The effect on periodontal and pulpal tissues of various cleansing procedures prior to replantation of extracted teeth

An experimental study in monkeys

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The effect of cleansing the root surface of extracted teeth in either saliva or tap water prior to replantation was studied in green Vervet monkeys (Cercopithecus aethiops). Maxillary central- and mandibular lateral incisors and mandibular first molars were extracted. The extra-alveolar period before replantation was 10 sec. During this period the root surface was cleaned with either saliva or tap water. The replanted teeth were examined histologically after 8 weeks. The histometric analysis showed significantly more normal periodontium and significantly less inflammatory root resorption in teeth cleaned in tap water than in teeth cleaned in saliva. The beneficial effect of this rinsing procedure in clinical practice in case of mature teeth is dubious, as inflammatory resorption is normally controlled by pulp extirpation and a root canal filling. Furthermore, the abovementioned finding is based on the use of tap water with only a moderate chlorine concentration, leading one to suspect that tap water with higher chlorine concentrations may be detrimental to healing. Provisional guidelines for the immediate treatment of avulsed teeth are suggested.

Key-words: Histopathology; histometry; root resorption; storage of avulsed teeth

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In most clinical situations, avulsed teeth can be stored temporarily in the oral cavity (i.e. in saliva), in tap water or in physiologic saline prior to replantation. Immediate replantation reduces the extra-alveolar period and thus increases the possibility of successful healing (3). As all avulsed teeth are expected to be contaminated, the problem arises concerning cleansing of the root surface before replantation. Actual sterilization of the root surface leads to extensive root resorption (5, 6, 8, 11). Two approaches thus remain, the patient replants the tooth after sucking it clean or after brief rinsing in cold tap water. To date, nothing is known about the effect of these two

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cleansing procedures on pulpal and periodontal healing. In a previous experiment, it was demonstrated that *prolonged storage* (i.e. 120 min) of monkey incisors in tap water led to extensive root resorption (3). However, some patients were examined by the authors where teeth had only been *rinsed* in tap water before replantation. These cases demonstrated periodontal healing without root resorption, indicating that brief rinsing in tap water possibly has a different effect on periodontal ligament cells from long-term storage. The other possibility is rinsing in saliva. Such a procedure also raises the question of effect on cells of the periodontal ligament and the pulp. In recent experimental studies, it was demonstrated that storage of teeth for periods up to 2 hours in saliva resulted in minimum root resorption (3, 10).

It thus appears that both cleansing procedures could be of value. In order to inform the public of appropriate handling of avulsed teeth prior to replantation, it is necessary to know the effect upon both periodontal and pulpal healing of the abovementioned treatment/storage procedures.

The purpose of the present study was, therefore, to compare periodontal and pulpal healing after brief cleansing of the root surface in either saliva or tap water prior to replantation.

MATERIAL AND METHODS

The material consisted of 6 green Vervet monkeys (Cercopithecus aethiops) weighing from 2.9 to 4.5 kg. Their incisor roots were completely formed. For the experiment the monkeys were anesthetized with phencyclidene (Sernylan®, Park Davis & Co., Detroit, USA) 2.5 mg/kg body weight, supplemented with pentobarbithone sodium (Mebumal®, DAK) 30 mg/kg body weight. The experimental teeth were the maxillary central- and mandibular lateral incisors and the mandibular first molars. The teeth were extracted with forceps using gentle luxating movements.

Altogether 6 pairs of contralateral teeth were extracted in each monkey. In two monkeys, it was not possible to replant the lower incisors, due to fracture during the extraction procedure. All pairs of teeth were distributed by lot into the following two experimental groups.

1. Immediate replantation after storage for 10 s in saliva. During this period, the tooth was held by the crown with forceps and pressed against the buccal mucosa. The cheek was then massaged against the root surface, imitating the sucking action for cleaning an avulsed tooth. The tooth was then replanted.

2. Immediate replantation after rinsing for 10 seconds in cold tap water. The tooth was held by the crown with forceps and placed under running cold tap water for 10 s and then replanted. The content of the tap water used appears in Table 1; the temperature was 10.1°C ± 0.85 S.D.

The teeth were left unsplinted. Following these procedures, the animals were allowed to resume normal activity and were maintained on a powdered diet (Standard diet for monkeys, Statens Seruminstitut, Copenhagen) supplemented with white bread and bananas.

The monkeys were sacrificed 8 weeks later. The anterior portion of the maxilla and mandible, containing the central- and lateral incisors, and the lateral portions of the mandible, containing the permanent molars, were removed. The tissue blocks were placed in 10 % neutral buffered formalin and demineralized in a 10 % solution of EDTA at pH 6.9. After double infil-

Table 1. *Chemical composition of tap water used in the present investigation*

Component	Concentration (mg/100 ml)*
Sodium	7
Chloride	12
Phosphorus	0
Calcium	11
Magnesium	2
Potassium	0.5
Fluorine	0.05
Iron	0.005
Sulfur	0
Chloramine	0.015
pH	7.5
Osmolarity	12**

* Values reported by the Copenhagen Water Supply Laboratory.
** Values reported by the University Hospital Pharmaceutical Laboratory, Copenhagen.

tration in celloidin-paraffin, the tissue blocks were sectioned perpendicular to the long axis of the incisors and molars at a thickness of 5 µm. The teeth were sectioned in step-serial sections at 500 µm intervals. At each sectioning level, one section was stained with hematoxylin-eosin.

A projection microscope (Reichert Visopan®) was used for the histometric study. Sections were projected on a 200 mm disc-type screen using a magnification of x 130 or x 520. All sections were evaluated blindly in relation to the clinical factors examined. A cross formed by 2 lines intersecting perpendicularly was placed over the projected cross-section of the root and oriented according to the labio-lingual axis of the tooth. At the intersections of the two lines and the root surface, corresponding to the mesio-distal and labio-lingual axes of the tooth, changes in the periodontium were noted, i.e. four measuring points were used for the incisors. For molars, each root was rated separately, whereafter the scores were pooled. The character of the periodontal changes was classified as follows: (1) normal periodontium, (2) inflammation in the periodontal ligament without root resorption, (3) surface resorption, (4) inflammatory resorption, (5) replacement resorption and (6) downgrowth of pocket epithelium below the cementoenamel junction. Details of this classification have been presented in a previous publication (2). An average of 15 sections were examined for each tooth. A pathology index was calculated for each tooth as follows: the number of measuring points showing a certain type of periodontal change was divided by the total number of measuring points on each tooth x 100 (2). The extent of vital pulp was registered at each sectioning level. In this context, the following 4 zones were registered, descending from the apical foramen to the coronal part of the pulp: (1) pulp with normal-appearing connective tissue cells and no inflammation, (2) pulp with normal-appearing connective tissue cells and inflammatory cells, (3) leukocyte zone without a stroma of connective tissue, (4) pulp tissue with evidence of necrosis (karyopyknosis, karyorrhexis, or lysis of cell nuclei). The extent of vital pulp tissue was defined as the combined length in µm of zones 1 and 2 from the apical foramen and was calculated from step-serial sections (3).

In the statistical analysis, a randomization test for two dependent samples was used; 5 % was considered as the critical probability value (12).

RESULTS

The results of the histometric analysis of the paired teeth are shown in Table 2. There was a significantly greater extent of normal periodontal ligament in the tap water group than in the saliva group ($p = 0.008$). This apparently corresponds to the significant decrease in

inflammatory resportion ($p = 0.04$) and the slight, but insignificant, increase in the length of vital pulp in the tap water group. No other histometric registration between the two groups showed any significant difference.

Table 2. *Pathology index related to experimental groups*

Experimental group	No. of teeth	Normal periodontium (%) $\bar{x}$ SD x*	Periapical inflammatory changes (%) $\bar{x}$ SD x*	Surface resorption (%) $\bar{x}$ SD x*	Inflammatory resorption (%) $\bar{x}$ SD x*	Replacement resorption (%) $\bar{x}$ SD x*	Downgrowth of pocket epithelium (%) $\bar{x}$ SD x*	Length of vital pulp tissue (mm) $\bar{x}$ SD
Saliva	16	67.9±27.54,16	5.1±9.55,8	8.5± 8.70,14	17.0±23.34,11	0.2±0.68,2	1.2±2.34,6	0.6±1.64
Tap water	16	77.9±19.58,16	1.8±2.90,7	9.5±11.09,12	8.7±12.35,10	1.4±3.91,2	0.7±1.14,5	2.0±2.93
Level of significance**		0.008	0.128	0.510	0.040	0.499	0.687	0.134

* = Number of teeth demonstrating a particular type of pathology.
** A comparison between the two groups according to a randomization test for two dependent samples.

DISCUSSION

In the present study, teeth rinsed in tap water prior to replantation demonstrated significantly more normal periodontium than teeth rinsed in saliva. Theoretically, several explanations could be suggested for this finding. First, the mechanical rubbing of the tooth against the oral mucosa in the saliva group could have had a detrimental effect upon healing. The lower temperature of the tap water could also influence healing. Finally, tap water rinsing could have reduced bacterial contamination of the periodontal ligament and in the apical zone of the pulp. It was not possible in the present study to establish which of these factors was responsible for the registered effect. However, when seen in the light of previous studies, it is possible that the elimination of bacteria by brief tap water rinsing led to increased pulp survival and thus reduced the extent of inflammatory root resorption (3,4,5).

As seen in Table 1, tap water has low electrolyte concentration and osmolarity. This was probably the cause of the detrimental effect on periodontal healing after replantation of long-term storage (120 min.) in tap water in a previous study using the same experimental model (3). The very brief exposure of the teeth to tap water used in the present study apparently did not damage cells in the periodontal ligament or pulp.

The observed beneficial effect of tap water rinsing on the reduction of in-

flammatory root resorption may not be of clinical value in the replantation of mature teeth, as the usual treatment in these cases is pulp extirpation and root canal treatment, which has been shown to be able to prevent or control inflammatory root resorption effectively (5). Of greater clinical interest would be enhanced pulp survival after tap water rinsing in replanted immature teeth where development of pulp necrosis and associated inflammatory root resorption usually compromise further root development and often result in loss of the tooth because of rapid progression of the resorption process. Another point which should be considered in relation to tap water rinsing is that the chlorine concentration in the tap water used was rather low. Chlorine concentration varies from one water supply to another and is often considerably higher than that seen here, something that could be detrimental to cell survival. Whether one would see the same detrimental effect after brief rinsing in highly chlorinated water is unknown at the present time and should be examined before general treatment guidelines for avulsed teeth are presented for public use. Until these studies are made, it is probably still safer to advocate storage of the avulsed tooth in the buccal vestibule until professional treatment can be obtained and the tooth replanted after brief rinsing in saline. This treatment appears to be supported by a recent experimental study in monkeys where storage of teeth in saliva for up to one hour resulted in only a slightly greater frequency of inflammatory resorption compared to immediate replantation (3).

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