Periodontal healing of replanted monkey teeth prevented from drying

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Root resorption of replanted teeth is dependent on the duration of the extra-alveolar period and on the storage environment. In the present investigation the significance of preserving the humidity of the periodontal ligament (PDL) during the extra-alveolar period was tested on isolated PDL cells and on replanted monkey teeth. The isolated PDL cells were tested with respect to cell viability (trypan blue exclusion test) and to cell recovery (number of cells after additional cultivation). About 70% of the cells were viable and 44% recovered after 1 h in a humid atmosphere. Practically no cells were viable or recovered after 1 h of drying. Replanted teeth that had been wrapped in plastic foil for 1 h before replantation showed no more resorption than immediately replanted teeth. This is in contrast to teeth dried in air for 1 h before replantation. They showed extensive root resorption on almost all root surfaces. Thus, prevention of evaporation of tissue fluid from the PDL must be considered a primary goal if the tooth cannot be replanted immediately. □ *Cell culture; periodontal ligament; replantation; root resorption; tooth*

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Root resorption in replanted teeth is dependent on the duration of the extra-alveolar period and on the storage environment (1–3, 5, 10, 17, 19–21, 24, 25). Exarticulated teeth should be replanted as soon as possible (5). Thirty to 40 min seems to be the limit of drying of the periodontal ligament (PDL) to avoid severe root resorption (10, 17).

Experimental storage of exarticulated teeth in Eagle's medium (11), saline (7), milk (15), or saliva (7, 15, 25) has been shown to be superior to dry storage (7, 15, 25). The composition of different available storage media has lately been studied (12, 16, 22). The importance of a physiologic osmolality, ionic strength, and macromolecular content without contamination of microorganisms has been emphasized (12, 22). In light of these investigations the optimal storage medium would be the tissue fluid of the PDL on the exarticulated tooth. The tissue fluid of the PDL of exarticulated teeth should thus be prevented from evaporating before replantation. By using a previously developed cell culture system (13), this theory could be studied in vitro by keeping PDL cells in a humid atmosphere, followed by viability and recovery studies.

The purpose of the present investigation was to compare such an in vitro study with an in vivo experiment in which the tissue fluid of the PDL was prevented from evaporating before replantation.

Materials and methods

General procedure. Isolated human PDL cells were stored in Eagle's medium, 100% relative humidity, or room atmosphere for 60 min. After the storage periods cell viability and cell recovery were determined. Extracted monkey teeth were wrapped in plastic foil to prevent fluid evaporation. After 60 min the teeth were replanted. Eight

weeks later the animals were killed, and periodontal conditions were evaluated by the method of Andreasen (2, 4). The results were compared with those of immediately replanted teeth and of air-dried teeth.

Cells. Human PDL cells were obtained as described earlier (14, 23). The cells were divided into three groups, each containing 10^5 cells, and were seeded in Linbro multidish trays (Falcon Plastics). After they had been cultivated for 3 days at 37°C in air + 5% CO_2 in Eagle's medium (18), supplemented to contain 4 mM L-glutamine, 10^5 IU/l penicillin, 100 µg/ml streptomycin, 10 µg/ml nystatin, and calf serum (10% v/v), the medium was soaked off, and one group of cell cultures was stored without medium in a hygrofore with 100% relative air humidity at 20°C for 60 min. A second group of cultures was stored in 1 ml Eagle's medium in the humid atmosphere at 37°C in air + 5% CO_2 for 60 min. The third group of cell cultures was stored in room atmosphere (60% relative air humidity) without medium at 20°C for 60 min.

Cell viability. After storage the dishes were washed twice with 2 ml physiologic salt solution without calcium and magnesium. One milliliter 0.025% trypsin + 0.02% EDTA were added to the dishes, and the cells were detached, soaked off, and centrifuged at 1000 *g*. Next, 0.2 ml Eagle's medium and 0.2 ml 0.2% trypan blue in Hanks' balanced salt solution were added to the tubes, and viable (non-stained) and non-viable (stained) cells were counted in a hemocytometer. The experiments were carried out in triplicate.

Cell recovery. After storage the PDL cells were cultivated for another 3 days in 2 ml Eagle's medium. The cultures were then trypsinized, and the total number of cells was counted. The experiments were carried out in triplicate.

Experimental animals. Eight monkeys (*Macaca fascicularis*) were used in the experiment, and the teeth of one additional monkey were used as control. The kidneys of the monkeys were used in the production of inactivated poliomyelitis vaccine at the National Bacteriological Laboratory. On the day of extraction and replantation the animals were only given bananas. From the day after replantation and throughout the experimental period the animals were fed with hard pellets (Astra-Ewos, Sweden) and fruit. During the extraction and replantation of the teeth the monkeys were anesthetized with 6% pentobarbital sodium (Nembutal®, Abbott), 30 mg/kg body weight.

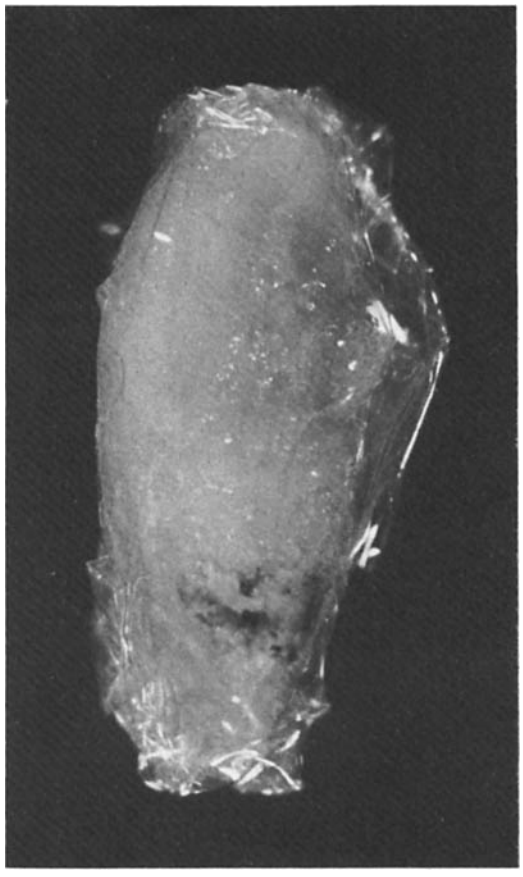

Fig. 1. Extracted maxillary lateral incisor wrapped in soft, thin plastic foil.

Teeth, storage conditions, and replantation. The four permanent mandibular and maxillar lateral incisors of each monkey were extracted under similar conditions with respect to time and trauma. All teeth had completed root formation. Immediately after extraction eight teeth, four mandibular and four maxillar, were wrapped in thin, soft plastic foil (polyethene, Essem Plast AB,

Sweden) and kept outside the mouth for 60 min at 21°C (Fig. 1). Fifteen teeth, seven mandibular and seven maxillar, were immediately replanted, and nine teeth, five mandibular and four maxillar, were allowed to dry in air at 55% relative humidity and 21°C for 60 min before replantation.

The teeth were replanted without removing the clotted blood in the alveoli. No splinting was used. After 8 weeks the the animals were killed, and the frontal parts of the jaws including the replanted teeth were removed *en bloc*. The same parts of the jaws were removed from the control monkey, whose teeth had not been extracted and replanted.

Histological procedure. The tissue blocks were immediately fixed in cold 10% neutral buffered formalin for 48 h. Demineralization was performed in 5% formic acid. After infiltration and embedding in paraffin the specimens were sectioned perpendicular to the long axis of the teeth. The blocks were sectioned in step-serial sections (5 μm) at levels 70 μm apart. The sections were stained with hematoxylin and eosin.

Evaluation. All sections were examined in a light microscope. For the morphometric evaluation 25–30 evenly distributed sections were examined from the level at which the root cervically is completely surrounded by bone to the apex of the root. The gingival part above the alveolar bone was excluded because of possible trauma from the forceps during extraction and of gingivitis in both experimental and control groups. The tissue reactions were evaluated by the method of Andreason (2, 4). The following groups were distinguished: 1) *Normal periodontium.* Histologically normal peridontal ligament in intact cementum. 2) *Inflammation in the PDL without root resorption.* Infiltrates of inflammatory cells in the PDL. 3) *Surface resorption.* Resorption lacuna on the root surface without inflammatory infiltrate. 4) *Inflammatory resorption.* Resorption lacuna on the root surface infiltrated with inflammatory cells. 5) *Replacement resorption.* Alveolar bone in contact with cementum or dentine.

A hair cross-fitted in the eyepiece of the microscope was placed over the center of the root canal. The four lines of the hair cross-intersected with the root surface at the buccal, lingual, or proximal surface. The periodontal conditions where the lines crossed the root surface were registered as described above. The frequency was calculated from the number of measuring points showing the previously defined criteria, divided by the total number of measuring points ×100.

Statistics. In testing differences of means, Student's *t* was used.

Results

Storage of cultured PDL cells in a humid atmosphere caused little cell damage compared with that to cells that were allowed to dry in room atmosphere. Almost all of the cells kept in room atmosphere were nonviable. Similar results were obtained in the in vivo study. Teeth that had been prevented from drying during the extra-alveolar period showed very little root resorption. In fact, the periodontal conditions in these teeth were similar to those seen in the immediately replanted teeth. The teeth that were allowed to dry in room atmosphere showed inflammatory resorption on almost the entire surface.

Cell viability. Less than 10% of the PDL cells survived 60 min of storage in room atmosphere. This was in contrast to storage in a humid atmosphere or in Eagle's medium. Sixty-eight percent of the cells were viable after 60 min of storage in a humid atmosphere without culture medium. Almost all of the cells kept in Eagle's medium were viable after 60 min (Table 1).

Cell recovery. No cells could be further cultivated after 60 min of storage in room atmosphere. Cell recovery after storage without medium but in a humid atmosphere was almost 50% of the total number of the control cells stored in Eagle's medium (Table 2).

Replanted teeth. No teeth were lost during the experiment despite the fact that no splinting was used. Student's *t* test showed no significant differences with regard to periodontal conditions between the teeth that had been wrapped in plastic foil for 60 min before replantation and the immediately replanted teeth (Table 3 and Fig. 2). Less than 10%

Table 1. Viability of PDL cells after storage without medium in a humid atmosphere, in room atmosphere, or in Eagle's medium. Three samples were analyzed

Storage conditions	Storage time (min)	Storage temp (°C)	Cell viability in % of total no. of cells in Eagle's medium	
			Mean values	Range
Eagle's medium	60	37	95	98–90
Humid atmosphere (rel. humidity 100%)	60	20	68	75–65
Room atmosphere (rel. humidity 60%)	60	20	7	11–1

Table 2. Recovery of PDL cells after storage without medium in a humid atmosphere, in room atmosphere, or in Eagle's medium and further cultivation for 3 days in Eagle's medium. Three samples were analyzed

Storage conditions	Storage time (min)	Storage temp (°C)	Cell recovery in % of total no. of cells in Eagle's medium	
			Mean values	Range
Eagle's medium	60	37	Controls (100)	
Humid atmosphere (rel. humidity 100%)	60	20	44	48–32
Room atmosphere (rel. humidity 60%)	60	20	0	

Table 3. Periodontal conditions of replanted monkey teeth after immediate replantation, storage in plastic foil or storage in room atmosphere, and an observation time of 8 weeks. Periodontal conditions are defined in Materials and methods. Values are based on the number of measuring points showing defined criteria divided by total number of measuring points × 100

Storage conditions	No. of teeth	Storage time (min)	Periodontal conditions, in %				
			1	2	3	4	5
Immediate replantation	15	—	88	5	3	4	0
Wrapped in plastic foil	8	60	96	0	2	2	0
Room atmosphere (rel. humidity 55%)	9	60	3	1	3	88	5

of the root surface in each tooth was undergoing resorption. Both surface resorption and inflammatory resorption were seen. All nine teeth that had been allowed to dry in room atmosphere for 60 min before replantation showed inflammatory resorptions on almost the entire root surface (Table 4 and Fig. 3). Replacement resorption was seen on 5% of the root surface in the teeth kept in room atmosphere. There were no significant differences between mandibular and maxillar incisors with regard to resorp-

Table 4. Comparison between ocurrence of inflammatory resorption and total root resorption (surface, inflammatory, and replacement) in teeth after immediate replantation, storage in plastic foil or in room atmosphere before replantation. The calculations are described in Materials and methods

Storage conditions	Storage time (min)	Inflammatory resorption, %		Total root resorption, %		Probability of root resorption after storage in room atmosphere being different from that after immediate replantation or wrapped in plastic foil	
		Mean	Range	Mean	Range	Inflammatory resorption	Total root resorption
Immediate replantation	—	4	23–0	7	23–0		
Wrapped in plastic foil	60	2	9–0	4	15–0	$P < 0.001$	$P < 0.001$
Room atmosphere (rel. humidity 55%)	60	88	100–56	96	100–85	$P < 0.001$	$P < 0.001$

tion frequencies. In nine teeth, regardless of the treatment, the most apical millimeter of the pulp seemed to be vital, while the other teeth showed no signs of vital pulpal rests. The non-replanted control teeth did not exhibit any resorption.

Discussion

Dry storage of exarticulated teeth before replantation results in massive resorption and subsequent ankylosis (3, 7, 10, 17). A vital PDL seems to be the most important factor for a successful healing without root resorption (6, 24). If the tooth cannot be immediately replanted, various storage media have been tested to preserve the vitality of the PDL. Physiological media have given the best results (7, 11, 15, 25), whereas unphysiologic media, such as tap water, may cause as much root resorption as dry storage (7). Most of the available storage media are artificial or have a composition that differs from the natural fluid surroundings of the tooth in the PDL. It would thus seem most natural to preserve the tissue fluid in the PDL of an exarticulated tooth rather than keeping it in some artificial storage medium. In the present study this hypothesis was tested both in vitro and in vivo. Isolated PDL cells could survive in high numbers in a humid atmosphere without culture medium. This result was obtained with the trypan blue exclusion test, which is a routine laboratory test of cell viability. However, this test does not give any information about the mitotic potential of the cells. This ability would seem to be critical for a successful healing. In the study of cell recovery, it was shown that this capacity was well preserved after humid storage.

In the in vivo study the results from the in vitro experiment was put into practical use. To preserve the tissue fluid in the PDL, the extracted teeth were wrapped in plastic foil. This procedure apparently prevented evaporation, since no more resorption was found in these teeth than in the immediately replanted teeth. The air-dried teeth in the reference group were covered by inflammatory resorption on almost all root surfaces.

Fig. 2. Normal PDL and intact cementum layer of mandibular lateral incisor that has been extracted and stored in plastic foil for 60 min before replantation. (×30.)

Surface resorptions that penetrate the intermediate cementum may develop into inflammatory resorption and become progressively aggresive owing to toxic influence of the necrotic pulp (8–10, 17). Endodontic treatment has been shown to result in replacement resorption in teeth that have been allowed to dry in air before replantation (2, 8). Replantation without any endodontic treatment would thus make the root surface more susceptible to resorption, since a primary injury or resorption will be amplified by the necrotic pulp. In the present study no trauma other than the extraction trauma was exerted on the immediately replanted teeth. It seems likely that the teeth kept in plastic foil for 60 min suffered no additional trauma other than the extraction trauma. These minor damages were not influenced by the necrotic pulp to any greater extent than the immediately replanted teeth, since they showed no more inflammatory resorption than these teeth (Table 4).

The results of the in vitro study of cell viability and cell recovery after storage in a humid atmosphere showed good correlation with the results of the in vivo study of periodontal healing of teeth in which fluid evaporation from the PDL was prevented during the extra-alveloar period. Apparently no other trauma was added to the extraction trauma during the extra-alveolar period, as indicated by the periodontal condition in both groups. Thus, prevention of evaporation of tissue fluid from the PDL must be considered a primary goal if the tooth cannot be replanted immediately.

The wrapping of traumatically exarticulated teeth in plastic foil may have a limited clinical application. It may be difficult to

Fig. 3. Extensive resorption and lacuna infiltrated with inflammatory cells on root surface of mandibular lateral incisors that have been allowed to dry in room atmosphere for 60 min before replantation. (×30.)

carry out in an emergency situation in such a manner that the PDL is not damaged and completely prevented from evaporation. On the other hand, the results are of considerable theoretical interest, since they emphasize the importance of a vital PDL for successful replantation. They also point at a useful method for further studies of PDL and of various aspects of the replantation procedure without the influence of any other storage medium than the tissue fluid itself.

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