



# The risk of ankylosis of 89 avulsed human teeth stored in saliva prior to replantation - A retrospective clinical follow up study

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## ABSTRACT

**Purpose:** The aim of the study was to evaluate the risk of ankylosis for avulsed human teeth stored in saliva preceded by various dry storage condition prior to replantation.

**Material and methods:** Data from patients with replanted permanent teeth stored in saliva prior to replantation were selected from a database at Copenhagen University Hospital. Periodontal healing related to ankylosis was analyzed.

**Results:** Seventy-four patients (54 male and 20 female) with 89 avulsed and replanted teeth, which all had been placed in saliva prior to replantation, were retrieved. The time the avulsed teeth were placed in saliva ranged from 1 to 150 minutes (mean time 35 minutes). Around 40 % of the saliva stored teeth showed healing without ankylosis. Dry storage for 5 minutes or less before saliva storage resulted in ankylosis in 40% of the teeth but when dry storage exceeded 5 minutes prior to storage in saliva, ankylosis was seen in 80%. Teeth with mature root development were significantly more often affected by ankylosis than teeth with immature root development. Ankylosis increased with increased storage time in saliva.

**Conclusion:** Storing a tooth in saliva prior to replantation decreases the risk of ankylosis compared with dry storage. Immature teeth have a better prognosis than mature teeth. Temporary storage in saliva should be encouraged if an avulsed permanent tooth cannot be immediately replanted or a suitable storage medium such as milk or saline are not immediately available at the place of accident.

*Keywords:* ankylosis, replantation, saliva, storage medium, tooth avulsion.

## SAMMANFATTNING

**Syfte:** Syftet med studien var att utvärdera risken för ankylos för exartikulerade (utslagna) permanenta tänder förvarade i saliv föregångna av olika torrförvaringstider innan replantation.

**Material och metod:** Patientdata med replanterade permanenta tänder som förvarats i saliv före replantation valdes ut från en databas vid Köpenhamns Universitetssjukhus. Parodontal läkning med avseende på ankylos analyserades.

**Resultat:** I studien inkluderades sjuttiofyra patienter (54 pojkar och 20 flickor) med 89 exartikulerade och replanterade tänder som alla hade förvarats i saliv före replantation. Den tid som de exartikulerade tänderna hade förvarats i saliv varierade från 1 minut till 150 minuter, med en genomsnittlig förvaringstid på 35 minuter. Cirka 40 % av de salivförvarade tänderna uppvisade läkning utan ankylos. Torrförvaring 5 minuter eller mindre före förvaring i saliv resulterade i ankylos hos 40% av tänderna men när torrförvaring översteg 5 minuter före förvaring i saliv sågs ankylos hos 80%. Tänder med slutet apex var signifikant mer påverkade av ankylos än tänder med öppet apex. Ankylos ökade med ökad förvaringstid i saliv.

**Slutsats:** Att förvara en tand i saliv innan replantation minskar risken för ankylos jämfört med att förvara tanden torrt. Tänder med öppet apex har bättre prognos än tänder med slutet apex. Tillfällig förvaring av tanden i saliv bör uppmuntras om tanden inte omedelbart kan replanteras eller om ett lämpligt förvaringsmedel som till exempel mjölk eller fysiologisk koksaltlösning inte finns tillgängligt direkt vid olycksplatsen.

*Nyckelord:* ankylos, exartikulerad tand, förvaringsmedium, replantation, saliv.

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# INTRODUCTION

## General

Traumatic dental injuries (TDI) are very frequently occurring (1–4). One of the most severe TDI is avulsion of permanent teeth (5). Tooth avulsion is when the tooth is completely luxated out of its socket (6). This type of injury occurs around 0.5-16% of all TDI (7–9). Avulsion is most frequently affecting the anterior teeth in the maxilla and avulsion is most common in 7 to 9-year olds. When a tooth is avulsed, the vascular supply is completely cut off from the pulp of the tooth and there is also a high risk of injury to the periodontal ligament (PDL) (7,10). The prognosis of the tooth is depending on what actions are taken immediately after the accident (5).

## Place of accident

Only permanent teeth should be replanted (5,7). Primary teeth should not be replanted because such a procedure can damage the underlying permanent tooth germ (11). When the permanent tooth is avulsed it is important to find the tooth and if possible, replant the tooth back in its socket as soon as possible. The avulsed permanent tooth should be picked up avoiding touching the root. If the tooth is contaminated it could be quickly rinsed in cold tap water, for maximum of 10 seconds, before replantation (5,7). After the tooth has been replanted it is important to seek the nearest dentist emergency for treatment. If the tooth for various reasons cannot be replanted it must be stored in a suitable storage medium to protect the PDL cells from drying out before the tooth can be replanted (5,7).

## Storage media

Although the tooth can be quickly rinsed in tap water, the avulsed tooth must never be stored in tap water (5,7). Storage in tap water destroys the PDL cells (7,10,12–14). The reason for this is the differences in osmolality, which means that the ion strength is different between the inner and outer cell environment, which leads to destruction of the cell membrane (7,10,12). If the tooth cannot be replanted at the site of accident it should preferably be stored in an isotonic or physiologic storage medium in which the osmolality is similar to tissue fluid (5,7,10). Examples of such good storage media are milk or physiologic (0.9%) saline (12,15,16). Numerous other suitable ways of storing have been suggested in the literature such as Hanks balanced salt solution, cell culture medium, egg-white, propolis, coconut water (12,17–22). The problem with many of these media are that they are not available at the site of accident. Isotonic saline solution may be difficult to find at the site of accident, but milk may often be found at, or close to, the site of accident so this has been recommended (5,12,13,15,21,23–29). Saliva is always available in the patients mouth and has therefore been recommended as storage medium (5,30,31). Saliva however does not have the same osmolality as milk or saline (12,27), but it has been reported as a suitable storage medium for shorter periods (12–14,16,25,26,32). Storage in saliva can be accomplished by putting the tooth in the mouth between the cheek and the molars (5,7,10). If the patient is not able to have the tooth in the mouth, the patient can spit saliva in a container and keep the tooth there (5,10).

## In vitro studies on saliva

Several studies have shown that if the tooth has been stored dry for 60 minutes or more the PDL cells are considered necrotic and hence healing with normal PDL is less likely (5,7,10,15,33,34). Some in vitro studies have presented that PDL cells can be stored in saliva

for a short period of time (12,16,26). One in vitro study showed that it was first at 2 or 3 hours that the damage to the PDL cells really increased during storage in saliva (12). The results from another in vitro study showed that no PDL cells were viable after storage in saliva for 3 hours. But the same study also showed that a short time of period in saliva and then storage in eugles medium increased the numbers of surviving PDL cells properly (28). The clonogenic capacity for PDL cells stored in saliva after 30 minutes have showed in an in vitro study the capacity of 7,6%. At 60 minutes the clonogenic capacity was 1.5% after storage in saliva (16). It has also been reported that if the clonogenic capacity is over 3% there is a chance of PDL healing (16).

An in vitro study pointed out that the low osmolality and ability to create high numbers of bacteria makes saliva a less suitable storage media at the extra-alveolar period for the tooth, before replantation (15). That study showed in detail that saliva contained four times more aerobic bacteria than milk and two times more anaerobic bacteria. In the same study, saliva stored monkey teeth showed a lot of adhering bacteria on the root surface, regardless of the time in saliva, while teeth stored in milk had few adhering bacteria on their periodontal ligament (15).

### **In vivo studies on saliva**

It has been shown that monkey teeth stored in saliva prior to replantation resulted in better healing of PDL and significantly lower ankylosis than dry storage of avulsed teeth (14). After 30 minutes of dry storage ankylosis could be seen (14). In teeth stored in saliva ankylosis initially was shown after 120 minutes of storage (14). In another study in monkeys the results showed that saliva results in good PDL healing for up to 2 h before replantation (13). Only a small area of ankylosis was seen when teeth were stored in saliva for two hours (13). A comparison of teeth stored in saliva for up to 2 hours showed almost as good result as immediately replanted teeth, with regards to periodontal healing (13). In a study where they investigated different storage media with different temperature prior to replantation of monkey teeth, it was found that a temperature of 37 degrees saliva showed no evidence of ankylosis after 60 minutes of saliva storage before replantation (32). A recent study has shown better wound healing in extraction sockets if saliva is present (35).

### **Clinical (human) studies and case reports on saliva**

There are very few clinical studies on teeth stored in saliva prior to replantation (36–38). There are some case reports that have shown teeth stored in saliva for several hours after the accident healed without root resorption after long term follow up (30,31). One example was a woman who had her avulsed tooth stored in the mouth for 1,5 h before replantation and with a three-year follow-up with normal healing without signs of ankylosis (31). Another case was a boy in a similar situation who had the tooth stored in the mouth for 5 hours before replantation and with a two-year follow-up with normal healing (30). A clinical long-term study from Copenhagen on 400 replanted teeth has indicated potential for long-term survival of teeth stored in different media (39). There is a need for more clinical studies on teeth stored in saliva prior to replantation.

### **At the clinic**

When the patient reaches the clinic, various treatment decisions will be taken depending on the status of the root development (open or closed apex) and the status of the PDL (5). The condition of the PDL is highly depending on how the tooth has been stored, especially for

how long time it has been stored dry (5,40,41). The tooth is replanted and splinted to the adjacent teeth (5).

### **Follow up and complications**

Complications in healing are related to the pulp and the PDL. Pulp infection must be avoided, otherwise infection from the pulp may trigger an inflammation on the root surface through the dentinal tubules. This results in infection related root resorption, earlier called inflammatory root resorption, which can destroy the root resulting in loss of the replanted tooth (7,13,42–44). For this reason, teeth stored dry for 60 minutes or more and root development is completed, revascularization of the pulp is unlikely and PDL cells are considered necrotic (5,33). So, the replanted tooth with closed apex should be endodontically treated within the first weeks (5,33). Today infection related resorption can be prevented or avoided (13,42,43,45,46). In teeth with open apex, there is a chance of revascularization, given the tooth has not been stored dry for 60 minutes or more, so in these teeth the pulp shall not be removed, but revascularization given a chance (5). It has also been suggested to remove the necrotic PDL prior to replantation (5,7).

If the PDL has been severely damaged, e.g. by drying for a long time, healing with normal PDL is less possible (5,7,10,14,33,36,47–51). Instead, bone will grow in contact with the root and the tooth will be fused with the alveolar bone, so called ankylosis (5,7,50,52,53). This is a progressive condition resulting in gradual replacement of the tooth by bone, so called replacement resorption. This will progress until the tooth is completely replaced by bone. There is no treatment for ankylosis and replacement resorption and hence this condition cannot be stopped. For this reason, ankylosis is the best predictor for non-successful healing and non-successful outcome (54).

In summary, some experimental and clinical studies have indicated that replanted teeth stored in saliva before replantation may improve PDL healing, possibly avoiding ankylosis. Other experimental studies did not agree with these findings (21). For this reason, there is a need for more clinical studies to learn more about the fate of avulsed teeth that have been stored in saliva prior to replantation.

### **Purpose/Aims**

The aim of the study was to evaluate the risk of ankylosis for avulsed human teeth stored in saliva preceded by various dry storage condition prior to replantation.

## **MATERIAL AND METHODS**

### **Literature**

To find relevant articles for the introduction in this field, reference search from literature Medline sources (Pubmed) and Web of Science (Scopus) was carried out. The search words were replantation, reimplantation, tooth replantation (Mesh), saliva (Mesh), storage and saliva. Additional search assured that articles on tooth avulsion and ankylosis were included. Moreover, when reading these articles additional references were found and added. In addition, the very recent comprehensive textbook “Textbook and Color Atlas of Traumatic Injuries to the Teeth” (55) was giving us additional references to complete our list of references for this master thesis.

## **Collection of data**

The material for this study was extracted from an existing database at the Department of Oral and Maxillofacial Surgery, at Copenhagen University Hospital (Rigshospitalet), which included 400 replanted permanent incisors from 322 patients. The data had been collected during the years 1965 to 1988. The permanent teeth were replanted and followed for up to 20 years (56). The methodology and results of this major material have been published elsewhere (39,56–59). The following section presents a summary of methods of relevance for the present study. For more details of the material and methodology, the author refer to the original, previously published article (56).

## **Copenhagen database - treatment protocol**

The clinical protocol for avulsed teeth at the University Hospital of Copenhagen was followed which stated that the avulsed teeth were placed in physiologic saline (0.9%) as soon as the patient arrived at the emergency ward. If the tooth was obviously contaminated, it was cleansed with a flow of saline from a syringe. The tooth was then replanted into its socket by digital pressure. No effort was made to remove the coagulum before replantation. Splinting was performed. Phenoxymethylpenicillin related to the weight of the patient, was administered by oral administration for four days, starting the day of the injury. In teeth with complete root formation, endodontic treatment was performed 7-14 days after the injury. Teeth with immature root development were observed, and endodontic treatment was performed only if there were signs of pulp necrosis or infection-related root resorption. One week after the injury, patients and parents were contacted to check and further supplement the information given at the initial emergency service to achieve the highest possible accuracy of data about the injury conditions, such as storage conditions and storage time.

The exclusion criteria were previous endodontic treatment, previous trauma, destruction of the crown caused by dental caries or restorations, the presence of a concomitant crown fracture with pulp exposure, root fracture or alveolar fracture.

The standard follow-up program included clinical and radiographic controls after 1 week, 2-3 weeks, 6 weeks, 6 months and 1, 2, 3, 5, 10, 15 and 20 years. In case of suspected root resorption, an additional control was performed after 4 weeks. The follow-up period was on average 5.1 years ranging up to 20 years.

## **Copenhagen database - clinical and radiographic registrations**

At the time of the injury, the following parameters were recorded: gender, age, cause of injury, date and time of injury, number of injured teeth, the condition of the supporting tissue, fractures of the teeth, type of storage medium and length of storage. Extra-oral dry time was defined as the time interval from when the tooth was avulsed out of its socket until the time when the tooth was placed in saliva.

For each tooth, clinical information from the time of injury and at follow-up examinations included tooth colour, tenderness to percussion, percussion sound, mobility of the tooth and position of the tooth in relation to the contralateral tooth (56).

The stage of root development was determined by evaluation of radiographs from the initial examination and classified into one of the following six stages described by Moorrees et al.



1963 (60): The material in the present study was divided into two subgroups: immature root development stages 1-5, or teeth with mature root development stage 6.

Ankylosis-related resorption was diagnosed if one or more of the following clinical signs were present:

- High-pitch percussion sound
- Absence of mobility
- Progressive infraposition of the tooth in growing individuals

In later stages, radiographic signs of replacement resorption verified the clinical diagnosis of ankylosis.

## **Present study**

For the present study, which focused on storage conditions and ankylosis, the following inclusion criteria were applied: tooth stored in saliva prior to replantation, presence of tooth-specific clinical information and radiographs from the time of injury and the subsequent controls according to a standardised protocol. Furthermore, a minimum one-year follow-up unless early complications had made extraction necessary before that time.

## **Ethical consideration**

All data used in the present study were obtained in a clinical context as part of a standardised treatment regime with full acceptance from the parents and is fully in line with the World Medical Association's Declaration of Helsinki from 2013. By Danish law, this study is considered a "quality assurance follow-up study" (all data were obtained in a clinical context and/or as part of a standardised treatment protocol), and the study thus does not qualify for evaluation by a research ethics committee in Denmark. In the present dataset it was not possible to track any individual patients.

## **Statistical methods**

The risk of ankylosis was estimated with the Aalen-Johansen method (61,62) accounting for the competing risk of tooth loss. We reported 3 years risks with 95% confidence limits separately for teeth with immature and mature root development in relation to dry-storage period. Due to lack of statistical software, confidence limits were not accounting for the dependencies of teeth placed in the same patients. Risk factors for ankylosis were analyzed by Cox regression analysis. Risk factors included in the analysis were: length of dry time and stage of root development. The effect of increasing the wet storage time on the rate of ankylosis was analyzed in the subset of the data including only teeth for which the dry storage time was less than 6 minutes. The level of significance was set at 5%. All analyses were performed with the statistical software R2.

## **RESULTS**

The material comprised 74 (54 male and 20 female) patients with 89 avulsed and replanted teeth which all had been placed in saliva prior to replantation. The mean age of the patients were 14.3 years ranging from 6 to 36 years. Table 1 shows the distribution of the saliva stored teeth related to tooth type and gender of the patients. Most teeth were central incisors and male were more commonly affected than female.

**Table 1. Type of avulsed tooth in different gender (n=89)**

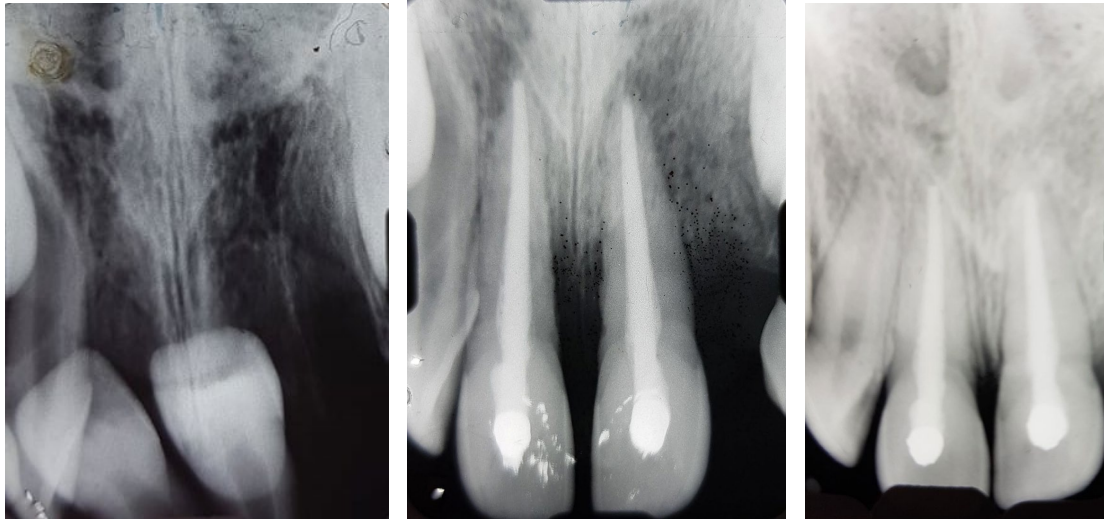
<b>Tooth type</b>	<b>Male</b>	<b>Female</b>
12	3	0
11	25	12
21	28	9
22	6	1
23	1	0
31	1	0
41	2	0
42	1	0
	<b>67</b>	<b>22</b>

The time the avulsed tooth was placed in saliva ranged from 1 to 150 minutes, with a mean time of 35 minutes. The distribution of mature and immature teeth is presented in Table 2. Mature teeth comprised 82% of the material.

**Table 2. Root development of the teeth (n= 89)**

	<b>n</b>	<b>(%)</b>
<b>Immature</b>	16	18%
<b>Mature</b>	73	82%

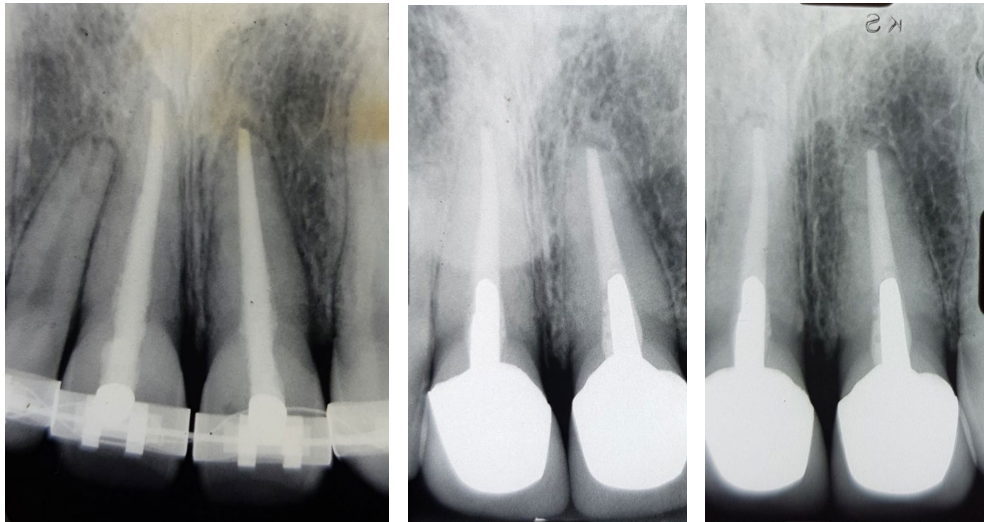
There were teeth stored in saliva for long periods without being ankylotic (Figure 1). Around 40 % of the teeth which had been stored in saliva prior to replantation showed healing without ankylosis. Figure 2 shows the overall risk of ankylosis over time. Ankylosis was in most cases diagnosed during the first year after replantation. However, some teeth were not diagnosed as ankylotic until 2-3 years after trauma. After 3 years there were no new cases of ankylosis diagnosed.



A

B

C



D

E

F

Fig. 1. 10-year-old girl with avulsion of the two central incisors. A. The roots were displaced out of the alveolar socket but remained attached to the palatal gingiva. The patient stored them in the mouth for 90 minutes before replantation. B. 2 months after replantation. C. 1 year after replantation. D. 5 years after replantation. E. 10 years after replantation. F. 20 years after replantation. No clinical or radiographic signs of ankylosis could be detected. Percussion sound and mobility were normal.

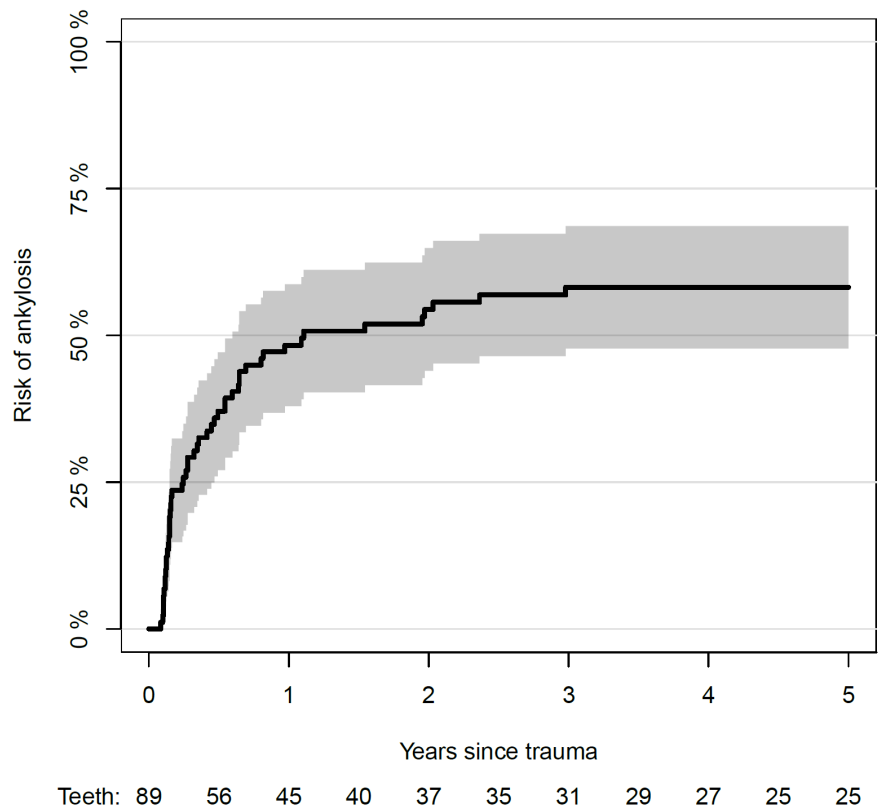


Fig. 2. Risk of ankylosis (y-axis) of avulsed permanent teeth stored in saliva prior to replantation. Number of remaining teeth over follow-up time (x-axis). N= 89.

Teeth with mature root development were significantly more often affected by ankylosis than teeth with immature root development ( $p=0.0436$ ). Figure 3 shows the risk of ankylosis related to stage of root development over time.

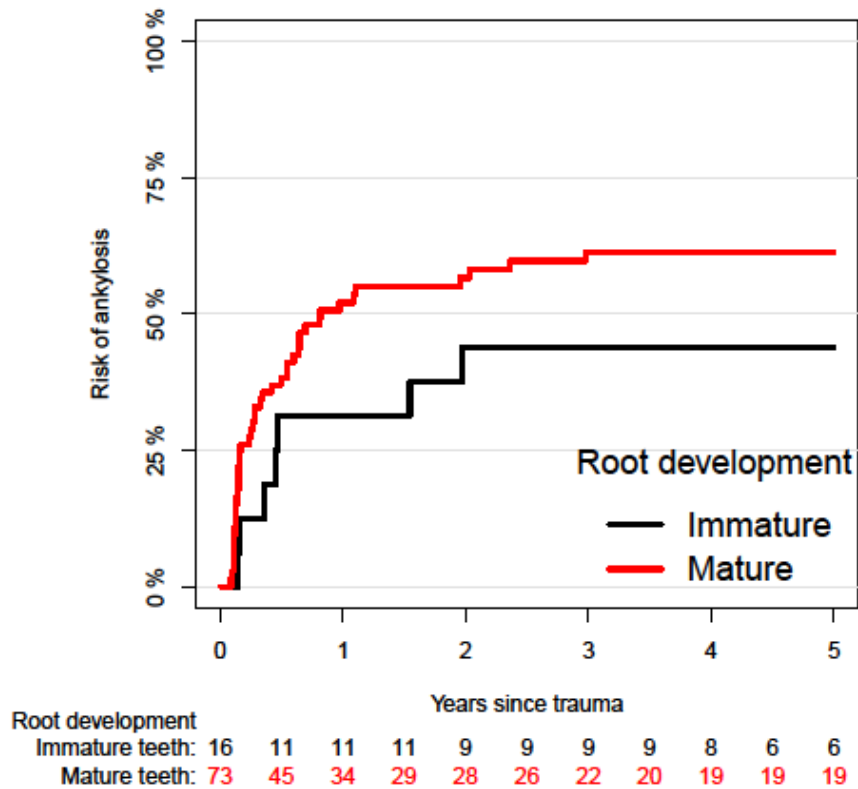


Fig. 3. Risk of ankylosis (y-axis) of avulsed permanent teeth stored in saliva prior to replantation related to root development. Number of remaining teeth over follow-up time (x-axis). N=89.

Fifty-three teeth had been stored dry 0-5 minutes before being placed in saliva. Twelve teeth were stored dry 6-15 minutes before being placed in saliva. Twenty-four teeth were stored dry more than 15 minutes before being placed in saliva. Table 3 and 4 shows the distribution of teeth stored dry for different times prior to being placed in saliva. When teeth had been stored dry more than 5 minutes before replantation there was a significant increase in the risk of ankylosis compared with teeth which had been stored dry for 5 minutes or less. (Table 4).

Table 3. Distribution of teeth stored dry prior to being placed in saliva.

<b>Variable</b>	<b>Level</b>	<b>Immature (n=16)</b>	<b>Mature (n=73)</b>	<b>Total (n=89)</b>
Dry-time group	0-5	9 (56.2)	44 (60.3)	53 (59.6)
	6-15	0 (0.0)	12 (16.4)	12 (13.5)
	>15	7 (43.8)	17 (23.3)	24 (27.0)

Table 4. Hazard ratio for replanted teeth stored in saliva prior to replantation related to dry time storage prior to placement in saliva and related to root development.

<b>Variable</b>	<b>Units (min)</b>	<b>HazardRatio CI.95</b>	<b>p-value</b>
Dry time group	0-5	Ref	
	6-15	2.25. [1.03;4.89]	0.0417
	>15	3.40. [1.81;6.37]	<0.001
Root development	Immature	Ref	
	Mature	2.37 [1.02;5.46]	0.0436

The risk of ankylosis was related to how long the tooth have been kept dry before the storage in saliva (Figure 4). If the teeth were stored dry for 5 minutes or less the risk of ankylosis was slightly higher than 40 %, whereas if the tooth had been stored dry for more than 5 minutes the risk of ankylosis increased to around 80%.

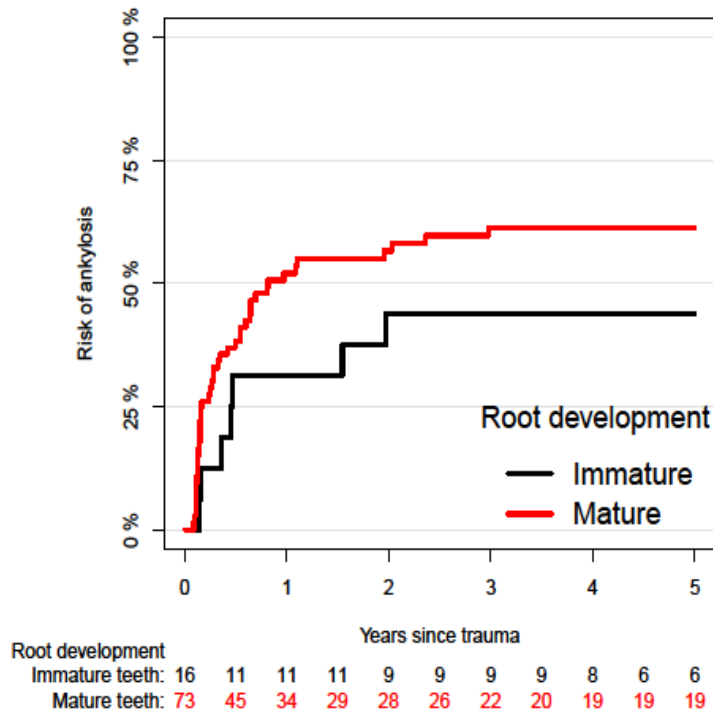


Fig. 4. Risk of ankylosis (y-axis) related to dry time storage (0-5, 6-15, > 15 minutes) prior to placing the tooth in saliva. Number of remaining teeth over follow-up time (x-axis). N = 89

A separate analysis was carried out for 53 teeth stored dry for 5 minutes or less before being placed in saliva. Figure 5 shows the frequency of ankylosis related to length of time the avulsed tooth was stored wet in saliva. Ankylosis increased with increased storage time in saliva (wet time). Avulsed immature teeth stored in saliva for more than 60 minutes before replantation showed ankylosis in less than 25%, whereas around half of the mature teeth stored in saliva for 60 minutes became ankylotic (although differences not statistically significant,  $p=0.192$  (Table 5). A linear trend was found showing that 1 minute more wet time increased the hazard rate for ankylosis by approximately 1% (CI= [0%,2%],  $p=0.052$ . (Figure 5).

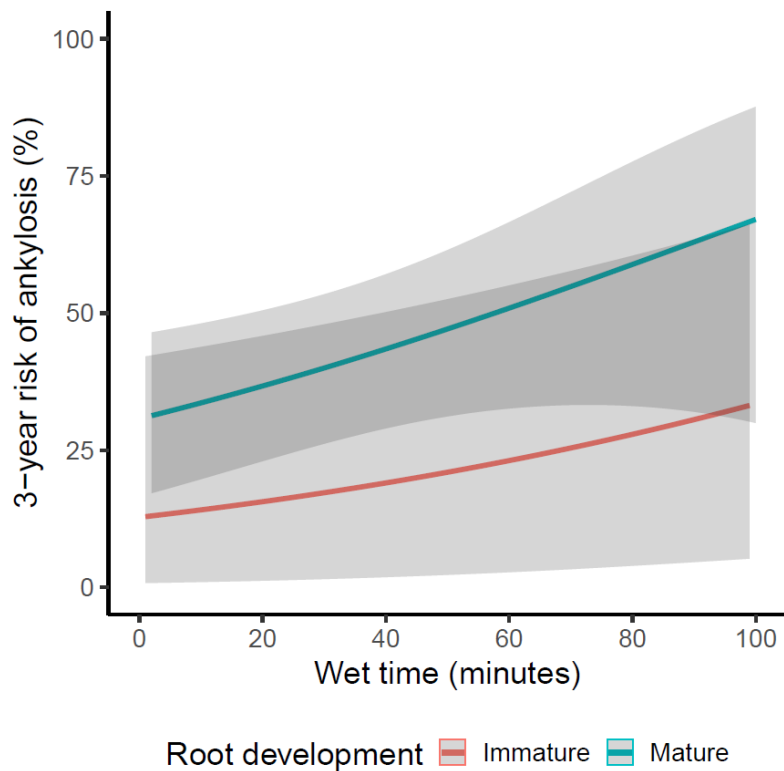


Fig 5. Trend of development of ankylosis during 3 years after replantation related to wet time in saliva for teeth with dry time 5 minutes or less. N=53.

Table 5. Risk of ankylosis estimated after 3 years after replantation

Root Development	Drytimegroup	Risk 3-years (CI)
Immature	0-5	23.7 [8.6;42.9]
Immature	6-15	45.7 [13.1;73.9]
Immature	>15	60.5 [29.6;81.2]
Mature	0-5	47.4 [32.8;60.7]
Mature	6-15	76.8 [45.7;91.5]
Mature	>15	89.3 [68.0;96.7]



## DISCUSSION

The results of this study have shown that saliva is a suitable storage medium, at least for a short time, for storing avulsed teeth prior to replantation, when teeth cannot be replanted or physiologic storage media such as milk or saline is not available at the site of accident.

### Discussion of methods

The strengths of this study is that the material of this study is the largest material found in the literature on saliva-stored human teeth prior to replantation. The patients included in the study were treated and followed according to standardized protocol. To ensure that the information regarding storage media and storage period was as accurate as possible, the patient was recalled in general after one week for a review of the information provided at the time of the accident. This was carried out by one person (Andreasen JO). In this way, misunderstandings and incorrect information were reduced. Another strength is that the healing of the replanted tooth has been followed during a long period of time.

A weakness in this study is that the recommended treatment may have change over time, for example, the fixation period for the traumatized tooth, varied from 6 weeks at the beginning of the study period compared to later recommendations of 1-2 weeks. It has been shown that functional stimulation during the first weeks of healing may have influence on development of ankylosis and hence a short time splinting has been suggested (5,7). However, this variation in splinting period affected the groups equally.

### Discussion of results

In most teeth ankylosis was diagnosed during the first year and all teeth with ankylosis were diagnosed within the first 3 years. This is in accordance with previous studies (7,39). Replacement resorption progressed with time which is in accordance with the literature (7,63)

Our results showed that storage in saliva prior to replantation has a positive effect on periodontal healing. Around forty percent, of the saliva stored replanted teeth showed healing without ankylosis. Our results are consistent with some previous experimental studies on animals and in vitro studies (12–14,16,26,32). In other studies where the teeth have been stored dry for 1h or more before replantation, ankylosis has been reported (13,14,25,36,46,63). One study with replanted dog teeth showed that it didn't matter if the teeth stored dry 20, 60 or 90 minutes before replantation because the PDL cells were already very damaged after 20 minutes and ankylosis had been reported (64). In another in vivo study with replanted monkey teeth, the results showed that it was a clear association between ankylosis, and teeth stored 30 minutes dry prior to replantation (14). Hence, ankylosis must be expected in many teeth when the dry storage period is long. A very recent study on replanted teeth stored dry more than 60 minutes prior to replantation showed that ankylosis can be expected in as much as 85 percent of the teeth (65). However, in the same study there were also some teeth that did not become ankylotic despite 60 minutes dry storage (65). The results of our study have indicated that teeth stored in saliva prior to replantation will result in a much better prognosis than teeth only stored dry prior to replantation. Moreover, dry storage prior to placing the tooth in saliva, will result in a significantly higher risk of ankylosis.

Our results show that teeth with immature root development have a lower risk of ankylosis compared to teeth with closed apex. The reason for this could be the thicker PDL layer in immature teeth, unlike the teeth with completed root development (7,39). Another factor may

be that immature teeth have an open apex where more vessels can provide a better blood supply.

The dry storage time before the tooth was placed in saliva showed to be a critical factor. Dry storage for 5 minutes or less before saliva storage resulted in ankylosis in 40% but when dry storage exceeded five minutes prior to storage in saliva, ankylosis was seen in 80% of the replanted teeth. This observation shows that the increased dry time for the avulsed tooth is a crucial factor even if the tooth is placed in saliva later. For this reason, dry storage should be minimized as much as possible before the tooth is placed in saliva.

Osmolality has been shown to be the most important factor for the PDL cells to survive. Saliva has been shown to have a reasonable high osmolality, although it is not as good as tissue fluid or milk (12,27). Blomlöf has shown in experimental *in vivo* studies that teeth stored in milk prior to replantation can heal with normal PDL if the replanted monkey teeth were stored in milk up to 6 hours. In saliva, the time was much shorter and Blomlöf suggested that saliva could function as a storage media for up to 2 hours (13). The results of our study suggest that saliva can be recommended as an immediate temporary storage media until a better storage medium such as milk or physiological saline is found near the site of accident. However, our findings show that saliva has limited ability to preserve the PDL during longer periods. This indicates that the saliva should be regarded as a temporary storage medium that should be replaced by a better, more physiological medium, as soon as possible. Our results indicate that there seems to be a linear trend with ankylosis increasing by around 1% per minute when the tooth is placed in saliva prior to replantation. This may be due to the suboptimal osmolality. Another reason for this can be bacterial contamination from the saliva that may damage the PDL cells on the root surface during the extra-alveolar period (15).

The results of this study indicate that avulsed teeth in humans have higher risk of ankylosis when they are stored in saliva prior to replantation compared to studies in animal teeth. The studies with replanted animal teeth stored in saliva prior to replantation seem to cope better with longer periods in saliva compared to human teeth (13,14,32). This may be because the studies in with animals in the laboratory are under more controlled forms compared with the clinical situations in humans.

There may be other beneficial factors for healing than osmolality explaining the findings in saliva stored teeth. One study has suggested that saliva is the major factor to accelerate the process in oral wound healing (66). Saliva creates a humid environment in the oral mucosa that prevents the oral tissues from dehydration, improved access to nutrition and increased the survival of the cells involved in the wound healing. All these factors together make re-epithelialization go faster. Saliva also contains many different molecules that improves the healing in the oral cavity, include proteins and peptides (66). One of the new discoveries is that saliva contains a lot of tissue factors and is probably a contributing reason to wound healing by speeding up blood clotting (67). Growth factors are also found in saliva e.g. epidermal growth factor (EGF), but it has been shown that most of the growth factors in human saliva are in very small amounts and sometimes in inactive form compared to saliva in animals (66,68–70). Histatins and Secretory leucocyte protease inhibits has also been found in saliva and helps accelerate wound healing (66). Another experimental study showed that saliva was important for rapid healing of the extraction socket (35).

In the clinical situation we should always aim at replanting the avulsed permanent tooth as soon as possible (5,7,10). If a tooth cannot be replanted, the tooth should be stored in an

optimal storage medium such as physiologic saline or milk as the first option (5,12,13,15,25–28). However, in many cases neither saline nor milk is available at the site of the accident. The advantage with saliva is that saliva is always available in the patients mouth, so quick placement of the tooth in saliva should be encouraged if the tooth cannot be replanted or stored in milk. This is best carried out by placing the tooth in the vestibule of the mouth between the cheek and teeth (7). Two studies have suggested that saliva also can be contained by spitting in a container and place the tooth there (5,10). Saliva should be regarded as a temporary storage medium and used during the time better storage alternatives can be searched and found near the place of accident.

## **Conclusion**

Storing a tooth in saliva prior to replantation decreases the risk of ankylosis compared with dry storage. Immature teeth have a better prognosis than mature teeth. Temporary storage in saliva should be encouraged if an avulsed permanent tooth cannot be immediately replanted or a suitable storage medium such as e.g. milk or saline are not immediately available at the place of accident.

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