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Study of the effectiveness of propolis extract as a storage medium for avulsed teeth

Ana Regina Casaroto[1], Mirian Marubayashi Hidalgo[2], Ana Maria Sell[3], Selma Lucy Franco[1], Roberto Kenji Nakamura Cuman[1], Eduardo Moreschi[4], Fausto Rodrigo Victorino[5], Vânia Antunes Steffens[6], Ciomar Aparecida Bersani-Amado[1]

[1]Department of Pharmacy and Pharmacology, State University of Maringá; [2]Department of Dentistry, State University of Maringá; [3]Department of Clinical Analysis, State University of Maringá; [4]Department of Dentistry, University Center of Maringá; [5]Department of Endodontics, Bauru School of Dentistry, University of São Paulo, Bauru, [6]Experimental Surgery and Biotery, State University of Maringá, Maringá; Brazil

Correspondence to: Prof. Dr Ciomar Aparecida Bersani-Amado, Universidade Estadual de Maringá, Departamento de Farmácia e Farmacologia, Av. Colombo, 5790, Zona 7, 87020–900 Maringá, PR, Brazil
Tel: +55 44 3261 4923
e-mail: cabamado@uem.br

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Abstract – The purpose of the present study was to evaluate the efficacy of propolis extract in maintaining the viability of human periodontal ligament (PDL) cells, and to radiographically analyze tooth replantation and the adjacent periodontium in dogs after storage in this extract. Human PDL cells were incubated with the experimental media propolis, milk, saliva, Hank's balanced salt solution (HBSS), and Dulbecco's modified Eagles medium (DMEM, positive controls), and distilled water (negative control). Cell viability was determined 0, 1, 3, 6, 12, and 24 h later by colorimetric MTT assay. Thirty incisors from dogs were divided into two storage time blocks (1 and 3 h) and were maintained in the experimental media. HBSS served as a positive control, and dry teeth (on gauze) as a negative control. The replanted teeth were radiographed once per month for 6 months. The radiographic images were standardized by the shortening/lengthening factor, and were both qualitatively and quantitatively analyzed. The *in vitro* results showed that the efficacy of propolis in maintaining functional viability of PDL cells was similar to that of milk. Propolis and milk were significantly better than controls from the 6-h time period. The *in vivo* results showed that teeth maintained in propolis medium exhibited replacement resorption with significant reduction in tooth length, similar to teeth maintained in saliva and dried teeth. This resorption was less intense with the 3-h storage time than the 1-h storage time. Conditions close to normal were found in teeth maintained in milk, similar to the HBSS control. Therefore, although propolis was effective in maintaining the viability of human PDL cells, resorption of the tooth replantation in dogs occurred under these experimental conditions.

Introduction

Dental avulsion is a consequence of injury that results in the complete displacement of a tooth from its alveolar socket, and may affect multiple tissues, such as the periodontal ligament (PDL), alveolar bone, cementum, dental pulp, and gingival mucosa (1). The reported incidence of complete avulsion ranges from 1 to 16% of all traumatic injuries to the permanent dentition (2). Trauma to the anterior teeth is the most prevalent (3), with sports and automobile accidents the most frequent causes (4). The most frequently involved age group is 7–11 years old (4), with at higher prevalence in males (4, 5).

When dental avulsion occurs, immediate replantation at the trauma site is the ideal procedure for maintaining the viability of PDL cells. However, immediate replantation is rarely achieved (6). The viability of PDL cells is dependent on the duration of extra-alveolar time, storage media of the tooth, and preservation of the root portion, all of which determine the prognosis for dental replantation (2, 5). Inflammatory resorption and replacement resorption subsequent to dental alveolar ankylosis are the most significant and common complications after replantation of avulsed teeth (7).

In vivo and *in vitro* research suggests the use of different storage media to maintain PDL cell viability and improve the prognosis for tooth replantation. These storage media include milk, saliva, and some cell-culture media (5, 8–10). *In vitro* studies also suggest formulations based on propolis as promising alternative media for maintaining PDL cells (11–13).

Propolis is a resinous substance derived from tree exsudates mixed with floral sap, salivary bee secretions, wax, and pollen. It is used by bees for thermally insulating, sealing, and protecting the hive against microorganisms (14, 15). Its complex chemical composition is dependent on the plant source and local flora (16, 17). The compounds comprising propolis include volatile oils (5–10%), waxes (30–40%), resins, balsams, and pollen grains, which are rich sources of essential elements such as magnesium, nickel, calcium, iron, and zinc (18). Polyphenols have been identified as the main organic constituents of propolis, mainly represented by flavonoids and accompanied by phenolic acids, esters, phenolic aldehydes, and ketones (19).

Dental studies have evaluated the biological activity of propolis, mainly with respect to the healing process (20), inhibition of dental plaque formation and

prevention of dental caries (21), and as an intracanal medication in endodontic treatment (22). Furthermore, an *in vitro* study by Ozan et al. (13) suggested that the antimicrobial, anti-inflammatory, and antioxidant properties of propolis may contribute to PDL repair of replanted teeth.

Although the biological properties of propolis have been widely investigated, no *in vivo* studies have assessed its effectiveness in periodontal healing and root resorption of replanted teeth compared with other storage media for avulsed teeth. Therefore, the purpose of the present study was to evaluate the efficacy of propolis extract in maintaining the viability of human PDL cells maintained in culture; and to radiographically analyze tooth replantation and the adjacent periodontium in dogs after storage in this extract, compared with other storage media for avulsed teeth.

Materials and methods

The *in vitro* experimental design with human PDL cell cultures was approved by the Ethics in Human Research Committee, and the *in vivo* design with dogs was approved by the Ethics in Animal Research Committee of the State University of Maringá (Brazil).

Preparation of propolis extract

Propolis samples were collected from the hives of honeybees (*Apis mellifera*) kept on the Iguatemi Experimental Farm of the State University of Maringá, Brazil, which is bounded by a eucalyptus plantation and a native forest. The propolis extract (10%) was obtained by turboextraction, using ethanol as the liquid extractor, based on a slight modification of Franco and Bueno (23). The extract was subjected to partial withdrawal of alcohol, under reduced pressure, using a rotator evaporator at a controlled temperature of 60°C.

Human PDL cell culture

The human PDL cell cultures used in this study were obtained from the Microbiology Laboratory, Bauru Dental School, University of São Paulo, Brazil. The cells were cultured in Dulbecco's modified Eagles medium supplemented with gentamicin (40 mg ml^{-1}, 20 μl per 80 ml), amphotericin B (5000 μg/ml, 1.2 μl per 80 ml), L-glutamine (2 mM), and 10% fetal bovine serum. The flasks were incubated in a humid atmosphere at 37°C in 5% CO2 and 95% air. Once confluent, the cells were detached using 0.25% EDTA Trypsin. Cells from passages 6 to 8 were used for the experiments.

Cell viability by MTT assay

Cell viability was determined by colorimetric MTT assay (3-[4,5-dimethylthiazolyl-2]-2,5-diphenyltetrazolium bromide) (24). For each experiment, 10^4 cells in supplemented DMEM were plated in 96-well tissue-culture plates and incubated at 37°C in 5% CO_2 and 95% air for 24 h. Subsequently, the medium was removed, and 200 μl of each of the different experimental solutions was added. The experimental storage solutions used in the experiment were propolis extract, whole ultrapasteurized milk, and human saliva. HBSS and supplemented DMEM served as the positive controls, and distilled water served as the negative control. The plates were maintained at room temperature (25°C) for 0, 1, 3, 6, 12, and 24 h ($n = 5$). After the appropriate times, the MTT solution (5 mg ml^{-1}) in DMEM medium was placed in each well, and the plates were incubated at 37°C in 5% CO_2 and 95% air for 3 h. The dimethylsulfoxide (DMSO) was then added to dissolve the purple formazan salt crystals released by the cells.

To quantify the viability of metabolically active cells, the optical density (OD) of the solubilized formazan product was measured by means of ELISA, at a 550 nm wavelength. Human saliva was collected from the same volunteer and centrifuged moments before the experiment.

Simulation of tooth avulsion

The simulation of tooth avulsion in dogs was based on the methods of Pettiette et al. (10) and Schwartz et al. (25) with slight modifications. A total of 30 upper and lower incisor teeth from six healthy young male mongrel dogs were used. The animals received an intramuscular injection of xylazine chlorhydrate (1.5 ml per 10.0 kg body weight) for muscular relaxation. After 30 min, they were anesthetized by intravenous administration of ketamine chlorhydrate (0.05 ml per 1.0 kg body weight).

Preoperative periapical radiographs taken with an adapted radiographic positioner were used for diagnosis, followed by tooth extraction. The teeth were extracted by turns between the upper and lower incisors, as atraumatically as possible, and immediately divided into two storage times of 1 and 3 h. The specimens were maintained at room temperature in the different experimental storage solutions ($n = 3$): propolis extract, whole ultrapasteurized milk, and human saliva. HBSS served as the positive control, and dry tooth (gauze) as the negative control.

After the appropriate storage time, the clotted blood was removed from the socket by saline irrigation, and the teeth were gently replanted in their original sockets. The teeth were splinted functionally for 15 days. The dogs were fed a diet of water and soft puppy food for the period of splinting. The replanted teeth were radiographed once per month for 6 months, using an adapted positioner and periapical radiographic film, with an exposure time of 0.7 s.

Qualitative analysis of radiographic images of tooth replantation and adjacent periodontium

The analysis was performed for the cervical, middle, and apical root thirds. The following radiographic characteristics were observed: alveolar bone crest, periodontal space, radiolucent image, root canal image, and inflammatory and/or replacement resorption. The analysis of the 1 and 3 h storage times in each of the experimental media was repeated twice at 1-week intervals by the same observer.

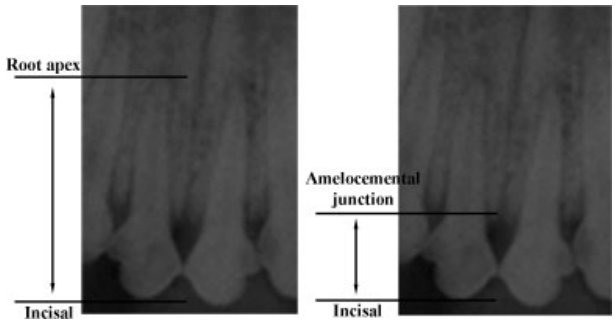

Fig. 1. Radiographs illustrating the tooth measurements used in the present study. (a) Incisor-apical distance used to calculate root resorption. (b) Incisor to enamel–cementum junction distance used to calculate the factor of radiographic shortening or lengthening.

Quantitative analysis of radiographic images of tooth replantation

The teeth were measured along their greatest length, from the incisal edge to the apex of the root, to compare the same tooth pre- and post-treatment and to evaluate possible apical root resorption occurring after replantation (Fig. 1a). These measurements were accompanied by 6 months of radiography. To obtain standardized images and considering the possibility of distortions of the pre- and post-treatment radiographs, the greatest distance from the incisal edge to the amelocemental junction was also measured on all of the radiographs (Fig. 1b). The correction of the radiographic distortion was calculated as a factor of shortening or lengthening used in the measurement of the incisoapical length of the teeth (Eqs 1 and 2) (26, 27). The same ruler (Triangular Scalimeter ME-15/1) was used in all of the measurements, which were repeated three times at 1-week intervals by the same observer. The arithmetic mean of the apical root resorption (mm) was converted into a percentage of the initial total length of the tooth:

$$\frac{\text{Initial total length}}{\text{X (expected total length)}} = \frac{\text{Initial crown length}}{\text{Final crown length}} \quad (1)$$

$$\text{X} - \text{final total length} = \text{Apical root resorption} \quad (2)$$

Statistical analysis

Cell viability data obtained by MTT assay were statistically analyzed by factorial ANOVA, Contrast test at a 5% significance level (STATISTICA v.7).

The radiographic characteristics of the qualitative analysis were evaluated using Cohen's κ coefficient (κ value, Statistical Analysis System [SAS] software) (28) to obtain inter-rater agreement of intra-observer readings. The radiographic data from the quantitative analysis were first evaluated by repeated-measures ANOVA with a 5% significance level to obtain the degree of agreement between the intra-observer measurements. The data were then statistically analyzed by factorial ANOVA, Contrast test with a 5% significance level.

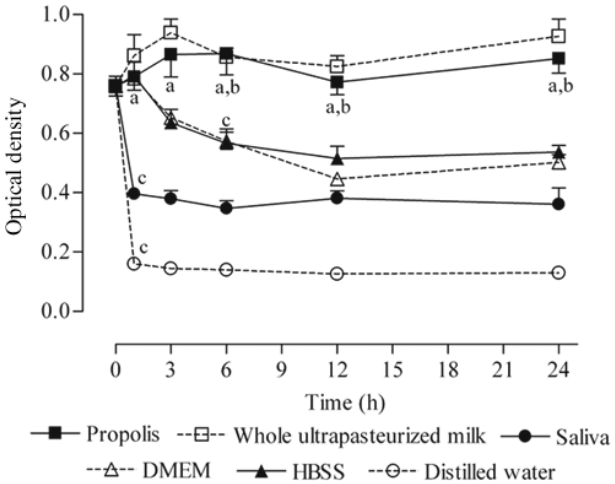

Fig. 2. Viability of PDL cells maintained in each experimental medium for a period of 24 h at room temperature. Each value represents the mean OD (550 nm) of five determinations. (a) $P \leq 0.001$, propolis compared with saliva and distilled water; (b) $P \leq 0.002$, propolis compared with DMEM and HBSS; (c) $P \leq 0.005$, saliva, DMEM, HBSS, and distilled water compared with viability at 0 h (Factorial ANOVA, Contrast Test).

Results

Cell viability by MTT assay

The OD results, which represent PDL cell viability in each experimental medium at room temperature, are presented in Fig. 2. The propolis-extract medium was effective at maintaining cell viability over the 24 h incubation, similar to the milk medium. Propolis maintained PDL cell viability significantly better than the DMEM and HBSS positive controls from the 6-h time period onwards. A significant reduction was observed in cell viability with DMEM and HBSS from the 6-h period onwards, compared with 0 h. At 1 h, propolis performed better than saliva, whereas saliva significantly reduced cell viability beginning with the first hour. Distilled water performed significantly worse than all other media in maintaining PDL cell viability, at all time periods.

Qualitative analysis of radiographic images

The two replications of intra-observer analysis at 1-week intervals were in agreement ($\kappa > 0.81$) (28), indicating the reliability of the radiographic descriptions.

Propolis extract

The teeth maintained in propolis at 1 h radiographically showed loss of the periodontal space in the three root thirds, and a continuous replacement resorption. The teeth also showed a radiolucent image, characterizing inflammatory resorption. The mandible teeth showed evidence of severe replacement resorption in the apical third, and evidence of severe inflammatory resorption in the cervical and middle thirds following discontinuous images of the root canal (Fig. 3A-b). These radiographic

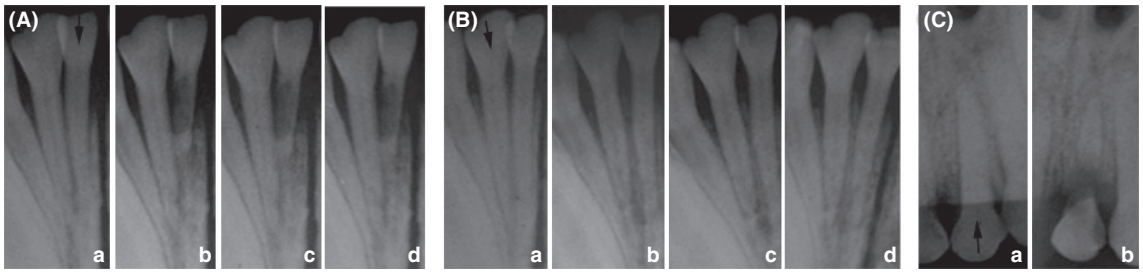

Fig. 3. Mandible tooth kept in propolis at 1 h (panel A) and 3 h (panel B), and maxillary tooth kept in propolis at 1 h (panel C) (arrow). In A: (a) initial radiographic image; (b) first, (c) third, and (d) sixth month post-replantation. Severe replacement resorption was observed in the apical third and severe inflammatory resorption in the cervical and middle thirds. In B: (a) initial radiographic image; (b) first, (c) third, and (d) sixth month post-replantation. Moderate replacement resorption was observed only in the apical third. In C: (a) initial radiographic image; (b) third month post-replantation. Severe inflammatory resorption was observed in the cervical and middle thirds.

signs were observed 1 month after replantation, and stabilized at 3 months (Fig. 3A-c). The maxillary teeth showed evidence of severe inflammatory resorption in the cervical and middle thirds, with gradual intensification of the radiographic characteristics over a period of months (Fig. 3C).

The mandible teeth maintained in propolis, after 3 h showed radiographic evidence of only moderate replacement resorption in the apical third (Fig. 3B-b). These radiographic signs were observed 1 month after replantation, and intensified in the following months (Fig. 3B-d). The maxillary teeth showed evidence of severe inflammatory resorption in the cervical third, similar to maxillary teeth maintained for 1 h.

Whole ultrapasteurized milk

The radiographic characteristics of the teeth maintained in milk at 1 h were similar to the 3-h storage time, in all months of observation. The mandible teeth showed radiographic evidence of only slight replacement resorption of the root apex during the first month after replantation (Fig. 4A-b). The maxillary teeth showed evidence of slight to moderate inflammatory resorption in several sites of root length. These characteristics of inflammatory resorption intensified over the months, and resulted in discontinuous images of the root canal (Fig. 4B-b).

Saliva

The mandible teeth maintained in saliva for 1 h showed radiographic evidence of moderate to severe replacement resorption in the apical third (Fig. 5A-b). These radiographic signs were intensified over the months. In contrast, the cervical and middle third showed no radiographic characteristics of resorption (Fig. 5A-c). Maxillary teeth showed evidence of severe resorption in the middle and apical thirds.

The maxillary and mandible teeth maintained in saliva for 3 h showed radiographic evidence of moderate replacement resorption in the apical third (Fig. 5B-b). These radiographic signs intensified over the months, and the teeth showed radiographically discontinuous images of the root canal (Fig. 5B-c).

HBSS (positive control)

The radiographic characteristics of the teeth maintained in HBSS for 1 h were similar to the 3-h storage time at all months of observation. The teeth showed radiographic aspects of normality, indicated by the presence of the periodontal space in most of the root length, and absent a radiolucent image, with continuity of the root-canal image. Infrequently observed signs included discrete loss of periodontal space and evidence of slight

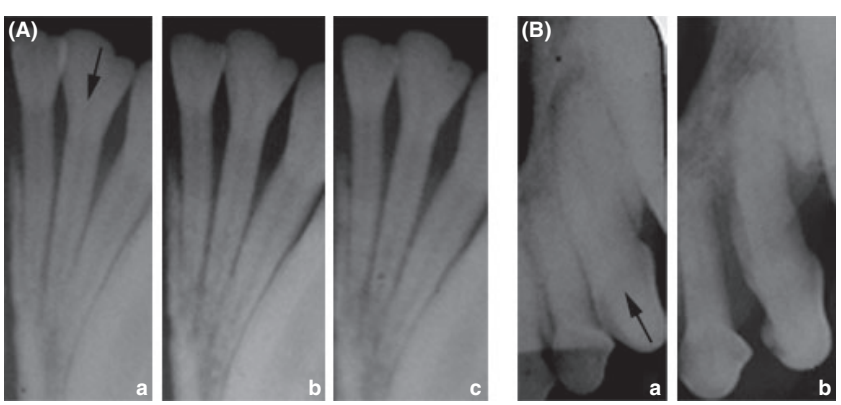

Fig. 4. Mandible (panel A) and Maxillary (panel B) tooth kept in milk at 1 h (arrow). In A: (a) initial radiographic image; (b) first and (c) sixth month post-replantation. Slight replacement resorption of the root apex was observed. In B: (a) initial radiographic image and (b) sixth month post-replantation. Slight to moderate inflammatory resorption was observed in several sites of root length.

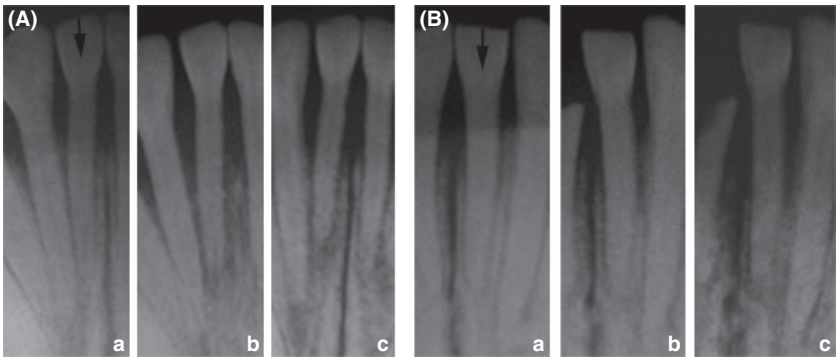

Fig. 5. Mandible tooth kept in saliva at 1 h (panel A) and 3 h (panel B) (arrow). In A: (a) initial radiographic image; (b) first and (c) sixth month post-replantation. Moderate to severe radiographic evidence of replacement resorption was observed in the apical third, and nonradiographic characteristics of resorption were observed in the cervical and middle thirds. In B: (a) initial radiographic image; (b) first and (c) sixth month post-replantation. Moderate replacement resorption was observed in the apical third together with radiographically discontinuous images of the root canal.

replacement resorption of the root apex. These signs were observed during the first month after replantation, and stabilized in the following months (Fig. 6A-b).

Dry tooth (gauze–negative control)

The radiographic characteristics of the teeth maintained in dry storage for 1 h were similar to the 3-h storage time at all months of observation. The teeth radiographically showed loss of periodontal space and replacement resorption at several sites of root length. These first signs were observed in the apical third, with moderate to severe evidence 1 month after replantation (Fig. 6B-b). Subsequently, they were observed in the middle third, with slight to moderate evidence 3 months post-replantation (Fig. 6B-c). The gradual intensification of the radiographic characteristics over the months resulted in discontinuous images of the root canal (Fig. 6B-d).

Quantitative analysis of radiographic images

The three replications of intra-observer analysis at 1-week intervals were in agreement, indicating the reliability of the radiographic descriptions.

Figure 7A and B show the results (%) for the degree of apical root resorption after measuring the radiographs of all tooth replantations in dogs, after storage in each experimental medium at room temperature.

With the 1-h storage time, the teeth maintained in the propolis medium showed increased apical resorption over the 6 months, and a significant reduction of the initial tooth length from the second month of observation. At 2 months, the resorption was significantly higher for the teeth maintained in propolis than for the teeth maintained in the milk and HBSS media. The teeth maintained in propolis reabsorbed significantly less than the teeth maintained in saliva between the first and fourth months of observation. At 5 months, the resorption of the teeth maintained in propolis was significantly more intense than in the teeth kept dry (negative control).

With the 3-h storage time, the teeth maintained in propolis medium showed increased apical resorption until the fourth month, and a significant reduction in the initial tooth length from this month onward. At 4 months, the resorption of these teeth significantly exceeded only that of the teeth maintained in milk medium. In addition, the teeth maintained in propolis medium and saliva reabsorbed significantly less with the 3-h storage time from the second and first month, respectively.

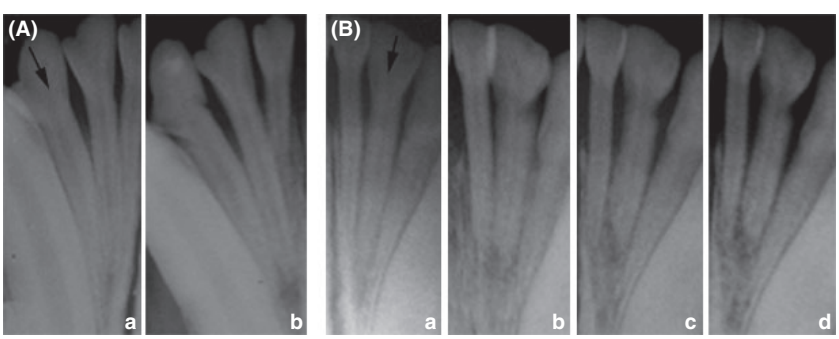

Fig. 6. Mandible tooth kept in HBSS (panel A) and maintained in dry storage (panel B) at 3 h (arrow). In A: (a) Initial radiographic image and (b) sixth month post-replantation. Slight evidence of replacement resorption of the root apex was observed. In B: (a) Initial radiographic image. (b) First, (c) third, and (d) sixth month post-replantation. Moderate to severe replacement resorption was observed in the apical third, and slight to moderate evidence of replacement resorption was observed in the middle third.

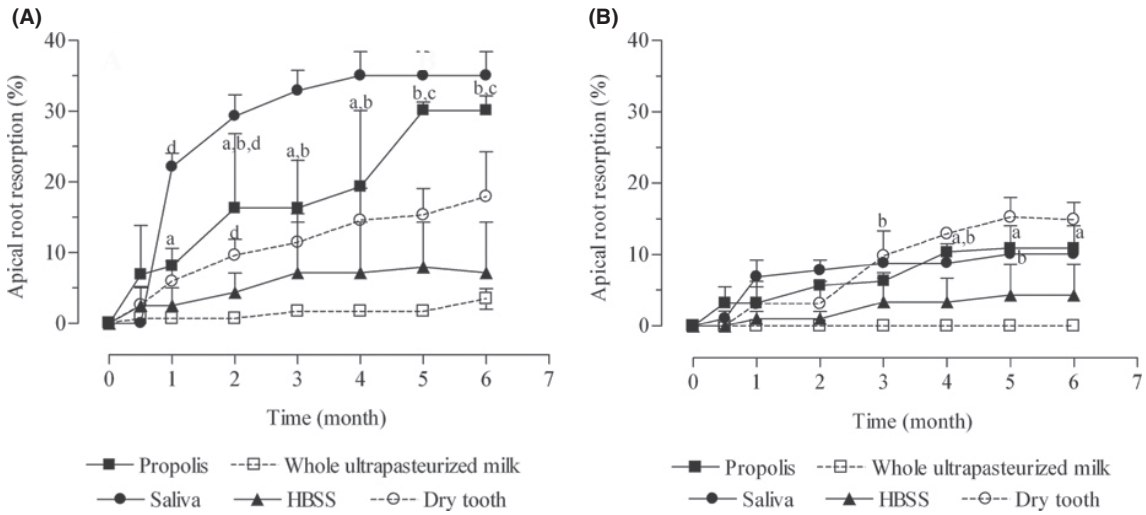

Fig. 7. Mean apical root resorption (%) of tooth replantation in dogs after being kept in each experimental medium for 1 h (figure A) and 3 h (figure B) at room temperature ($n = 3$). Monthly monitoring for 6 months. In A: (a) $P \leq 0.005$, propolis compared with saliva. (b) $P \leq 0.01$, propolis compared with HBSS and milk. (c) $P \leq 0.002$, propolis compared with dry medium. (d) $P \leq 0.036$, propolis, saliva, and dry medium compared with the 0 month. In B: (a) $P \leq 0.025$, propolis compared with milk. (b) $P \leq 0.033$, propolis, saliva, and dry medium compared with the 0 month (Factorial ANOVA, Contrast Test).

Discussion

The present data showed that the propolis extract was effective in maintaining the functional viability of human PDL cells, but did not prevent root resorption of avulsed and replanted teeth in dogs.

The *in vitro* results showed that the efficacy of propolis in maintaining the functional viability of PDL cells was similar to that of milk, a medium traditionally indicated for the storage of avulsed teeth (29, 30). The cell viability observed with the propolis medium over 24 h was superior to the DMEM and HBSS controls (12, 13).

Al-Shaher et al. (11) studied the viability of PDL and dental pulp cells *in vitro* when they were maintained in a low concentration of propolis, and observed low toxicity with maintenance of cell viability greater than 75%. Moreover, Sonmez et al. (15) showed that a high concentration of propolis was cytotoxic to gingival fibroblasts. Ozan et al. (13) observed that 10% propolis extract in DMEM culture medium was more effective than milk medium and HBSS in maintaining PDL cell viability for 24 h. These reports are consistent with the present results, which showed that a low concentration of propolis extract was effective in maintaining functional cell viability.

Traditionally, milk has been presented as the ideal storage medium for avulsed teeth, being better than DMEM (31), HBSS (6), and saliva at maintaining cell viability (32). Consistent with the observations of Chamorro et al. (30), both milk and HBSS had a low percentage of apoptotic cells and no significant difference after 24 h when used as storage media. In the present study, to simulate the circumstances of storage of avulsed teeth, the media were maintained at room temperature (25°C) and not incubated at 37°C in 5% CO_2 and 95% air, which are ideal conditions for maintaining cell cultures. The lack of suitable CO_2 and humidity conditions can explain the decrease in cell viability observed with the DMEM and HBSS controls after 3 h of incubation.

Saliva proved to be the least satisfactory medium, and showed a strong reduction in the number of functionally viable cells after 1 h incubation, as described previously (32, 33).

Studies on the composition of different media suggest that the viability of PDL cells is more closely linked to the osmolality of the solution than to its chemical composition (31, 32, 34). According to some studies (32, 35), media between 290 and 330 mOsm kg^{-1} produce more reliable cell growth than do media with lower osmolality. Additionally, media devoid of microbial contamination (8, 32, 33) and near physiological pH are more favorable for cell viability (2).

Therefore, milk was used in this study as a potential transport medium for maintaining avulsed teeth (31, 35) because of some of its specific characteristics, such as osmolality of 270–284 mOsm kg^{-1} (2, 36, 37), pH 6.7 to 7.3 (32, 37), low microbial content, and lack of active toxic components that would be harmful to PDL cell viability (29, 36, 38). Additionally, the epidermal growth factor present in milk can stimulate osteoclasia and decrease the probability of dental alveolar ankylosis and possible replacement resorption (1).

Hank's balanced salt solution is a storage medium with the ability to maintain PDL cell integrity (39). It is non-toxic, has a neutral pH, contains many essential nutrients (31, 39), and has an osmolality (270–320 mOsm kg^{-1}) that is suitable for cell growth (36). A disadvantage of HBSS is that it may not be readily available in many locations where tooth avulsions are likely to occur (36).

The saliva medium, despite its obvious availability, has characteristics that are harmful to PDL cell integrity, such as low osmolality (66 mOsm kg^{-1}) (32) and the

presence of enzymes, microorganisms, and bacterial toxins (40). Bacteria or their products may potentiate cell damage because the cell membrane has already been damaged by the hypotonic solution, and can consequently accentuate the postreimplantation inflammatory response (32, 33).

The water medium and keeping avulsed teeth dry appear to be less suitable conditions for storage. Water is hypotonic in the intracellular environment, which explains the death of PDL cells maintained in this solution (34). Teeth kept dry for extended periods may have the root covered with necrotic and dying cells. These may be potent stimulators of inflammation, with replacement resorption as the ultimate result (10).

In the present study, the propolis medium showed the best results with regard to the functional viability of PDL cells. Propolis has been reported as possessing biological properties that may support its use as a storage medium (11, 12) and for healing replanted avulsed teeth (41). Propolis medium has remarkable antibacterial (22, 42), antifungal (22), anti-inflammatory (22, 43), antioxidant (44), and immunomodulatory properties (19).

Nevertheless, no previous study has investigated the *in vivo* effectiveness of propolis in periodontal healing of avulsed teeth, thus justifying the radiographic analysis of tooth replantation in dogs and comparing the results for propolis to those for other storage media in the present study. The dog model is one of the most frequently used in root resorption research of teeth replantation after avulsion (10, 41, 45–47).

With regard to the methodology used in the present study, endodontic treatment was not performed (25, 45, 48). Subsequent pulp infection may have played a role in the inflammatory root resorption observed within the groups. However, because the status of the pulp was found to be essentially the same in all groups, the observed differences were most likely attributable to the different storage conditions used (25). Additionally, the use of young dogs increased the possibility of pulp revascularization because the apical delta was not intensely mineralized (34).

As demonstrated by the qualitative and quantitative radiographic analyses, the teeth maintained in propolis medium showed intense replacement resorption. The resorption process advanced during the months of observation, with decreased initial tooth length from the second month of observation with teeth from the 1-h storage time. In contrast, the resorption process of the teeth from the 3-h storage time was less intense than the 1-h storage time, and the tooth length did not change during the last 3 months of observation. These results suggest that maintaining avulsed teeth for a longer period of time in propolis medium allowed propolis' biological properties (anti-inflammatory, antimicrobial, and antioxidant) to perform more efficiently.

The main organic constituents of propolis, mainly flavonoids, are able to neutralize oxygen radicals and preserve the cell membrane through lipid peroxidation (41). As anti-inflammatory agents, the flavonoids are known to help heal bleeding periodontal tissue and stimulate enzymes that fortify the walls of the blood vessels in the periodontium (11). Additionally, propolis contains iron and zinc, which are important for the synthesis of collagen and may increase the healing effect of epithelial tissue (14).

Gulinelli et al. (49) assessed the influence of 15% propolis as a root surface treatment on the healing process after delayed tooth replantation. These authors observed active replacement resorption and inflammatory resorption in several specimens of teeth treated with propolis solution.

In this radiographic study, conditions close to normal were found in teeth maintained in the milk and HBSS media. The images observed in the first month showed slight replacement resorption of the apical root segment of teeth, which was then controlled over the months. These teeth did not show a significant decrease in initial length, consistent with previous reports (8, 9, 38).

The radiographic analysis showed that the saliva medium exhibited unsatisfactory qualitative and quantitative performance, regardless of the storage time (1 or 3 h), similar to the dry storage negative control. Previous research has shown that teeth kept dry in a room atmosphere showed inflammatory resorption on almost the entire surface (50). Dental specimens allowed to air dry for 1 h had half of the periodontal root surface involved by ankylosis (46) and showed a high incidence of replacement resorption (9). Dry storage, independently of time and temperature, is harmful to PDL cells and should be avoided (25).

The present study showed radiographically that the resorption was more intense for the maxillary teeth, supporting the hypothesis that the degree of bone remodeling is higher in the maxilla than in the mandible, and that the reimplanted maxillary teeth participate more easily in the remodeling process (51). Only one other published report has shown the degree of success of tooth replantation to be superior in mandibular teeth than in maxillary teeth (52). In other studies, similar results may have been masked because the groups of teeth included both maxillary and mandibular teeth in the same group.

Inflammatory resorption was observed in the cervical third of the maxillary teeth, independent of the storage medium. When the teeth were maintained in the propolis and saliva media, fracture and loss of crown or total loss of the tooth occurred, unlike teeth maintained in milk and HBSS, which showed controlled resorption over several months. The resorption process in this zone may also be explained by the action of the active extremity of the forceps, which causes trauma to the cervical root at the moment of dental extraction (37).

The present results showed that the propolis extract was able to maintain the functional viability of PDL cells, but it enabled the occurrence of the root resorption process after teeth replantation in dogs under the present experimental conditions. Therefore, further studies should be performed to assess the response of adjacent tissue to apical foramen and improve the propolis extract before it can be used as a storage medium for avulsed teeth.

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