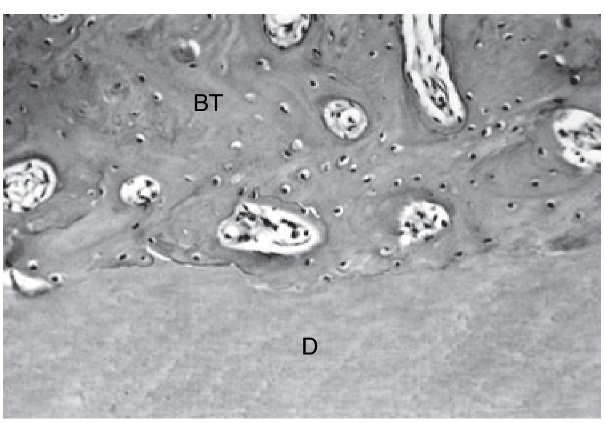

Fig. 2. Group I (saline). Replacement root resorption. Dentin (D); Bone tissue (BT). H&E. Original magnification: ×160.

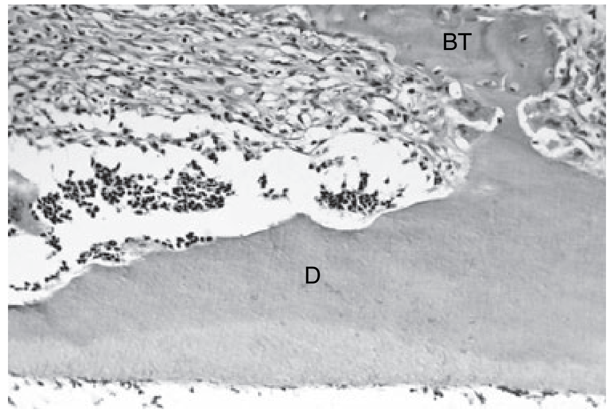

Fig. 3. Group I (saline). Root resorption with the presence of inflammatory cells. Dentin (D); Bone tissue (BT). H&E. Original magnification: ×160.

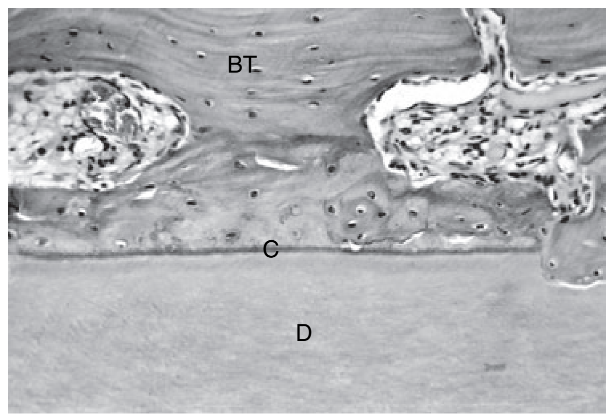

Fig. 4. Group I (saline). Ankylosis area. Dentin (D); Bone tissue (BT); cementum (C). H&E. Original magnification: ×160.

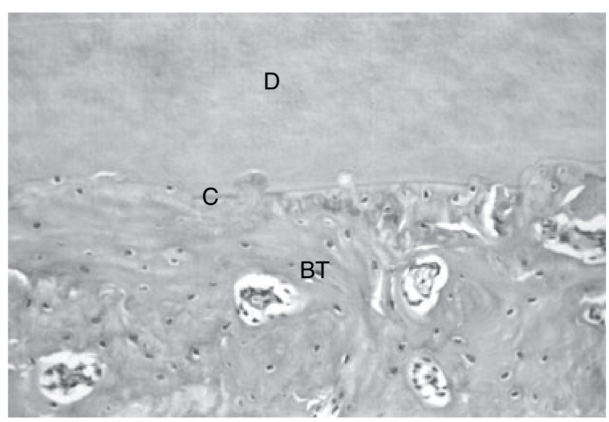

Fig. 5. Group II (fluoride). Periodontal ligament space filled by newly formed bone tissue (BT), showing areas of direct contact with the cementum (C). Dentin (D). H&E. Original magnification: ×160.

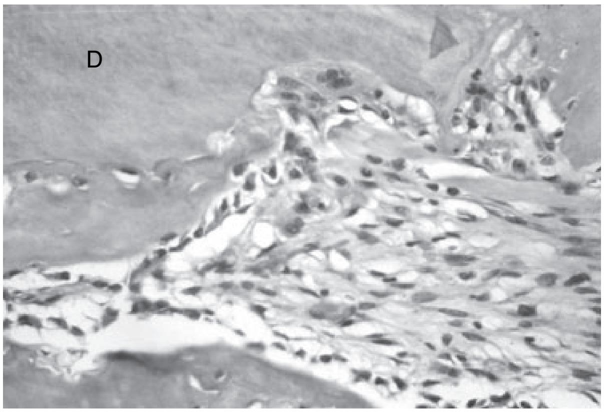

Fig. 6. Group II (fluoride). Active replacement resorption areas, with presence of multinuclear cells in the middle third. Dentin (D). H&E. Original magnification: ×250.

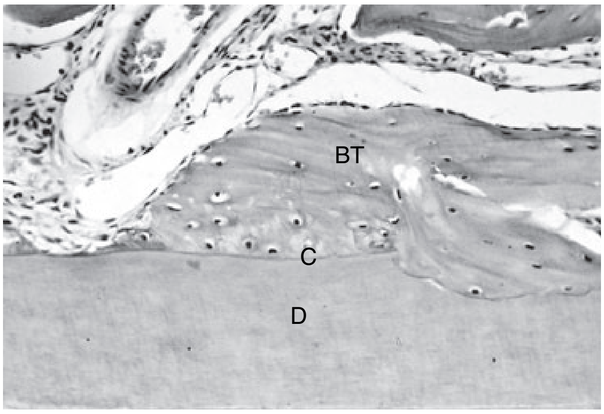

Fig. 7. Group II (fluoride). Ankylosis area. Dentin (D); Bone tissue (BT); cementum (C). H&E. Original magnification: ×160.

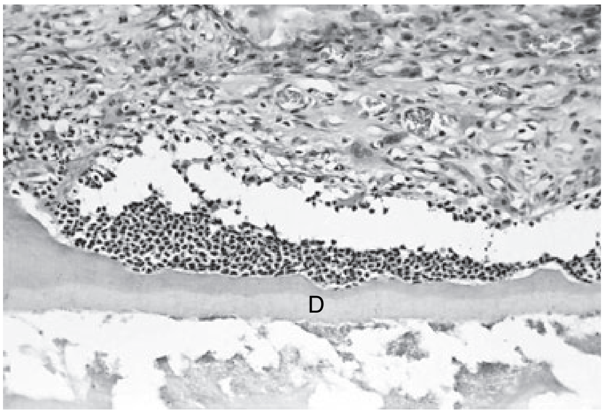

Fig. 8. Group II (fluoride). Inflammatory root resorption areas, with presence of numerous lymphocytes. Dentin (D). H&E. Original magnification: ×160.

some areas, dentin resorption with a large number of lymphocytes was present (Fig. 8). The alveolar wall showed bone apposition, causing narrowing and/or filling of the PDL space. Newly formed bone trabeculae were observed at the bottom of the socket.

Group III (Propolis)

In all specimens, the gingival mucosa epithelium was below the cementoenamel junction, presenting fibroblasts and few lymphocytes. In few areas of the root surface, the PDL space was filled by a fibrous connective tissue, with the fibers disposed parallel to the root surface (Fig. 9), while in other areas, it was filled by newly formed bone tissue (Fig. 10). In five cases, cementum and dentin presented some areas of active replacement resorption in all root thirds (Fig. 11). Four specimens showed resorption with several lymphocytes (Fig. 12). In few areas, the bone tissue was in intimate contact with the cementum. Except for the apical third, the alveolar wall exhibited bone apposition and, in several specimens, almost the whole PDL space was filled by bone tissue in contact with the root surface. Newly formed bone trabeculae were observed at the bottom of the socket.

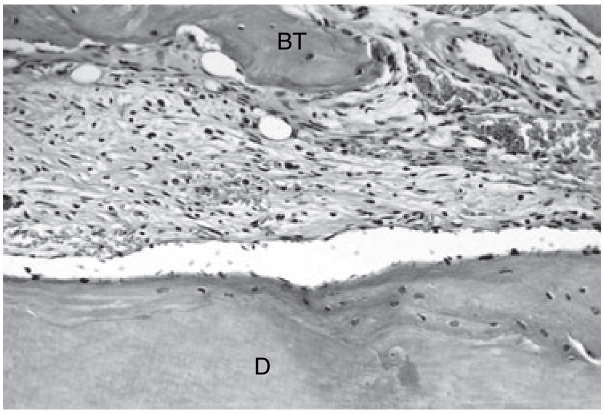

Fig. 9. Group III (propolis). Periodontal ligament space filled by fibrous connective and fibers parallel to the root surface. Dentin (D); Bone tissue (BT). H&E. Original magnification: ×160.

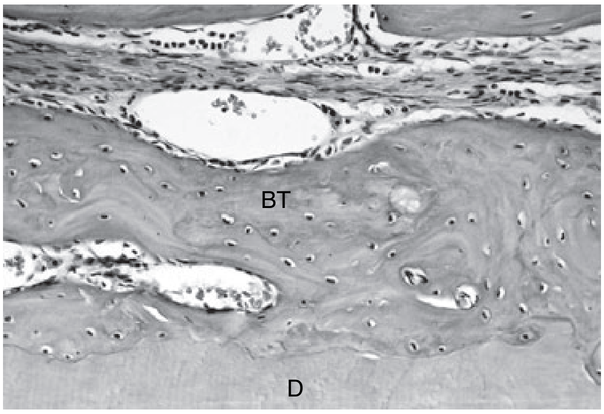

Fig. 10. Group III (propolis). Periodontal ligament space filled by newly formed bone tissue. Dentin (D); Bone tissue (BT). H&E. Original magnification: ×160.

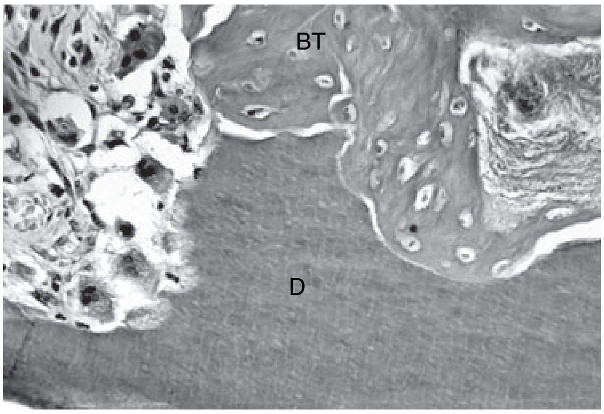

Fig. 11. Group III (propolis). Active replacement resorption. Dentin (D); Bone tissue (BT). H&E. Original magnification: ×250.

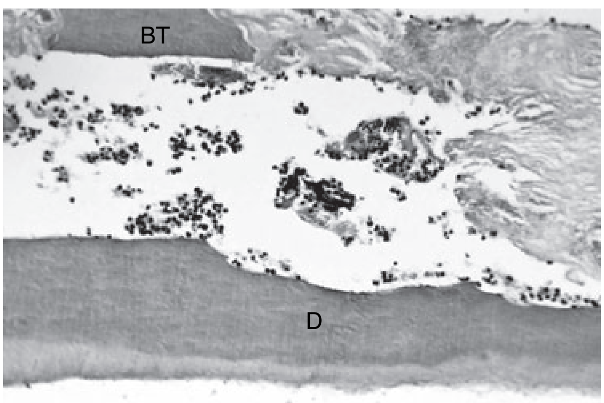

Fig. 12. Group III (propolis). Root resorption with presence of numerous inflammatory cells. Dentin (D); Bone tissue (BT). H&E. Original magnification: ×160.

Statistical analysis

Although all groups exhibited inflammatory resorption, replacement resorption and ankylosis, there was no statistically significant difference among them ($P > 0.05$) (Tables 1 and 2).

Table 1. Significance (*P* value) and Kruskal-Wallis values in the tissue healing events after tooth replantation

Type of resorption	*P* value	Kruskal–Wallis
Ankylosis	0.1797	4.894
Replacement resorption	0.6666	7.174
Inflammatory resorption	0.1490	5.333

Table 2. Mean scores (± SD) for the histological events in each group

Histological events	Group I	Group II	Group III
Inflammatory resorption	2.0 ± 0.92	1.25 ± 0.46	1.25 ± 0.46
Replacement resorption	1.37 ± 0.51	1.75 ± 0.46	2.0 ± 0.53
Ankylosis	1.25 ± 0.46	1.5 ± 0.53	1.62 ± 0.51

Discussion

The rat was the experimental animal model used in this study because of its ease of handling and because of the fact that several dentoalveolar trauma studies have been conducted with this animal (4, 9, 11, 13, 14, 16, 17). The use of a well established methodology is important for comparison and discussion of the results.

The survival of replanted teeth is directly related to the presence, type and velocity of root resorption process (1, 3, 28). Some conditions might favor this phenomenon and should therefore be controlled. Among them, extra-alveolar time, storage medium, inadequate root handling and pulp necrosis are key factors (1, 2, 9, 11, 13, 14, 16, 17).

Long extra-alveolar periods eliminate any possibility of preserving PDL cell vitality (2, 3), which makes unfeasible the reattachment of PDL fibers to root surface. In the present study, a 1-h extra-alveolar period was used to simulate the clinical conditions under which most teeth are replanted (29).

Avulsion causes rupture of the neurovascular bundle, leading to pulp necrosis, which facilitates bacterial contamination. Root canal therapy is thus required in cases of avulsed teeth (30, 31). The association between bacteria lodged in the root canal system or dentinal tubules and cementum/PDL damage triggers an inflammatory root resorption process (32, 33). In this study, a calcium hydroxide-based paste was used as an intracanal dressing (30) mainly because of well-known antimicrobial and toxin-neutralizing properties of calcium hydroxide (34, 35), which would control the contamination and hence the inflammatory root resorption (33).

In addition to pulp necrosis, another route of contamination is root surface exposure to the environment during the extra-alveolar period. In these cases, contamination may be tentatively controlled by two ways: systemic antibiotic therapy (31) and treatment of the root surface (5, 7, 9, 10, 30). All animals in this study received a penicillin-based antibiotic injection intramuscularly, according to the protocol proposed by Flores et al. (30) for teeth with delayed replantation. Despite all care, however, inflammatory resorption was present in all groups; somewhat greater in Group I (saline), but not statistically significant when compared with the other groups. A possible explanation to this would be the fact that, although the fluoride and propolis solutions used for root surface treatment have antimicrobial properties (19, 36, 37), their spectrum of action may not reach all bacteria present on root surface and the persistent microorganisms are capable of maintaining the resorption process.

The root-adhered necrotic PDL remnants were mechanically removed with a scalpel blade as part of the root surface treatment. This technique is easy to perform and preserves the cementum layer, which has been considered an important barrier for the control of external root resorption (38). PDL necrosis leads to the loss of important structures, such as the epithelial rests of Malassez, cementoblasts and precementum, which seem to have a key role on the preservation of the PDL space (39).

The replacement of cementoblasts by osteoblasts, which have a receptor for the parathyroid hormone, together with the bone/cementum/dentin interface allow the tooth participating in the bone remodeling process, gradually starting its replacement by bone (39, 40). This type of resorption was observed in all groups, with a slight predominance in Group III.

In view of these biological events, the occurrence of root resorption is anticipated during the healing of teeth with delayed replantation. Therefore, root surface treatment should be performed in an attempt to prevent, delay or reduce occurrence of root resorption (4–17). In this study, the substances used for such purpose were saline, 2% acidulated-phosphate sodium fluoride solution and 15% propolis solution.

Saline was used as a control because it has no properties that could favor the healing of teeth with delayed replantation. This was confirmed by the histologic analysis, which revealed a more intense inflammatory resorption compared with the other groups.

The 2% acidulated-phosphate sodium fluoride solution has been widely used for the root surface treatment (4, 9, 10), yields good results and is part of the routine protocol for teeth with delayed replantation these constitute the rationale for its choice (30, 31). The replacement of the hydroxyapatite layer by a fluorapatite layer is expected to make the root surface more resistant to the resorption processes (10), in addition to its osteoclast-inhibiting and antimicrobial properties (37). In this study, Group II (fluoride) had results similar to those of Group III and those published elsewhere (4, 9), with predominance of replacement resorption and ankylosis.

The rationale of using a propolis solution for root surface treatment of teeth with delayed replantation relies on its biological properties (18–27, 36, 41–43). Propolis is composed of a mix of viscous resin from peels and shoots associated with flower sap, bee saliva, wax and pollen (36). It is a product of great value for bees, being used as a defense means for their survival and for maintenance of the internal temperature and aseptic conditions of the beehive. Propolis is also used to close the beehive openings, making it waterproof, and may be used for mummification of dead invaders that cannot be removed from the beehive, preventing their deterioration (36).

The commercially available propolis solutions usually have an alcoholic vehicle (20, 21). To avoid any possible damages to the tissues, the propolis solution evaluated in this study was prepared using propyleneglycol as a vehicle to reach a 15% concentration. Its antibacterial, anti-inflammatory and antiviral actions are attributed to the presence of flavonoids, aromatic acids, esters, ferulic and cafeic acids (19, 36). Although these properties were not sufficient to arrest root resorption, the analysis of the qualitative data revealed that the inflammatory resorption was less intense in Group III (propolis) compared with Group I (saline) and that replacement resorption and ankylosis were similar to those observed in Group II (fluoride). These findings seem to result from the antimicrobial activity of the propolis solution (19, 36), which, combined with the endodontic treatment and systemic antibiotic therapy, reduced the occurrence of inflammatory resorption, underscoring both replacement resorption and ankylosis because the PDL was not present.

In view of the current knowledge on the healing process in the delayed tooth replantation and how the biological events take place in the absence of PDL (28, 38–40), the therapeutic approach should be based on prevention or arrestment of the inflammatory resorption, by neutralizing the contamination with endodontic treatment, systemic antibiotic therapy and root surface treatment.

The outcomes of this study indicate that contamination control by treating the root surfaces of avulsed teeth makes a difference because root canal and systemic antibiotic therapy were similar in all groups and inflammatory resorption was more frequent in Group I. The lack of complete control of inflammatory resorption may be attributed to the mechanism of action (36, 37) and to the spectrum of action of both propolis and fluoride solutions, which were not able to eliminate all bacteria. Antibiosis assays with propolis against 10 gram-positive and 20 gram-negative bacterial species found that the antibacterial activity of propolis is more effective against gram-positive bacteria (44).

Replacement resorption and ankylosis are anticipated events in cases of tooth replantation as long as there is no substitute for the PDL that can actually accomplish its functions with the same characteristics. Research on tooth avulsion and replantation should, therefore, focus on the search for treatments that increase root surface resistance to delay the resorption process as much as possible.

Based on the outcomes of this study, it may be concluded that the proposed root surface treatments (15% propolis solution, 2% acidulated-phosphate sodium fluoride solution and saline) yield similar external root resorption in teeth with delayed tooth replantation.

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