## ORIGINAL ARTICLE



# Risk of ankylosis of avulsed teeth immediately replanted or stored under favorable storage conditions before replantation: A long-term clinical study

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## **Abstract**

**Background/Aim:** There are few long-term clinical follow-up studies on human teeth replanted immediately or after storage in a suitable storage medium prior to replantation. This study aimed to assess the risk of ankylosis in avulsed human teeth replanted immediately or after storage in physiological media for a short time.

Material: Data from 116 patients with 145 replanted avulsed permanent teeth were selected from a comprehensive dental trauma database in Copenhagen University Hospital. The following teeth were selected: Group 1 comprised 36 teeth replanted immediately (dry time <6 min; wet time <6 min). Group 2 comprised 61 teeth replanted after physiologic storage media (saliva and saline) (dry time <6 min; wet time >5 min; wet time ranged from 7 to 170 min, and mean wet time was 59 min). Group 3 (control) included 48 teeth replanted after dry storage (dry time > 60 min).

**Method:** Clinical and radiographic registrations were carried out according to a standardized protocol; follow-up ranged from 7 months to 23 years. Ankylosis was diagnosed by percussion test and radiographs and related to the conditions prior to replantation and stage of root development.

Results: The overall risk of ankylosis was 17.2% [95% CI: 4.61; 29.79] for immediately replanted teeth, 55.3% [95% CI: 42.54; 68.00] for teeth stored in physiologic media before replantation, and 85.7% [95% CI: 75.70; 95.73] for teeth stored dry more than 1 h. Mature teeth showed a significantly higher risk of ankylosis than immature teeth. Conclusion: This clinical long-term study has verified earlier experimental studies showing that immediate reimplantation has the lowest risk of ankylosis. Physiologic storage media are good alternatives that also reduce the risk of ankylosis compared to dry storage, where ankylosis is more likely although not always seen. Mature teeth are significantly more likely to develop ankylosis.

#### KEYWORDS

ankylosis, avulsion, replacement resorption, replantation, root resorption, traumatic dental injury

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#### 1 | INTRODUCTION

An avulsed permanent tooth is a serious traumatic dental injury (TDI), which preferably should be dealt with immediately. The prognosis is very much decided at the place of the accident and the time immediately after the avulsion has taken place. <sup>1-6</sup> The preferred treatment for an avulsed permanent tooth is replanting the avulsed tooth back into its socket. This should be carried out as soon as possible to have the best outcome. <sup>7-10</sup> If the tooth cannot be replanted immediately, it must be stored, and the storage conditions are of importance. <sup>11,12</sup>

Experimental in vitro studies have shown that the time of dry storage and periodontal ligament (PDL) cell viability seems to be the most important factor for the outcome. 13,14 In vivo studies carried out on monkeys showed that PDL cells were largely nonviable if the dry time exceeded 60 min, and more resorptions have been reported in teeth stored for 60 min compared with teeth stored for 45 min. 15,16 Moreover, other in vivo studies found that replantation within 15-20 min showed a higher possibility for periodontal healing.<sup>17-19</sup> One experimental study in dogs suggested not to replant after 20 min of dry storage. 20 However, a recent study in humans showed that some teeth stored dry can heal with normal PDL even after 60 min of dry storage. <sup>21</sup> Replanted teeth that have been stored dry for less than 15 min have a higher rate of periodontal healing compared to teeth stored dry for 60 min or more, which showed a higher rate of healing with ankylosis and less survival. 5,6,22 A retrospective clinical study concluded that teeth that have been stored dry for less than 20min before replantation showed a lower risk of ankylosis and replacement resorption than teeth stored for 40, 60, and more than 60 min, indicating a relation of ankylosis with increasing dry storage time.<sup>21</sup> Another retrospective study showed that more root resorption was associated with teeth replanted after being stored for more than 30min, and higher survival rate was associated with mature (closed apex) replanted teeth.<sup>23</sup> However, in a one-year clinical follow-up study, immature (open apex) teeth showed less root resorption compared to mature teeth.<sup>5</sup>

If the tooth cannot be replanted immediately, it should be stored in a favorable physiologic storage medium until it is replanted—namely, an isotonic storage medium with an osmolality close to that of the tissue fluid. The aim is to preserve the PDL cells on the root until replantation can be carried out. Several storage media have been suggested in the literature, such as saliva, milk, saline, Hanks' balanced salt solution, propolis, egg white, and coconut water. 11,12,15,24 Most studies on storage media are experimental and are not yet verified in clinical studies. However, a long-term clinical study has recently verified that saliva may be a suitable temporary storage medium in cases where the tooth cannot be immediately replanted. 25

Complications such as infection-related root resorption can today be prevented or managed by endodontic treatment. However, when the PDL is severely damaged, ankylosis of the replanted tooth is expected and the tooth will be gradually replaced by bone. <sup>26–30</sup> For this reason, diagnosing ankylosis is the most accurate way of assessing the long-term outcome of replantation. <sup>27–31</sup>

In summary, our knowledge on tooth avulsion and replantation is mainly based on experimental cell studies and animal experiments. There is a need for more long-term clinical follow-ups, preferably with more data, to confirm or reject the findings from experimental studies on teeth replanted after storage in favorable and unfavorable conditions. Therefore, the present study aimed to perform a long-term clinical assessment of the risks of ankylosis-related resorption in different storage conditions.

#### 2 | MATERIALS AND METHODS

The material used in this study originates from previously collected data on 400 replanted teeth from 322 patients registered in Copenhagen University Hospital during the period 1965–1988. This data has been described in previous articles on pulp survival and periodontal and pulp healing after replantation. <sup>1-4</sup> Information regarding general demographic data (age and gender), the affected tooth, stage of root development (registered as completed root development or not), history of the trauma, and data gathered from diagnosis and follow-up visits were registered in a specific trauma database at the hospital. Further, clinical and radiological examinations and follow-up registrations were carried out according to a standardized protocol. <sup>2,32</sup> Follow-up periods ranged from 7 months to 23 years.

From the 400 replanted teeth in the original data, teeth stored in nonphysiologic storage media prior to replantation (e.g., tap water, disinfection solutions, etc.) were excluded. The remaining 145 replanted teeth (in 116 patients) were divided into the following groups for the present study.

Group 1: 36 immediately replanted teeth (dry time <6 min and wet time <6 min).

Group 2: 61 teeth stored in physiologic wet media prior to replantation; these included teeth stored in saliva or saline for more than  $5 \, \text{min}$  with a dry time of less than  $6 \, \text{min}$ . Specifically, wet time ranged from  $7 \, \text{to} \, 170 \, \text{min}$ , and the mean wet time was  $59 \, \text{min}$ .

Group 3 (control): 48 teeth stored dry in air for more than 60 min before replantation.

Ankylosis was diagnosed clinically by using a percussion test and radiographically by observing a resorption cavity filled with bone (replacement resorption), and it was registered in the database as presence or absence without grading. 1-4.32 All the indices and the methods used for diagnosis and evaluation have been described in previous articles. 1-4.32 The findings were correlated to the storage conditions prior to replantation and to the stage of root development (immature = open apex vs. mature = closed apex).

#### 2.1 | Statistics

The risks of ankylosis over time were estimated using the Aalen-Johansen method<sup>33</sup> accounting for the competing risk of tooth loss. We reported the 3-year risk of ankylosis with 95% confidence limits separately for teeth with immature and mature root development and in relation to the dry-storage period. The level of significance was set at 5%. All analyses were performed with the statistical software RR Core Team 2020. 34,35

#### 2.2 | Ethical considerations

All data used in the present study were already obtained earlier as part of a standardized treatment regime with previous full acceptance from the patients, or in cases of children, from the patient's parents, and the study fully follows the World Medical Association Declaration of Helsinki 2013. The Danish database originates from trauma registration forms from Copenhagen University Hospital. Access to the patient's individual records was not possible.

### 3 | RESULTS

The distribution data of the remaining 145 replanted teeth (for 116 patients) is presented in Table 1. Male patients were more common than females, and for patients over 15 years of age, this difference was significant (p = .03). Almost 25% of the included 145 teeth were replanted immediately, 42% were stored in a physiological medium, and 33% had been stored dry. The distribution of mature and immature teeth is seen in Table 2. Mature teeth were more common in the study material. Risk figures for ankylosis in relation to root development and different storage conditions are presented in Table 3. Mature teeth tended to have a higher risk of ankylosis than immature teeth. The overall risk of ankylosis for immediately replanted teeth was 17.2% [95% CI: 4.61; 29.79]. For teeth stored in physiologic media before replantation, the overall risk was 55.3% [95% CI: 42.54; 68.00]. For teeth stored dry for more than 1h, the overall risk of ankylosis was 85.7% [95% CI: 75.70; 95.73]. Figure 1 summarizes the risk of ankylosis related to immature and mature root development for teeth replanted immediately, for teeth stored wet in physiologic media, and for teeth stored dry prior to replantation. The risk of ankylosis was lowest in immediately replanted teeth. Teeth that had been stored in physiologic storage media prior to replantation showed a higher risk of ankylosis than immediately replanted teeth, whereas teeth that had been stored dry before replantation showed the highest risk of ankylosis.

**TABLE 1** An overview of the material with regards to age and sex. The number (*n*) and percentage (%) of patients distributed by age and gender.

Age	Male <i>n</i> = 79	Female n=37	Total <i>n</i> = 116	p Value
≤10	21 (26.6%)	12 (32.4%)	33 (28.4%)	
11-15	19 (24.1%)	16 (43.2%)	35 (30.2%)	
>15	39 (49.4%)	9 (24.3%)	48 (41.4%)	.03

## 4 | DISCUSSION

This long-term clinical study showed that avulsed teeth which have been immediately replanted healed successfully and had less risk of ankylosis, compared with teeth stored in various physiological media. Teeth stored dry for a long period prior to replantation had less chance to heal successfully and had the highest risk of ankylosis. We also found that immature teeth had a lower risk of ankylosis than mature teeth. In the present study, we have only included immediately replanted teeth and teeth stored in favorable storage media (saline and saliva) for a short time. We excluded all other storage media and teeth with longer storage times listed in the database. The times were chosen to enable us to investigate favorable storage conditions. We have already analyzed teeth that were stored dry between 5 and 60 min in a previous study using the same database. <sup>21</sup>

Our knowledge of replantation is mainly based on in vitro and in vivo studies, so it is important that these findings are verified or rejected in future long-term clinical studies. The present study material is unique in the world due to being a large clinical material on humans collected over a long time. Currently, there are very few clinical studies on immediately replanted teeth or teeth replanted after a short time with long follow-up periods and a reasonable number of participants. 21-23,25 In this study, we aimed to assess the risk of replanted teeth becoming ankylosed after a replantation done under favorable conditions (immediate replantation or replantation after storage in physiologic wet storage media, i.e., saline or saliva); we also compared it with the risk of ankylosis in teeth replanted after unfavorable conditions (dry storage before replantation) that was reported in a previous study. 21 At the time the material was registered, milk and other recently recommended storage media were not used in Denmark, so data on milk is so far based only on experimental studies. Therefore, long-term clinical studies are needed to verify that milk is a suitable storage medium. On the contrary, the positive effects of saliva in humans have been recently reported in a longterm clinical follow-up study.<sup>25</sup>

The literature recommends the immediate replantation of avulsed teeth to achieve successful healing. 7,10,37,38 This is supported by the findings of our long-term clinical study. Our findings show that immature and mature teeth that are immediately replanted (dry time <6 min and wet time <6 min) have the highest chance to heal normally and the least risk of ankylosis among the other groups, mainly because the PDL is expected to be viable in immediately replanted teeth due to short dry time and less exposure to the environment, which will, eventually, promote healing and reduce the risk of ankylosis. Consistent with these findings, other clinical and in vivo studies have reported that avulsed teeth should be replanted immediately to have the best possible outcome and should not be kept in dry storage. Furthermore, if replanting directly is not possible, avulsed teeth should be kept in physiological media until replantation. The earlier the replantation is carried out, the higher chance for the PDL to be viable. 5,13,15,17,39 Our results confirm these assumptions. In addition, our results show that the risk of ankylosis increases when storage media are used. Therefore, immediate

Condition before replantation	Immature n = 37	Mature n = 108	Total n = 145
Immediate replantation	12 (32.4%)	24 (22.2%)	36 (24.8%)
Physiologic storage	16 (43.2%)	45 (41.7%)	61 (42.1%)
Dry storage	9 (24.3%)	39 (36.1%)	48 (33.1%)

TABLE 2 Distribution of immature and mature teeth, in numbers (*n*) and percentage (%), for different storage conditions.

Root development	Storage prior to replantation	Risk of ankylosis after 3 years, expressed in percent % [CI 95%]
Overall	Immediate replantation	17.20 [4.61; 29.79]
Overall	Physiologic medium	55.27 [42.54; 68.00]
Overall	Dry	85.71 [75.70; 95.73]
Immature	Immediate replantation	8.33 [0.00; 23.97]
Immature	Physiologic medium	38.64 [14.27; 63.00]
Immature	Dry	66.67 [35.87; 97.46]
Mature	Immediate replantation	21.88 [4.76; 38.99]
Mature	Physiologic medium	61.10 [46.56; 75.64]
Mature	Dry	89.74 [80.22; 99.27]

**TABLE 3** Risk of ankylosis 3 years after replantation related to root development and condition before replantation.

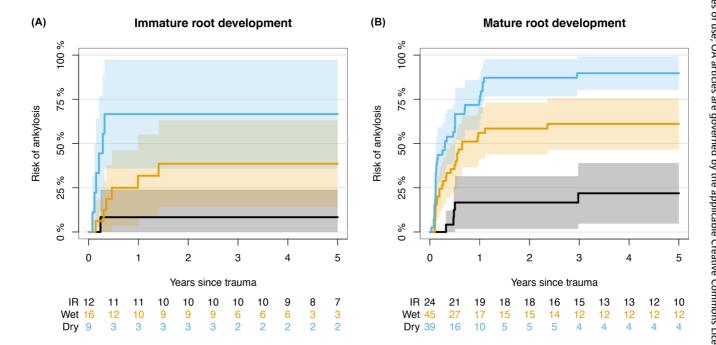


FIGURE 1 Risk of ankylosis related to immature (A) and mature (B) root development for teeth replanted immediately (black line), teeth stored in physiological medium (orange line), and teeth stored dry (blue line) prior to replantation. Shaded areas show the confidence interval for each group of teeth.

replantation is recommended for the best chance of a good outcome and the least risk of ankylosis. In our study, less than 25% in the immediately replanted group became ankylosed, which could be explained by improper tooth handling before replantation, contamination, or damage to the PDL. Hence, there is always a risk for ankylosis after replantation; however, the risk very much decreases with proper handling and immediate replantation.

Unfortunately, immediate replantation is not always feasible because of handling difficulty (e.g., replantation in young individual or

lack of knowledge) and people's assumptions that it would be better and easier to keep their avulsed teeth in physiological media and later replant them by professionals in a dental office. Therefore, it is important to further improve public knowledge and awareness about immediate replantation to reduce the risk of ankylosis and losing teeth. In most cases, unless the patient is unconscious, it is possible to replant at the place of the accident. We suggest that this information is stressed to the public to ensure they replant the tooth immediately at the site of the accident if possible.

This study showed that teeth stored in physiologic storage media (storage time > 5 min and with dry time < 6 min) had almost twice the risk of ankylosis compared to immediately replanted teeth; this may be due to the isotonic difference in the storage media, which may affect the preservation of PDL cells and lead to ankylosis, especially with long storage time. There are many different types of physiologic storage mediums-such as milk, saliva, Hanks' balanced salt solution, propolis, egg and coconut solution—that can be used to store an avulsed tooth depending on cost or ease of handling and availability on the site of trauma. 12,24 According to the guidelines of the International Association of Dental Traumatology, saline, saliva, and milk are considered physiological media that preserve the PDL before replantation. 10,40 The storage media used in the physiological storage group in this study were saliva and saline. There were no teeth stored in milk in this material because it was collected during the years 1966-1988 when milk as a storage medium had not yet been fully implemented clinically in Denmark. A recent study on replanted teeth stored in saliva prior to replantation showed that saliva can be used as an alternative storage medium if there is no medium with appropriate osmolality available on site.<sup>25</sup> Osmolality is one of the main factors in appropriate physiologic media; it is important for successful healing because the water absorption process is crucial for PDL cells' viability. The osmolality of appropriate storage media ranges between 230 and 400 mmoL/Kg. 11 Furthermore, storage media are exposed to bacteria, which may have virulent factors that could affect the PDL. Saliva may contain different types of bacteria. 15 However, a study on 196 replanted teeth in humans reported that teeth stored in saliva showed more normal PDL healing than teeth stored in dry storage, and there was no difference between teeth stored in milk or teeth stored in saliva or saline.<sup>23</sup> Thus, it seems that the risk of ankylosis in teeth stored in physiologic storage media is considerably lower than the risk in teeth stored in dry storage. 41 This result is consistent with studies that reported that teeth stored in a physiological medium had less resorption than teeth stored dry or in a nonphysiological medium. Physiological media with proper isotonicity can conserve the thickness and viability of the PDL on the avulsed teeth until replantation, which consequently increases the chance of successful healing. 1,17,19

Other results of the study showed that teeth stored dry for >60 min had the highest risk of ankylosis. This is consistent with several studies reporting that avulsed teeth stored in dry storage or exposed to the air for a long time lose PDL viability, which causes teeth resorption after replantation and reduces the chance for normal healing with healthy PDL. 7.13,21,26,42

Previous guidelines have suggested that teeth stored dry for >60 min should be cleaned from the necrotic tissue remnants attached to them before replantation. 40,43 However, the material used in this study was collected in the period 1965–1988, when there was no such recommendation, so all teeth in the present material were replanted without the intention to remove the attached necrotic PDL before replantation. 40 Since some of the teeth could still heal without ankylosis despite being stored dry for >60 min, we argue

that removing tissue remnants should not be recommended. If such tissue had been removed prior to replantation, these teeth would have ankylosed. Namely, leaving the tissue in place without removing it may have been the reason for healing without ankylosis, and this should be studied further in the future. Our results are in accordance with the recent guidelines of the International Association of Dental Traumatology, where active removal of PDL exposed to drying for more than 60 min is no longer recommended. <sup>10</sup>

In immature teeth, considerably less ankylosis was seen. This is probably due to a higher capacity of regeneration in teeth with an open apex or possibly because the PDL is thicker and with more wide apices rich in blood supply, which made immature teeth more susceptible to revascularization and, in turn, less risk of ankylosis. 4.5.21

The findings in our study also illustrate the importance of always replanting all teeth even if the dry storage time is long. In contrast with our findings, in a study on premolar teeth extracted from dogs and stored dry for either 20 min or 60 min, where some teeth had Emdogain applied to them, the researchers reported that after 20 min of dry storage the replanted teeth were ankylosed and there was no chance to avoid ankylosis.<sup>20</sup> In our study, there were some teeth healing without ankylosis even after 60min of dry storage, which proves there is a chance for normal healing without ankylosis. Their findings may be related to the type of teeth included in the study; in general, premolar teeth may have more than one root and may experience a more traumatic way of extraction, which is confirmed in the aforementioned study as the researchers reported a loss of 10 roots due to fracture during extraction.<sup>20</sup> Studies on replanted teeth in monkeys reported that difficulty in extraction and luxation time affects the PDL attached to the teeth, which leads to resorption after replantation. <sup>18,26</sup> In addition, the avulsion situation in anterior teeth differs completely. Even more, in that study the researchers performed hemisecting, and the endodontic treatments were done extraorally.<sup>20</sup> All these factors could cause damage and loss of the remnant PDL, which will lead to more ankylosis.

The current study showed that the replantation of immature teeth presented less risk of ankylosis compared to mature teeth in all groups. On the contrary, some studies on human replanted teeth reported that immature teeth had a lower survival rate because immature teeth have lower calcification and short roots, which make them more susceptible to inflammation and resorption. 23,39,44 Furthermore, younger patients have a higher rate of bone remodeling when the replacement resorption starts. <sup>23,39,44</sup> This controversial result may also be due to factors like the timing of endodontic treatment because the revascularization of immature teeth (assumed to be one of the factors for a higher normal healing rate) needs a long time to happen and, during this time, teeth may be exposed to infection, which indicates endodontic intervention and subsequently reduces the chance for revascularization. Today, infection-related resorption is not an expected complication because we know how to proactively prevent and treat it. As a result of this proactive approach and increased knowledge among dentists, the vulnerable are less of a major problem today, although it is still seen now and then. However, if infection-related resorption occurs, it can be managed

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by endodontic treatment. The main long-term problem today is ankylosis and progressive replacement resorption.

In the current study, all studied groups presented some ankylosed teeth, ranging 10%–90%. Therefore, ankylosis should be expected in all replantation cases, even with teeth replanted immediately. Because of this risk, all replanted teeth should be carefully monitored for a long time after replantation. Ankylosis may cause teeth to inhibit growth and development in the region of replantation. Therefore, it is important to conduct proper follow-up of replanted teeth that become ankylotic and intervene in good time using different treatment options, such as decoronation. <sup>27,45</sup>

The strength of this study is that it utilizes a large clinical material (145 teeth) with long follow-up periods (in some cases up to 23 years), which is unique for a clinical study on replantation. Another strength is that all patients were treated according to a well-developed standardized protocol in the same clinic. Moreover, all patients were contacted the week after the trauma to check on the validity of data, for instance, to resolve difficulties in remembering some information such as actual time since avulsion, the extraoral storage time, and storage medium.

In addition, in the current study, the success rate assessment of teeth replantation was carried out by assessing the replanted teeth that risked ankylosis or healed successfully without ankylosis under different conditions. The success rate is a more accurate method of assessing the condition of the replanted teeth than the survival rate, which depends mainly on the presence of replanted teeth in the oral cavity whether it is ankylosed or not.

The follow-up periods ranged from months up to 23 years for the total material. When the material is split into groups, the number in some groups will be too small to make meaningful statistical comparisons. Consequently, we regarded 5 years as a suitable and accurate way to present the material, using the Aalen-Johansen method for long-term comparisons between groups, and 3 years to estimate the risk of ankylosis related to the risk factors shown in Table 3. Almost all ankylotic teeth were diagnosed in the first 2 years after trauma. Ankylosis is a progressive condition, and teeth diagnosed as ankylotic will be ankylotic for the rest of their life. Hence, the diagnosis of ankylosis will not change over time. We have chosen 3 years as a timepoint because all cases of ankylosis were diagnosed after 3 years. This allowed us to include a high number of teeth in the study and get the best risk estimate. If we had chosen 23 years as a timepoint, there would have been fewer teeth, and our results would have been less precise.

A weakness of a study such as this is that it is retrospective. It is important to build prospective follow-up studies using standardized registration in the future. If this can be carried out worldwide, we will have access to larger materials and can compare treatment outcomes in different centers. Another weakness may be the long period of data collection; collecting the data over several decades may result in that treatment recommendations may change over time, such as the timing and quality of the endodontic treatment, the endodontic retreatment, splinting techniques, and time of splinting. However, differences in treatment will most likely equally affect the variables studied.

The results of this study on clinical material with long-term follow-up verify that we should further encourage avulsed permanent teeth to be replanted immediately if possible. Storage in physiologic media is still a good alternative, although a somewhat higher risk of ankylosis is expected.

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Abdulaziz Gul initiated, planned and designed the study, carried out literature review, analyzed the results and wrote the manuscript. Eva Lauridsen planned and designed the study, guided us in the database, analyzed the results and took part in the scientific writing. Thomas A. Gerds carried out the statistical analyses and took part in the scientific writing, Lars Andersson, planned and designed the study, analyzed the results and took part in the scientific writing.

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No funding was provided to complete this study.

#### CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest related to the study.

#### DATA AVAILABILITY STATEMENT

If you want to have access to data please contact us. Our database is in the Copenhagen University Hospital and may be available at your request.

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