Dental Traumatology

Dental Traumatology 2008; **24**: 510–514; doi: 10.1111/j.1600-9657.2008.00648.x

Periodontal healing of replanted dog teeth stored in milk and egg albumen

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Accepted 5 July, 2007

Abstract – The type of storage medium used to store avulsed teeth prior to replantation has been shown to be a decisive factor in periodontal ligament (PDL) healing. The aim of the present study was to investigate the effect of storage medium on periodontal healing. Thirty teeth from three dogs were endodontically treated to prevent subsequent inflammatory root resorption. The teeth were atraumatically extracted and randomly stored in milk or egg albumen for 3, 6 and 10 h at 4°C. All teeth were splinted for 1 weeks after replanting. After 2 months animals were sacrificed using vital perfusion-fixation and teeth were histologically prepared and evaluated following Andreasen's method. It was found that teeth stored in egg albumen for 6 and 10 h had significantly higher incident of PDL healing than those treated with milk for the same period ($P < 0.05$). . The highest incidence of PDL healing was observed in teeth stored in egg albumen for 6 h. The least surface resorption was also evident in this group ($P < 0.05$). The result of this study shows that egg albumen is an excellent storage media for up to 10 h considering its likely availability at most accident sites.

Avulsion, a serious injury to teeth, is probably more common than dental caries (1). On one hand, avulsion can affect the pulp and periodontal ligament (PDL) tissues as well as dental hard tissues, alveolar bone and gingiva. On the other hand, progressive root replacement resorption is a complication associated with the replantation of avulsed teeth (2). The status of periodontal ligament and pulps is critical for the healing of replanted teeth. Several factors including length of the extra alveolar time, use of suitable storage media and the type and duration of splinting are important in replantation success rate (3–6). Dry storage of avulsed teeth leads to death of PDL cells attached to the root. To slow down this process, it is essential to keep these teeth in a protective medium. Presently, several media including milk, Viaspan® (Dupont Pharma, Wilmington, Denmark), Hank's balanced salt solution (HBSS), saliva, water and many others are recommended for this purpose. Many studies have focused on the effect of these media on periodontal healing of avulsed teeth. Among these media, HBSS and Visapan® have been proved to be superior to milk (2, 6–8). Visapan has also shown potential value as a superior long-term storage medium (9). The American Academy of Endodontists has recommended the use of HBSS as an acceptable storage medium for avulsed teeth because of its capability to maintain vitality and proliferative capacity of PDL cells for up to 48 h at room temperature (10, 11). In Germany and Switzerland, a tooth rescue box (Dentosafe; EMT tooth saver) has been introduced and distributed in all schools (12).Nevertheless, synthetic media are seldom available near the site of an accident rendering their use rather impractical and only of academic interest. Therefore, it would be very useful to find an easily accessible storage medium, which offers a suitable environment for PDL cells. Egg albumen seems to be a good choice because of its high amount of protein (albumen), vitamins, water, and additional lack of microbial contamination (13, 14).

The objective of the present study was to evaluate the effectiveness of egg albumen as a storage medium for avulsed tooth in dogs.

Materials and methods

Three dogs were used in this experiment. All six incisors and four premolars from both jaws with healthy periodontal tissues, no apparent caries, and closed apices were chosen. To prevent inflammation resorption, root canal treatment was performed under aseptic condition before tooth extraction. Access cavities were prepared using a diamond taper bur. Canals were instrumented using K files and subsequently irrigated with 5% NaOCL solutions followed by water. Obturation was carried out with gutta–percha (Aria dent, Tehran, Iran) and AH26 sealer (Dentsply Detroy GMBH, Dentsply International Inc, York, PA, USA) by the lateral condensation technique. Pulp chambers were filled with amalgam (Aristaloy, Enagelhard Clauk, UK). Teeth were then atraumatically extracted and randomly stored in milk or

egg albumen for 3, 6, 10 h at 4°C. At the end of the storage periods, root surfaces and sockets were gently rinsed with 5mL of normal saline to remove debris and blood clot and then smoothly replanted in their sockets. As positive controls, three teeth were replanted after drying for 1 h without using any storage media whereas negative control included same number of teeth replanted immediately after extraction. Therefore, of total 30 teeth, 24 teeth in experimental groups and 6 in positive and negative controls were used. Periapical radiographs were taken to check the correct repositioning of the teeth. Teeth were then splinted with wire and resin composite for 1 weeks. Animals were given soft diet with no additional drugs such as antibiotics or supplement. Two months after removing the splints, and following permission by the ethical committee, dogs were sacrificed using vital perfusion-fixation (15). Animals' upper and lower jaws were removed and sectioned with 5 μm thickness at 500 μm intervals from apical foramen. Four sections were prepared from each tooth and stained by the Oral Pathology Department of the Torabinejad Research Center and subsequently examined for histological findings using the Andreasen criteria (16). The following points were noted:

1 Normal periodontium (PDL)
2 Surface resorption (SR)
3 Inflammatory resorption (IR)
4 Replacement resorption (RR) or ankylosis (A)

Each surface was divided into eight areas and in each area, the dominant condition was determined. In sum, 32 divisions were evaluated in each group of four teeth and incidents of any of the above conditions were determined (Fig. 1). Comparative effects of milk and egg albumen on PDL healing including resorption and replacement, were analysed using SPSS software (version 11, SPSS Inc., Chicago, IL, USA) and the chi-squared tests.

Results

During the experiment, one tooth form each of following groups was lost: 6 and 10 h storage in milk, 6 h storage in egg albumen, positive and negative controls. Histometric results are shown in Table 1 and Fig.2. After 3 h storage, PDL healing seemed to be higher in teeth stored in milk than in egg albumen, although the difference was not significant ($P > 0.05$). In contrast, PDL healing in positive controls (teeth dried for 1 h before replanting) was significantly lower than in other groups ($P < 0.05$). The highest incident of RR was evident in positive controls ($P < 0.05$), whereas teeth stored in egg albumen for 3 h exhibited no RR. After 6 h, teeth treated with egg albumen showed significantly higher levels of PDL healing compared with those treated with milk ($P < 0.05$). Storage in egg albumen for 10 h resulted in significantly higher PDL healing and lower amount of IR and SR. ($P < 0.05$).

Table 1. Periodontal ligament (PDL) healing related to type of media and duration of storage

	Milk			Egg albumen		
	3 h	6 h	10 h	3 h	6 h	10 h
PDL healing	64.9	11.5	11.5	59.4	71.9	59.4
Surface resorption	14.1	35.4	39.4	14.1	12.5	28.1
Inflammatory resorption	10.2	30.2	24	7	5.2	1.6
PDL inflammation	10.2	22.9	24	19.5	10.4	3.9
Replacement resorption	0.8	0	1	0	0	7

Values are expressed in percentage.

In sum, teeth stored in egg albumen showed the highest incidence of PDL healing, while those stored without any media exhibited the highest incidence of RR and SR. No RR was evident in teeth stored in milk or egg albumen for 6 h, although those treated in egg albumen for 3 and 10 h did show some levels of RR. IR was also much less for teeth treated in egg albumen ($P < 0.05$). Significantly higher inflammation of PDL was recorded for teeth stored in milk for 6 and 10 h, although surprisingly no significant difference was observed between teeth stored for 3 h in milk or in albumen.

Negative and positive controls showed no evidence of IR. Yet, both controls especially positive controls displayed significantly higher levels of RR compared with milk- and albumen-treated samples (Table 2).

Discussion

The prognosis of avulsed and replanted teeth is dependent on the periodontal healing and amount of

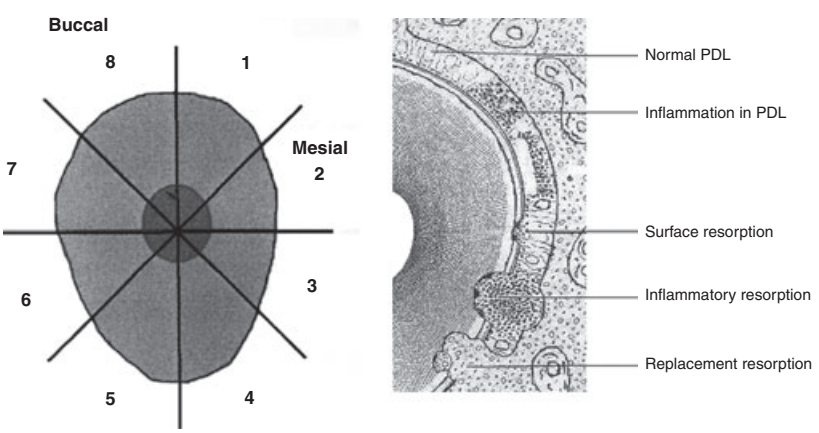

Fig. 1. Schematic presentation of analysis of replanted roots with drawing four lines on cross-sectioned teeth (22).

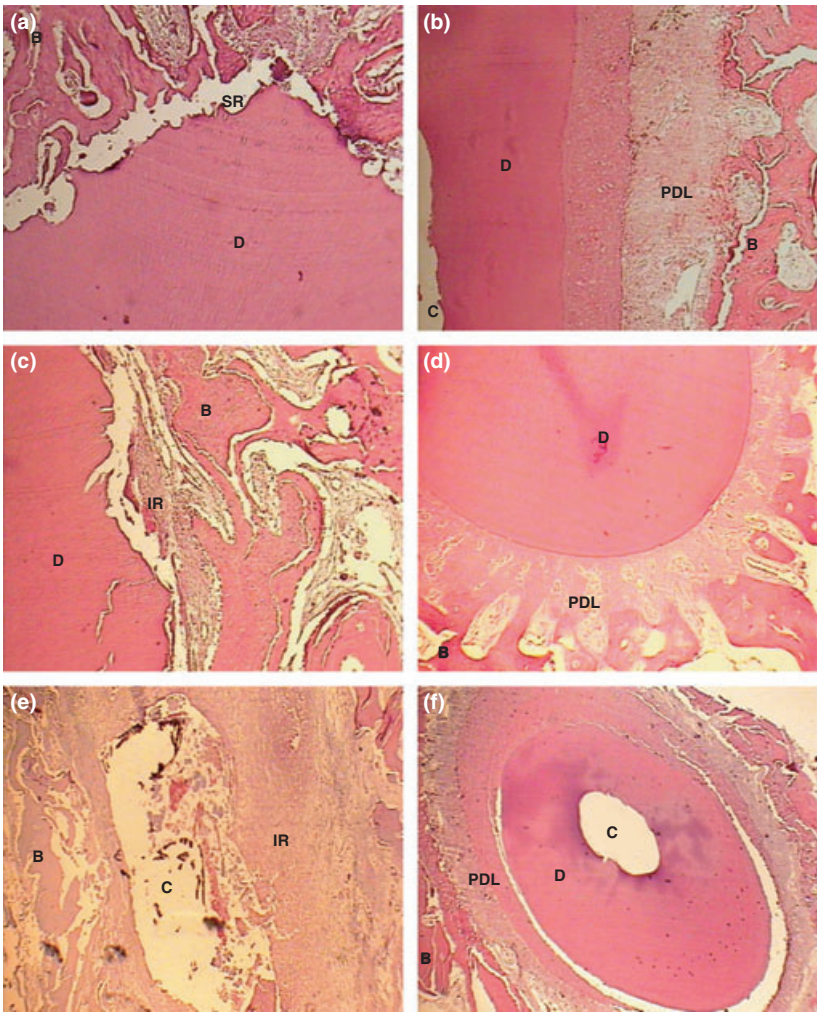

Fig. 2. Photomicrographs of the various histopathological scoring of the periodontal ligament (PDL) of the replanted teeth stored in milk and egg albumen for periods of 3, 6 and 10 h. (a) Surface resorption (3 h storage in milk). (b) Normal PDL (3 h storage in egg albumen). (c) Inflammatory resorption (6 h storage in milk). (d) Normal PDL (6 h storage in egg albumen). (e) Inflammatory resorption (10 h storage in milk). (f) Normal PDL (10 h storage in egg albumen). C, canal; D, dentin; PDL, periodontal ligament; SR, surface resorption; IR, inflammatory resorption; A, ankylosis; B, bone.

Table 2. Periodontal healing related to type of storage media

	Milk	Egg albumen	Positive group	Negative group
PDL healing	32.8	62.8	0	62.8
Surface resorption	28.1	18.8	31.3	17.2
Inflammatory resorption	20.3	4.5	0	0
PDL inflammation	18.1	11.4	0	12.5
Replacement resorption	0.6	2.6	68.8	7.8

Values are expressed in percentage.

root, PDL, and surface resorption. Hammer (17) first claimed the importance of PDL viability prior to replantation when he demonstrated that the amount of viable periodontal membrane directly affects the length of survival of a replanted tooth. Researchers have made many attempts to find storage media that are able to preserve PDL cell viability and prevent unpredictable squeal of inflammatory root resorption or replacement resorption post replantation. To this end, several materials including milk, normal saline, saliva, HBSS have been also recommended each with certain advantages and disadvantages. This study aimed to investigate the effect of different storage media on the above criteria following the method used by Trope and Friedman (2).

In consideration of earlier published data, to prevent inflammatory root resorption resulting from infection of root canal, all teeth used in this experiment were subjected to root canal treatment prior to extraction (18). During this study, two teeth belonging to milk-treatment groups and one belonging to positive controls were lost. The loss of these teeth was presumably caused by severe inflammatory reaction and as it occurred in the milk-treated teeth after periods of 6 and 10 h, it is probably indicative of milk limitation as a storage medium for long storage.

Teeth replanted after drying for 1 h (positive control group) showed an extremely high incidence of

replacement resorption, but no inflammatory root resorption. This finding was in agreement with previous studies (2, 6, 19–21). In contrast, teeth immediately replanted (negative control group) showed no complication and only small isolated areas of SR (17.2%), RR (7.8%) and PDL inflammation (12.5%). No evidence of inflammatory root resorption was observed in this group. The incidence of PDL healing was extremely high for this group (62.8%).

In this study, parallel to previous studies, milk was found to be a suitable storage medium for a period of 3 h (4, 7, 8). During this time, incidence of PDL healing was extremely high (64.8%), while storage in milk for 6 and 10 h showed only 11.5% PDL healing. Previous studies recommended milk as an excellent storage medium for up to 6 h considering its availability at most accident sites (2, 22, 23).

In this study, egg albumen showed extremely high incidents of PDL healing after 6 and 10 h, similar to the negative group. It could be explained that, the environment of socket between 24 and 48 h might have resulted in surface reactions that interfered with the superior capacity of egg albumen to maintain the health of periodontal ligament cells. Generally, the incident of RR was rather low in this study, a fact which is probably explained by short-term splint (1 week) compared with other studies with 2 weeks splint (13). Khademi et al. investigated the viability of fibroblast cells in PDL stored in milk and egg albumen. According to their results, the viability of egg albumen was similar to that of HBSS after different periods of time (14). In another study by Blomlof with a type of milk (Filmjolk, Alra Foods Inc., Sweden), PDL cells survived for only 1 h, which was probably because of the low pH milk (4.2–4.5). In an *in vitro* study by Rozenfarb et al. (24), viability of skin fibroblasts in several culture media including milk, egg albumen, saliva and MEM (a culture medium) was investigated. No significant difference was observed amongst MEM, egg albumen and milk, although all were superior to saliva. It was also found that the osmolarity of the MEM, milk and egg albumen ranged from 251 to 298 mOsm kg^{-1}, whereas the saliva was hypotonic, with an osmolarity of 73 mOsm kg^{-1}, which could explain the observed difference. This *in vitro* investigation was conducted for periods of only 15, 45, and 90 min, whereas in this study, longer storage times were applied. Therefore, it could explain the discrepancy between our study and that by Rozenfarb. It is also worth noting that fibroblasts from skin are different from those derived from PDL. In another study by Hiltz and Trope, although the vitality of human lip fibroblasts in milk maintained a high percentage after 6 h (68.2%), Viaspan was the most effective storage medium at all observation periods. It even displayed 37.6% cell survival after 168 h storage (9). Previous studies have also shown that fibroblasts from the gingiva, lip and skin have similar morphology, but are different in amount of growth, producing of protein, collagen, alkaline phosphates (6, 8, 25).

Based on the results of this study, it can be concluded that egg albumen is a favourable storage medium for up to 10 h considering its availability at most accident sites. More studies need to be conducted for further information.

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