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Ankylosis of experimentally reimplanted teeth related to extra-alveolar period and storage environment

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Abstract

*This study was designed to determine if avulsed permanent incisors which have been kept dry for a period and then immersed in an isotonic solution prior to implantation have an increased healing rate. The permanent maxillary incisors of six beagle dogs were grouped by lot in six treatment classes so that each type of incisor was included in each class. After extraction, the teeth of a treatment class were stored either dry, wet, or first dry and then wet. One week after reimplantation, splints were removed and all root canals cleansed and filled with calcium hydroxide. The dogs were sacrificed after three months and the teeth with premaxillas were removed and exposed to routine histologic treatment. Transverse sections were evaluated according to earlier described methods. All root surfaces of a treatment class were regarded as a homogeneous risk mass. The percentage of sites with ankylosis increased with increased dry time. Subsequent wet storage in Hank's solution decreased the frequency. Storage in just a wet environment for up to 60 minutes resulted in a negligible percentage of ankylosis. It was concluded that avulsed teeth which have been kept dry for 15 minutes or longer should be conditioned in an isotonic solution for about 30 minutes prior to reimplantation. When the dry time exceeds 30 minutes, there is a greatly increased risk of ankylosis after reimplantation. **

It is well-known that the risk of ankylosis in reimplanted teeth increases with extension of the extra-alveolar period, particularly if the teeth are stored dry during this period (for review see Andreasen[1]).

In a retrospective clinical study by Cvek et al.[2] the occurrence of ankylosis was analyzed systemically for the first time in relation to extra-alveolar period, with interaction between dry and subsequent wet storage as the modifying factor. Although the statistical evaluation revealed no more than trends, it was concluded that healing might be good after reimplantation of avulsed permanent incisors provided the teeth have been kept dry for not more than 40 minutes, and provided that those which have been kept dry for more than 15 minutes are placed in an isotonic solution for about 30 minutes prior to reimplantation. This presupposes that teeth with necrotic pulp are given adequate endodontic treatment with calcium hydroxide paste as a temporary root-filling material. Under such circumstances complications other than ankylosis need hardly be expected.

The material referred to in Table 1 has been rearranged according to the premises of the present investigation. It consisted of mature as well as immature teeth but did not cover all clinically relevant combinations of dry and wet storage. Therefore, an experimental series was performed in order to confirm or reject the assumptions based on the results from the retrospective study.

Methods and Materials

Six healthy beagle dogs, 15 months of age (two females and four males) previously included in a study on experimental gingivitis[3] were used. The permanent maxillary incisors (six teeth per dog) were selected for the experiment. All teeth demonstrated complete root formation as judged from intraoral radiographs. Each dog

Table 1. Rearrangement of the data from Cvek et al.[2] on the occurrence of ankylosis of 34 teeth reimplanted after accidental total dislocation, treated endodontically with calcium hydroxide due to pulpal necrosis.

Extraoral/time in minutes			Ankylosis	
dry +	wet*	Number/of teeth	n	%
–	0–60	9	1	11
10–15	0–65	6	1	17
20–40	0–10	6	4	67
20–40	25–60	4	0	0
≧60	0–60	9	9	100

* storage in isotonic environment
The rearrangement was made in order to create a basis for the present study.

* The present study, designed to experimentally test the results of the 1974 study by Cvek et al., closely agrees with their findings.[2]

contributed one tooth to each treatment class in such a way that the class always comprised six different incisors. These groups of teeth were assigned by lot to the particular treatments.

All procedures in the dogs were performed by one operator (LM) with the animals anesthetized by intravenous injection.[a] Prior to the experiment impressions were taken of the premaxillary area of the dogs, and acrylic splints were made which included the maxillary incisors and canines. The teeth were extracted gently with a forceps and stored either dry, wet, or first dry and then wet (Table 2). For dry storage the teeth were placed between gauze sponges at room temperature. For wet storage they were kept in a sterile flask containing Hank's solution[4] at a temperature between +5° and +10°C. Every five minutes the flasks were shaken gently in order to wash off autolyzed cell material from the root surface.

After the extraoral period, the blood clot that had formed in the sockets was removed with a sterile excavator, the teeth were carefully reimplanted, and the acrylic splint was fixed using phosphate cement. For two weeks postoperatively the maxillary front teeth were washed daily with 0.2% chlorhexidine solution.

One week after reimplantation, the splints were removed and the teeth were cleansed with pumice and a rotating rubber cup. A rubber dam was applied, then this and the teeth were washed with 3% hydrogen peroxide and 0.5% chlorhexidine in 70% alcohol. Palatal cavities were prepared and root canal treatment was performed in all experimental teeth. The canals were cleansed mechanically, rinsed with sterile saline, and filled with calcium hydroxide.[b] The cavities were sealed with cement.[c]

Three months after the experiment the dogs were sacrificed by drug overdose.[d] The premaxilla with all experimental teeth was removed. The specimens were fixed in 10% neutral formalin, decalcified in 10% solution of EDTA at pH 7.4, and embedded in paraffin. The specimens were prepared as described by Andreasen[5], so that the blocks were oriented perpendicular to the long axis of the teeth and cut in step-serial sections at about 500 μm intervals. A few sections 5 μm thick were cut at each level, and two of these were stained with hematoxylin-eosin. The best preserved section was used for the analysis.

The analyses were performed according to Andreasen[5] in a light microscope by one evaluator (LM) using a cross-ruled grid. The sections were magnified 20x. The center of the cross was placed in the center of the cross-sectioned root, oriented buccolingually. Thus, there were four points of intersection between grid line and periodontal ligament, constituting the sites of analysis at each tooth level. The analysis was made at a magnification of 40x (objective 4x, ocular 10x).

Root surface conditions were studied at each site of analysis, and classified as follows: (1) no resorption, (2) resorption with ankylosis, and (3) resorption without ankylosis (Figure 1). About 20 sections from each tooth were examined (about 80 sites of analysis) without knowledge of treatment used. The percentage of sites with each condition was calculated for each type of extraoral treatment. Therefore, all teeth in a treatment class were pooled and the root surfaces regarded as a homogeneous risk mass (about 480 sites of analysis at most), since each type of incisor tooth was included in the class by the lot system.

Differences in occurrence of ankylosis between treatment classes were subjected to the test of the difference between two percentages.

Results

The results are presented in Table 2. Sites of analysis without resorption ranged from 82–86% in treatment Classes 1, 2, 5, and 6, while a dry period of 30 minutes followed by 30 minutes wet storage decreased to 75% (Class 3). Increasing the preceding dry period to 60 minutes decreased the number to 50%.

Storage in a wet environment for 30 minutes (Class 2) after 15 minutes dry (Class 1) decreased the percentage of sites with ankylosis from four to zero. The difference is statistically significant at the 0.1% level ($Z = 4.15$). Increasing the dry time to 30 minutes followed by the same duration of wet storage (Class 3) gave rise to 5%

[a] Pentothal-Sodium®, Abbot Inc., Belgium.
[b] Calacept®, Scania Dental AB, Sweden.
[c] IRM Cement®, Caulk, USA.
[d] Pentothal-Sodium®, Abbot Inc., Belgium.

Table 2. Percentage of sites with external root resorption in experimentally reimplanted beagle teeth exposed to different combinations of extraoral storage.

Treatment class	Extraoral time in minutes: dry +	wet	Number of exp. teeth*	% sites with no resorption	% sites with resorption: without ankylosis	with ankylosis
1	15	–	5	84.8	11.4	3.9
2	15	30	6	85.7	14.4	0
3	30	30	6	75.3	19.9	4.9
4	60	30	5	49.8	36.9	13.3
5	–	30	5	83.2	16.6	0.3
6	–	60	3	82.5	17.4	0

* Because of failure during extraction procedure the number of teeth was less than six in some treatment classes.

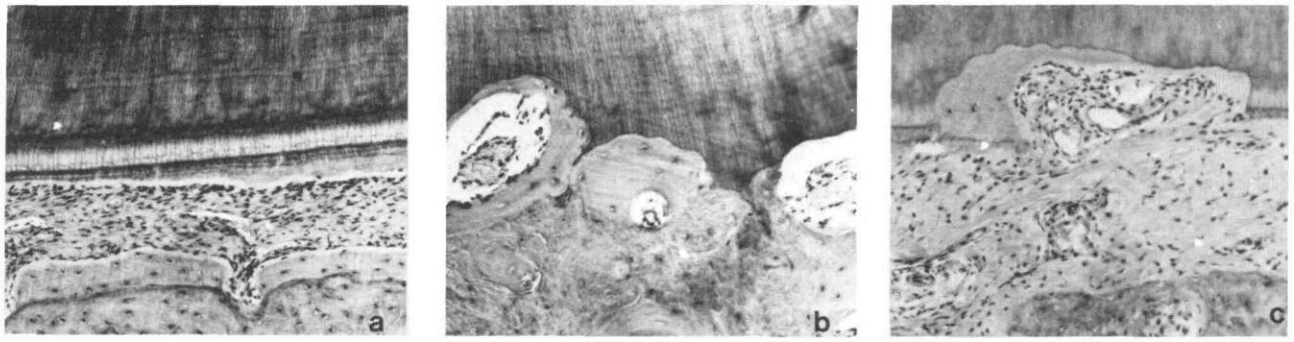

Figure 1. Sections from reimplanted teeth showing: (a) normal area, (b) resorption with ankylosis, and (c) resorption without ankylosis (110x).

ankylosis, which increased to 13% when the dry time was 60 minutes (Class 4), a difference that is statistically significant at the 0.1% level ($Z = 4.29$). Storage in a wet environment only (Classes 5 and 6) resulted in a negligible percentage of ankylosis.

Discussion

To express the risk of ankylosis in avulsed teeth in clinically relevant terms it is necessary to perform proper endodontic treatment of nonvital pulps. Cvek[6] has shown that external root resorption, with osteolysis of adjacent bone, will heal after such treatment provided the damage to the periodontal tissues is not so pronounced as to constitute prerequisites for ankylosis. This means that leaving root canals with untreated necrotic pulp influences the frequency of root resorption, with or without ankylosis. An osteolytic process caused by necrotic pulp tissue can be presumed either to prevent the occurrence of ankylosis, or to increase the periodontal destruction and facilitate the development of ankylosis after endodontic treatment.

The results of the present study agree closely with those of Cvek et al.[2] (A future study might include additional treatment classes, at least with dry periods of 30 and 60 minutes without subsequent wet period.) Combining the information from the two investigations warrants the following conclusions: when a tooth to be reimplanted has been out of its socket for 15 minutes or more, it is advantageous to condition it in an isotonic solution for about 30 minutes; and when the dry time exceeds 30 minutes, there is an increased risk of ankylosis.

For various reasons we did not attempt to interpret the frequency of resorption in terms of healing. One reason is that observations made after three months might not be representative of the long-term clinical outcome as evaluated from: (1) radiographs, (2) mobility and percussion tests, and (3) position of the teeth in the dental arch. In this context, the frequency of resorption without ankylosis creates a lot of uncertainty. Clinically, healing implies a reestablished periodontal space bordered by lamina dura as seen in the radiograph, and normal conditions with respect to the above criteria. Therefore, the methodology used in the present study for evaluating the effect of the different treatment procedures merely serves to elucidate the relative risk of ankylosis. The histometric technic applied is quite sufficient for this purpose (Andreasen[7] found that using four registration locations per section is only slightly inferior to 24 or more).

Conclusions

It seems to be an advantage to condition avulsed teeth which have been kept dry for 15 minutes or more in an isotonic solution for about 30 minutes before reimplantation. When the dry time exceeds 30 minutes there is a greatly increased risk of ankylosis, even if the teeth are conditioned in isotonic solution.

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