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Propolis as storage media for avulsed teeth: microscopic and morphometric analysis in rats

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Abstract – The maintenance of the avulsed teeth in appropriate media for preserving the cellular viability has been important for repairing the periodontal ligament and preventing the root resorption after tooth reimplantation. Propolis is a substance capable of preserving cellular viability. This study aimed to analyze the propolis substance as a storage media for maintaining the avulsed teeth, besides to determine the ideal time period for keeping the tooth inside it. Thus, 60 maxillary right central incisors of rats were extracted and divided into five groups. In groups I and II, teeth were kept in propolis for 60 min and 6 h, respectively; in group III, teeth were kept in milk for 6 h; in group IV, teeth were kept dry for 60 min; and in group V, they were immediately reimplanted. All teeth had their root canals filled with calcium hydroxide paste. Following, teeth were reimplanted in their sockets. After 15 and 60 days, animals were killed and the obtained samples were processed in laboratory for microscopic and morphometric analyzing. The results showed that the occurrence of inflammatory resorption, dental ankylosis and the formation of the connective tissue parallel to the root surface were similar among groups. It could be verified a greater occurrence of replacement resorption in group IV when comparing to other groups. In groups I and IV, the presence of periodontal ligament-like connective tissue was substantially smaller than the other groups. Regarding to the cementum amount over the root, it could be observed that this was present in smaller amount in groups I and IV. Group II was similar to groups III and IV. Therefore, according to the results of this study, the use of propolis as a storage media for maintaining avulsed teeth could be highlighted, and the 6-h period was more appropriate than the 60-min period.

Tooth avulsion, characterized by complete displacement of the tooth from its socket, accounts for about 0.5–16% of all cases of dental trauma (1). After avulsion, the tooth should be repositioned in the socket in an attempt to re-establish normality (1). Immediate reimplantation (1–3) or maintenance of the avulsed tooth in storage media compatible for survival of these cells before reimplantation (1) is an imperious procedure.

Propolis is a natural, non-toxic resinous substance that has been collected from several types of plants by bees for covering and protecting the hive (4, 5). Currently, propolis has been employed in Medicine and Dentistry because of its anti-inflammatory (6), antiseptic (6, 7), healing and antimicrobial (8, 9) properties. Additionally, to its characteristics, propolis contains elements that have been essential during collagen synthesis (10).

Martin & Pileggi (11) observed that propolis solution had the capacity of preserving the cellular viability for 45 min, statistically overcoming Hank's balanced solution, milk and saline. Al-Shaher et al. (7) verified that 75% of the periodontal ligament cells had been kept viable after 20-h maintenance in propolis solution. Ozan et al. (12) observed that 10% propolis solution had preserved the periodontal ligament cells viability for until 24 h.

Therefore, this study aimed to evaluate the effect of propolis as a storage media for maintaining avulsed teeth and to determine the ideal time period for keeping the tooth inside it.

Materials and methods

The study was conducted on 60 male rats (*Rattus norvegicus albinus*, Wistar) weighing approximately 250–300 g. All experimental procedures were approved by the Animal Research and Ethics Committee of Araçatuba Dental School, UNESP, Brazil. (Process 2007–002503). The animals were kept in cages identified according to the group and study periods. The cages were cleaned daily. The animals received a grained solid food before and during the study, except for 12 h before and after surgery, and water *ad libitum*.

For surgical interventions, the animals were anaesthetized with a mixture of ketamine (Dopalen; Sespo Industria e Comércio Ltda, Jacareí, São Paulo, Brazil) and xylazine (Anasedan; Agribrands do Brasil Ltda, Paulínia, São Paulo, Brazil), to intramuscular, in a dose of 0.05 ml per 100 g of weight for each substance. Anaesthesia was applied with disposable insulin syringes. Assepsis of the anterior portion of the maxilla was

realized with Periogard® (Colgate-Palmolive Indústria Brasileira, São Bernardo, São Paulo, Brazil). In addition, extraction of the maxillary right central incisor was realized, simulating a case of tooth avulsion.

The extracted teeth were divided into five groups according to storage media:

1 Group I: teeth were immersed for 60 min in 30 ml of 20% propolis solution (YOD – Comércio de Produtos Naturais Ltda, Campinas, São Paulo, Brazil);
2 Group II: teeth were immersed for 6 h in 30 ml of 20% propolis solution;
3 Group III: teeth were immersed for 6 h in 30 ml of milk (Lider Alimentos do Brasil Ltda, Lobato, Paraná, Brazil);
4 Group IV: teeth were kept dry for 60 min;
5 Group V: teeth were immediate reimplantation.

After times for each group and before of tooth reimplantation, the dental papillae of the teeth was excised with a #11 surgical blade (Embramac Exportação e Importação, Ribeirão Preto, São Paulo, Brazil), for exposure of root canals. The pulp was removed via apical foramen, with a slightly curved #15 Flexofile (Dentsply-Maillefer, Ballaigues, Switzerland). Root canal instrumentation was completed using #20 and #25 Flexofiles. Canals were copiously irrigated with a 1% hypochlorite solution (Probem Produtos Farmacêuticos e Odontológicos Ltda, Catanduva, São Paulo, Brazil) using a Luer Look syringe and a 30 × 4 gauge needle. Then, root canals were dried with sterile absorbent paper points (Tanariman Industrial Ltda, Manacapuru, Amazonas, Brazil) and filled with calcium hydroxide paste (S.S. White Artigos Dentários LTDA, Rio de Janeiro, Rio de Janeiro, Brazil).

Reimplantation procedures were followed with each tooth in its respective sockets. No contention was applied (13, 14). The animals received a single dose of 20 000 U.I. of benzatine G penicillin (Eurofarma Laboratórios Ltda, São Paulo, São Paulo, Brazil), via intramuscular injection.

Six animals of each group were killed at 15 and 60 days after reimplantation with an excessive dose of anaesthetic. The right side of the maxilla was separated from the left in the median line with a #15 surgical blade. The maxilla was further cut nearby the third molar in order to loosen the hemimaxilla containing the tooth reimplanted.

The specimens were fixed in 10% buffered formalin for 7 days and decalcified in 4.13% EDTA (pH 7). The specimens were then processed and embedded in paraffin to show transversal cuts of the cervical, middle and apical thirds of the tooth. Sections of 5 μm were taken at each 50 μm with a microtome, totalling 20 sections for each specimen. The sections were stained by haematoxylin–eosin.

For microscopic analysis, the following parameters were considered: integrity of dental structure, presence of cementum, characteristics of the connective tissue formed in the periodontal space (parallel or perpendicular), presence of dental ankylosis (union of the root and bone, with absence of connective tissue between those) and occurrence of lacunae of inflammatory resorption or lacunae of replacement resorption (substitution of the tooth for bone). In addition, morphometric analysis was also performed, with the aid of the IMAGETOOL (UTHSCSA, version 3.0, University of Texas Health Science Center, San Antonio, TX, USA), specific for measuring areas and perimeters. Fifteen histological sections of each study period were photographed with a digital camera (Canon, Tokyo, Japão) attached to a microscope (Carl Zeiss, Axiolab, Jena, Germany) connected to a PC computer. The images were stored as figures (JPEG) for analysis in the IMAGETOOL software. The perimeter of areas showing ankylosis, connective tissue formed in the periodontal space, the amount of remaining cementum and the areas of inflammatory or replacement resorption were measured.

Microscopic and morphometric analysis were assigned by a blinded and experienced examiner. The identification of the histological sections was occulted and the results recorded on specific tables. Statistical analyses of the results were carried out using Tukey's test, with a significance level set at 5% ($P \leq 0.05$).

Results

Group I (propolis, 60 min)

Analysis of histological sections of specimens in group revealed formation of a dense connective tissue parallel to the root surface in most cases at 15 days (Fig. 1). This tissue presented few collagen fibres, areas of blood clot and inflammatory cells. At 60 days, this tissue was better organized, presented a larger amount of collagen fibres and absence of inflammatory cells (Fig. 2). Tooth ankylosis was observed at all study periods. Examination of the root surface revealed the presence of cementum covering great part of the root surface, being significantly reduced at 60 days ($P < 0.05$). Lacunae of inflammatory and replacement resorption were observed at study periods (Figs 1 and 2).

Group II (propolis, 6 h)

Analysis of histological sections of specimens in group revealed formation of a dense connective tissue parallel

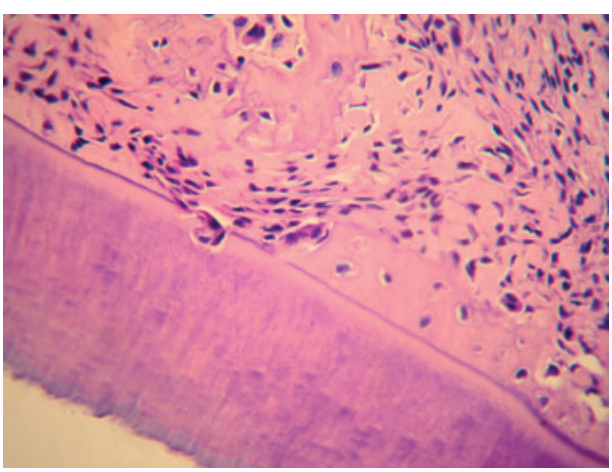

Fig. 1. Group I (propolis, 60 min), 15 days: presence of cementum, connective tissue parallel to the root, dental ankylosis and lacunae of inflammatory resorption. Original magnification 157.5 ×.

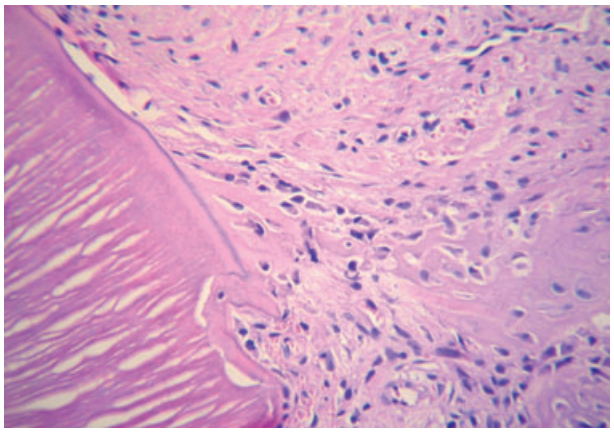

Fig. 2. Group I (propolis, 60 min), 60 days: presence of cementum, connective tissue parallel to the root, dental ankylosis and lacunae of replacement resorption. Original magnification 157.5 ×.

to the root surface in most cases at 15 and 60 days (Figs 3 and 4). The tissue perpendicular to the root was observed at 15 days ($P < 0.05$; Fig. 3). Tooth ankylosis was observed at study periods (Fig. 4). Examination of the root surface revealed the presence of cementum covering great part of the root surface (Figs 3 and 4), being significantly reduced at 60 days ($P < 0.05$). Small lacunae of inflammatory resorption were observed at study periods (Fig. 3). Lacunae of replacement resorption were insignificant at study periods (Fig. 4).

Group III (milk, 6 h)

Analysis of histological sections of specimens in group revealed formation of a dense connective tissue parallel or perpendicular to the root surface in most cases at 15 and 60 days. Tooth ankylosis was significantly increased at 60 days ($P < 0.05$). Examination of the root surface revealed the presence of cementum covering great part of

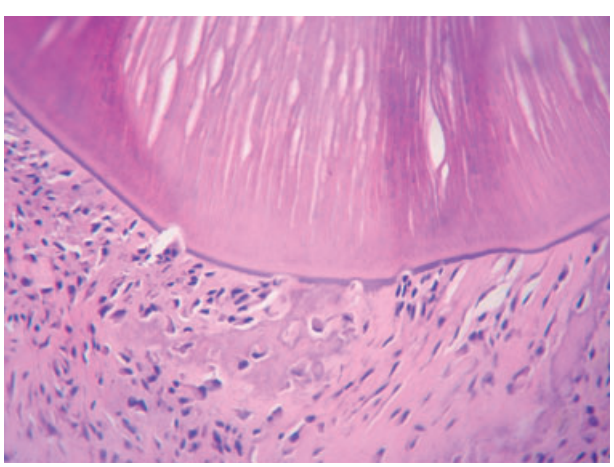

Fig. 3. Group II (propolis, 6 h), 15 days: presence of cementum, connective tissue parallel and perpendicular to the root and lacunae of inflammatory resorption. Original magnification 157.5 ×.

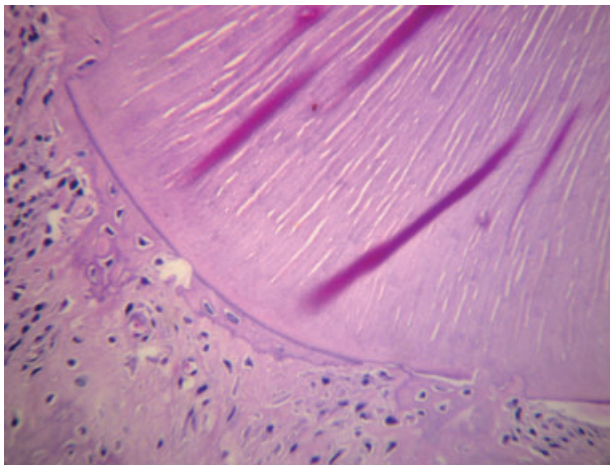

Fig. 4. Group II (propolis, 6 h), 60 days: presence of cementum, connective tissue parallel to the root, dental ankylosis and lacunae of replacement resorption. Original magnification 157.5 ×.

the root surface, being significantly reduced at 60 days ($P < 0.05$). Lacunae of inflammatory were observed at study periods (Figs 5 and 6). Small lacunae of replacement resorption were noted in 60 days.

Group IV (dry, 60 min)

Analysis of histological sections of specimens in group revealed formation of a dense connective tissue parallel to the root surface in most cases at 15 and 60 days (Figs 7 and 8). Tooth ankylosis was present in study periods (Fig. 8). Examination of the root surface revealed the presence of cementum covering part of the root surface (approximately 72%). Lacunae of inflammatory and replacement resorption were observed at study periods (Figs 7 and 8), being significantly increased at 60 days ($P < 0.05$).

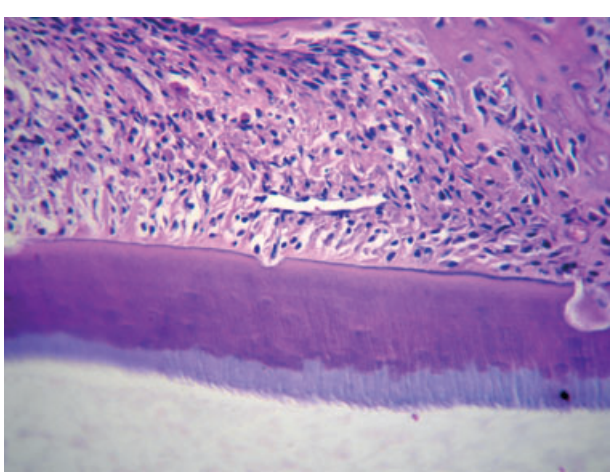

Fig. 5. Group III (milk, 6 h), 15 days: presence of cementum, connective tissue perpendicular to the root and lacunae of inflammatory resorption. Original magnification 157.5 ×.

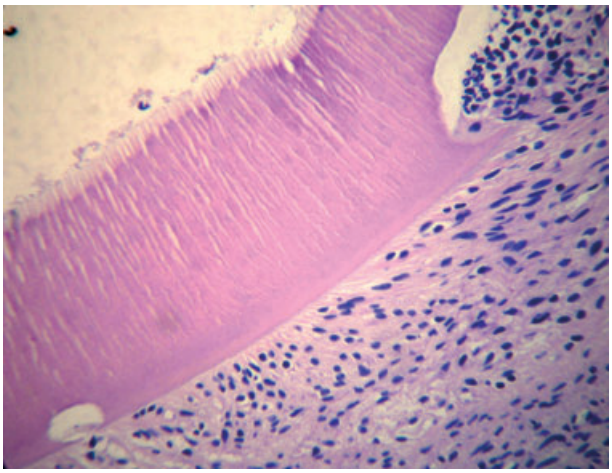

Fig. 6. Group III (milk, 6 h), 60 days: presence of cementum, connective tissue perpendicular to the root and lacunae of inflammatory resorption. Original magnification 157.5 ×.

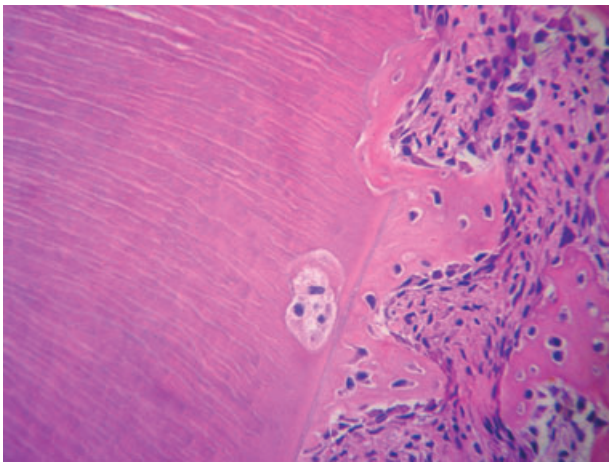

Fig. 8. Group IV (dry, 60 min), 60 days: presence of connective tissue parallel to the root, dental ankylosis and lacunae of inflammatory and replacement resorption. Original magnification 157.5 ×.

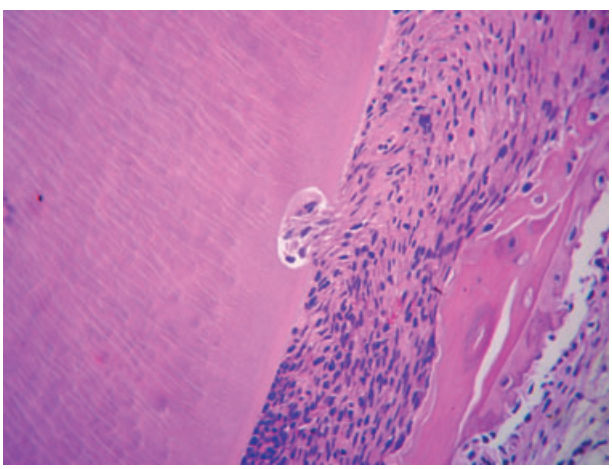

Fig. 7. Group IV (dry, 60 min), 15 days: presence of connective tissue parallel to the root and lacunae of inflammatory resorption. Original magnification 157.5 ×.

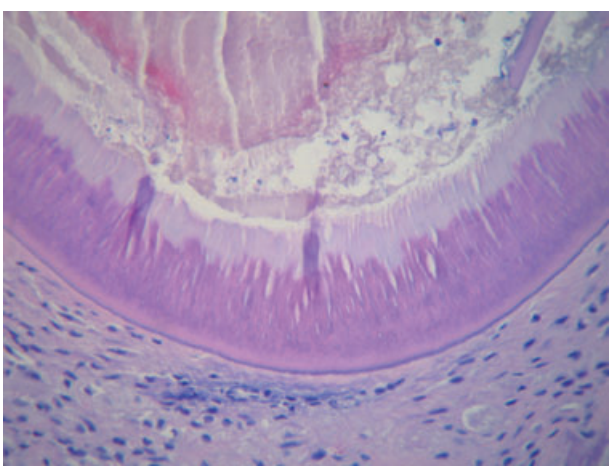

Fig. 9. Group V (immediate reimplanted), 15 days: integrity of dental structure, presence of cementum and connective tissue parallel to the root. Original magnification 157.5 ×.

Group V (immediate reimplanted)

Analysis of histological sections of specimens in group revealed formation of a dense connective tissue parallel (Fig. 9) or perpendicular (Fig. 10) to the root surface in most cases at 15 and 60 days. Tooth ankylosis was noted in study periods. Examination of the root surface revealed the presence of cementum covering great part of the root surface (Figs 9 and 10), being reduced at 60 days ($P < 0.05$). Small lacunae of inflammatory were observed at study periods, being increased at 60 days. Small lacunae of replacement resorption were noted.

Comparison between the experimental groups

The tissue perpendicular to the root (periodontal ligament-like) was significantly different in groups ($P < 0.05$). This tissue was null in group I and insignificant in group IV (Table 1). These groups were different at group III. The groups II, III and V were not different between them. Analysis of the root surface reveals that a larger amount of cementum was in groups II, III and V. The presence of cementum was equal between them (Table 1). These groups was significantly different of groups I and IV ($P < 0.05$). In groups I and IV, the present of cementum was less frequent (Table 1). Concerning the replacement resorption, there was more frequent in group IV and was statistically difference of other groups ($P < 0.05$). The others histological events were statistically similar.

Discussion

In order to achieve tooth reimplantation success, the maintenance of the vitality of cells present on the root is essential (1–3). American Association of Endodontists has recommended that avulsed teeth should be immediately replanted, because the preservation of the cells viability would be significantly higher (15, 16).

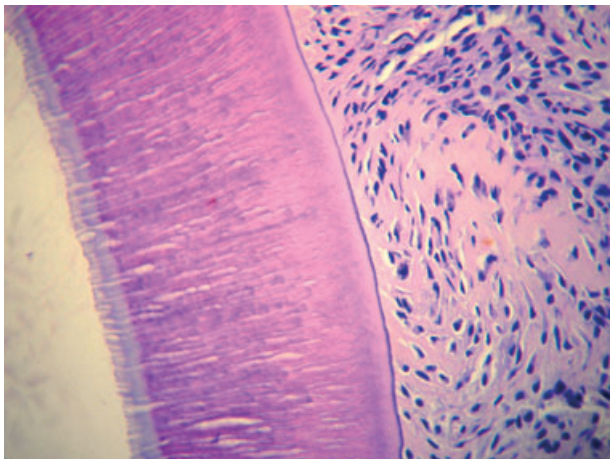

Fig. 10. Group V (immediate reimplanted), 60 days: integrity of dental structure, presence of cementum and connective tissue perpendicular to the root. Original magnification 157.5 ×.

Table 1. Mean values of percentages of the histological events occurrence among experimental groups

Histological events	Experimental groups				
	Group I	Group II	Group III	Group IV	Group V
Inflammatory resorption	1.83	3.06	1.73	5.32	2.78
Replacement resorption	0.07[2]	0.009[2]	0.22[2]	2.10[1]	0.40[2]
Presence of cementum	77.24[1]	92.13[2,4]	92.32[2,4]	72.47[3]	89.08[4]
Dental ankylosis	43.67	43.10	44.01	31.10	38.91
Connective tissue parallel	57.48	46.67	48.61	68.42	57.68
Connective tissue perpendicular	0[2]	2.69	7.26[1]	0.38[2]	3.82

[1]Significant statistical difference from [2]($P < 0.05$); [3]significant statistical difference from [4]($P < 0.05$).

When immediate reimplantation has been realized, there would be the possibility of the periodontal ligament repair, and, the root integrity would be kept, with the presence of cementum over it (1, 3, 17, 18). In some cases, there could be the appearance of small areas of root resorption and dental ankylosis (17–19). These histological events have indicated that the immediate reimplantation would be satisfactory and it could extend the conservation of the replanted tooth in its socket.

In this present study, the presence of small areas of root resorption and dental ankylosis was observed in the group of immediate reimplantation; however, in the majority of the histological sections, the presence of cementum recovering the root surface and the root's integrity were observed. These events were similar to those found on other scientific studies (17–19).

When the execution of tooth reimplantation has not been immediately possible, the tooth should be kept in any storage media. The placement of the tooth inside this media has had the objective of preserving the vitality of the cells present on the root surface for a long period of time and, in some cases, even stimulating their proliferation (1). Milk has been recommended as a storage media for avulsed teeth by the most of the researchers (20–25). Milk effectiveness for preserving cellular viability could be related to osmolarity, the presence of nutritional substances and growth factors, and to the low bacterial content because of the pasteurization process (25). The avulsed tooth maintenance inside milk should not exceed 6 h (22, 25), considering that after this period its effectiveness would substantially decrease (24).

In our study, it was verified the milk effectiveness as a storage media for avulsed teeth. In most of the histological sections, small lacunae of root resorption and presence of cementum recovering the root were observed. In some cases, the presence of periodontal ligament-like tissue connective was noted. These results were similar to those found on literature (20–25).

Unfortunately, in most cases, avulsed teeth have been kept dry, either wrapped in plastic or paper sheets or immersed in solutions unsuitable for cells surviving (26, 27). The appropriate maintenance of the avulsed tooth could lead to the development of dental ankylosis and root resorption, undesirable consequences of delayed tooth reimplantation (1, 17, 18). In our study, in delayed reimplantation, it could be observed the presence of dental ankylosis and the lack of periodontal repair, in the majority of the sections. The occurrence of root resorption was also described. These results were similar to those found on other studies, in which the avulsed teeth had been kept dry for a mean period of 60 min (17, 18, 28, 29).

The search for a storage media for avulsed teeth that would increase the repair chances and inhibit the root resorption occurrence has currently been a research target.

Propolis is a complex mixture of substances that aims to seal hives. Bees collect the substances from the plants' flowers, leaves and stalks, then fabricate propolis and deposit it into their hives. This mixture has been constituted of resin containing vitamins, mineral salts, phenolic compounds as flavonoids, fatty acids, aromatic acids and esters, 30% of waxes, 5% of pollen, 4–15% of volatile substances and strange materials and 13% of unknown substances (4). Among these substances present in propolis, flavonoids have been highlighted, which have been indicated as responsible for the anti-inflammatory, antimicrobial and antifungal actions (30, 31). Several studies have demonstrated that the preparations based on propolis would be capable of accelerating the wounds repair process, in the more diverse tissues types (8, 9).

Because of these features, propolis has been tested on the periodontal ligament cells. Martin & Pileggi, in 2004, evaluated the viability of cells kept in two propolis concentrated solutions (50% and 100%), Hank's balanced saline solution (HBSS), saline solution or milk, for 45 min. Both concentrations of propolis solution were efficient in preserving the cellular viability and were more effective than HBSS, milk and saline, for that purpose (11).

Özan et al., evaluated the ability of either 10% or 20% propolis solution, as storage media for avulsed teeth cells, for the periods of 1, 3, 6, 12 and 24 h. The authors also suggested the execution of studies on animals' models for confirming propolis as a storage media (12).

Propolis solution was tested, in our study, on the periods of 60 min and 6 h. These were compared with immediate reimplantation and milk storage for 6 h, conditions which have been accepted as satisfactory, and to delayed reimplantation after 60 min, situation which has been frequently found in dental offices and clinics.

This study's results highlighted the efficiency of propolis as a storage media for maintaining avulsed teeth for 6 h. It was observed that when the teeth had been kept in propolis for 6 h, there was a higher preservation of both the cementum layer and root surface. When this group was compared with both the immediate replantation and milk for 6 h, it was verified similarity between them. However, when propolis was evaluated as a storage media for 60 min, the results were not that stimulating. It could be observed the reduction of the cementum presence on the root and the lack of periodontal repair after tooth reimplantation in this group. This demonstrated that the maintenance of the avulsed tooth in propolis for 6 h was more effective. It has been believed that there would be the best action and the highest incorporation of the active principles of propolis which have been responsible for the antimicrobial, anti-inflammatory and healing actions when the tooth has been kept in propolis for 6 h.

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