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Evaluating Emdogain® and healing of replanted teeth using an intra-individual experimental–control study design

Margareta Fridström[1,2], Jens Schollin[3], Claes-Göran Crossner[1,4]

[1]Department of Pediatric Dentistry, Postgraduate Dental Education Center, Örebro; [2]Department of Pediatric Dentistry, Specialisttandvården, Mälarsjukhuset, Eskilstuna; [3]Department of Pediatrics, Örebro University Hospital, Örebro, Sweden; [4]Institute of Clinical Dentistry, Faculty of Medicine, University of Tromsø, Tromsø, Norway

Correspondence to: Claes-Göran Crossner, Institute of Clinical Dentistry Faculty of Medicine University of Tromsø, N-9037 Tromsø, Norway
Tel.: +47 776 49121
Fax: +47 776 49101
e-mail: claes.crossner@fagmed.uit.no

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Abstract – The purpose of the present investigation was to use an intra-individual experimental–control study design to explore if application of Emdogain® prior to re-plantation after a dry extra-alveolar period of 60 min would promote a favorable healing of the periodontal ligament cells. Ten patients, for whom already decisions had been taken to extract two maxillary premolars because of crowding, participated in the study. The teeth were extracted and endodontic treatment was performed extra orally. The experimental tooth and its alveolar socket were covered with Emdogain® prior to replantation. The contra lateral tooth served as a control and was replanted without any prior treatment. The teeth were stabilized with a retainer for 3–7 days and the patients were followed up every third week. After 13 weeks, the teeth were finally extracted and prepared for histological examination. Radiographs were taken before the study period, at day 29 and prior to the final extraction. The results were in favor of Emdogain®, but the overall difference between the Emdogain®-treated tooth and its control was rather small, and it seemed questionable if the registered differences could be of any obvious practical clinical importance. Histologically, all the teeth showed some degree of pathology after such a long dry extra-oral time and the outcome seemed to be more correlated to the individual than to the treatment. Given more favorable conditions regarding storage medium and/or extra-oral time, Emdogain® might still be of value for an uncomplicated healing after replantation.

Traumatic dental injuries are common in children and teenagers and already at the age of 12, 20–30% would have suffered some kind of dental trauma in the permanent dentition (1, 2). The majority of these injuries involve the anterior teeth, especially the maxillary central incisors (3) and 0.5–16% are reported to be exarticulated (1). If the avulsed tooth is not fractured or damaged it is recommended to be replanted immediately at the site of the injury (1, 3) and the long-time prognosis is usually good (4). The periodontal ligament (PDL) cells will maintain their viability, allowing them to re-attach after re-plantation, only causing a minimal destructive inflammation because of the crushing injury, and healing with new replacement cementum is likely to occur after the initial inflammation has subsided (5).

For obvious reasons, it is not always possible to replant a tooth immediately after the accident and a prolonged extra-oral time may lead to irreversible damage of the PDL cells, particularly of the cementoblasts (6), and the overall treatment outcome is reported to be strongly dependent on the length of the dry extra-alveolar period (4, 7). According to some authors, the critical time limit of dry extra oral storage is 15 min (8, 9). However, if the extra-alveolar period is less than 1 h, complete or partial healing of the PDL cells may still be possible (4, 8, 10). If an immediate replantation is not possible, the avulsed tooth should be stored in a physiologic storage media like milk, saline or saliva until the patient has reached a dentist for replantation (3). Several storage media have also been developed which offers protection to PDL for a more extended time [e.g., Viaspan® (Barr Laboratories, Pomona, NY, USA) or other cell culture media (11)]. If the PDL is damaged, complications like surface resorption (repair-related root resorption or necrosis-related resorption), inflammatory resorption (infection-related root resorption), or replacement resorption (ankylosis-related root resorption or endosteal root resorption) may occur (8, 12). Surface resorption has no further clinical consequences, while the inflammatory resorption may lead to rapid loss of the tooth. In the case of replacement resorption, bone is fused to cementum and dentine. Consequently, as there is no protecting PDL the root will be replaced by bone within 3–7 years (4).

It is important that the avulsed tooth is given the best opportunity to promote the healing of the PDL and if it is not possible to immediately replant the tooth at the site of injury and if a suitable storage medium is not available, an alternative would be to stimulate the healing process. In the mid 1980s, an important discovery

revealed that enamel-related proteins mediate the formation of acellular cementum on the root of the developing tooth providing a foundation for all necessary tissues associated with functional periodontal attachment (13). Based on this observation, an enamel product named Emdogain® (BIORA AB, Malmö, Sweden) has been developed with the purpose of promoting regeneration of the periodontium that has been lost because of marginal periodontitis (13). Emdogain® has been shown to significantly improve PDL regeneration after periodontal surgery in adults (14, 15). As a replanted tooth usually has areas of lost or destroyed PDL, Emdogain® might be a choice of treatment to optimize the PDL healing and avoid ankylosis-related root resorption.

The purpose of the present investigation was to use an intra-individual experimental–control study design to explore if application of Emdogain® prior to replantation after a dry extra-alveolar period of 60 min would promote a favorable healing of the PDL and subsequently improve the long-time prognosis of avulsed teeth with a prolonged extra-oral time.

Material and methods

Patients in need of the orthodontic treatment, including extraction of two contra lateral maxillary premolars were chosen to participate in the study. The intent was to extract the two teeth, keep them in a dry environment for 60 min, treat one of them with Emdogain®, replant them, and finally extract and investigate them 3 months later.

Material

Ten participants were recruited among patients referred to the Department of Orthodontics, Postgraduate Dental Education Center, Örebro, Sweden. Participation was voluntary and the decision to extract two maxillary premolars because of crowding had already been taken. All included patients were between 12 and 15 years of age. They were primarily examined by a pediatric dentist as well as by a pediatrician and were found to have healthy dentitions and no adverse medical findings. The Ethics Committee of Örebro County Council had approved the study and all participants together with their parents received both verbal and written information of the purpose and the content of the study.

Methods

Prior to extraction, an impression was taken on the upper jaw and a maxillary wrap-around plastic splint, a so-called Essix retainer was made for fixation. The experimental tooth was randomly chosen by throwing a dice. If the number was even, the premolar in the first quadrant was chosen to be treated with Emdogain® and if the number was uneven the premolar in the second quadrant was chosen. The teeth were conventionally extracted under local anesthesia (Xylocain-Adrenalin Dental®, Astra, Sweden) and endodontic treatment was then performed extra orally. To avoid contamination

Fig. 1. Gentle handling of the root surface during the extra oral time including the endodontic treatment.

and minimize damage to cementoblasts and other PDL cells adhering to the root surface, the teeth were held with a special device during the endodontic treatment (Fig. 1). The pulps were accessed occlusally using a diamond burr and were extirpated with Hedström files. The root canals were irrigated with Dakin's solution, dried with paper points, and filled with guttapercha and sealer (Apexit®, Ivoclar/Vivadent; Schaan, Liechtenstein). The access cavities were filled with light-cured, resin-modified glass–ionomer material (Photac-Fil® Quick; 3M ESPE, MN, USA). After 60 min, the alveolar sockets were rinsed with saline before replanting the teeth and stabilizing them with the Essix retainer. The experimental tooth was covered with Emdogain® prior to re-plantation and the alveolar socket was filled with Emdogain®. The contra lateral tooth served as control and was replanted without any prior treatment. All patients were recommended to rinse the mouth with Chlorhexidine (1mg/ml Hexident®, Ipex, Sweden) for 7 days and the retainer was removed after 3–7 days. Clinical follow-up examinations were performed every third week including registration of pain or discomfort, tooth mobility, percussion sound, reaction to percussion, and gingival pocket depths. Updated radiographs of the teeth were available at the start of the study and additional radiographs were taken on day 29 and 92, and pathological changes like widened PDL, root resorption, and infection-related resorption of adjacent bone were recorded. After 13 weeks (day 92), the teeth were finally extracted and prepared for histological examination and the preplanned orthodontic treatment was instigated.

Histological examination

Immediately after extraction, the teeth were placed in phosphate buffered formalin until the clinical part of the study was finished, i.e., several weeks. After this period, the crowns of the teeth were removed by means of a diamond band saw. This was made under constant

irrigation with tap water and care was taken not to damage the root surface. The roots were then decalcified with sodium citrate and formic acid, dehydrated in ethanol, and finally embedded in paraffin. Five-μm thick semi-serial sections were then taken perpendicular to the long axis of the teeth. The distance between each section was 700 μm. The microscopic examination was made in a light microscope Leica DMRB (Wetzlar, Germany). Microphotographs were taken with Nikon Digital Still Camera DXM 1200 (Excel Technologies inc., Enfield, CT, USA) and transferred to a computer. Quantitative evaluation was made in Olympus BH2 (GMI-inc., Ramsey, MN, USA) equipped in such a way that two examiners could simultaneously evaluate the image. Each cross-section was divided into the mesial, distal, buccal and lingual surfaces, and the surfaces in the axial corners. Thus, the following parameters were registered on eight different surface areas according to Fridström 2005 (16):

1 Status of the periodontal ligament:
a) PDL with collagen fibers attached to the cementum.
b) PDL with collagen fibers detached from the cementum.
c) Loss of PDL.
2 Status of cementum:
a) Cementum present.
b) No cementum.
3 Root resorption:
a) No resorption.
b) Superficial resorption limited to the cementum and peripheral dentine not reaching the dentinal tubules.
c) Deep penetrating resorption reaching the dentinal tubules.

The registration was not based on the appearance in a single spot but on the dominating parameter in the different areas. The relative area (%) occupied by the different parameters was calculated. For example, if there were 20 registrations for no resorption, 40 for superficial resorption, and 20 for deep resorption, these figures were recalculated to represent a percentage of the surface. Thus, in this case, the figure for no resorption was 20/80 = 25%, superficial resorption 40/80 = 50%, and deep resorption 20/80 = 25%. In this way, it was possible to compare the registrations from the different teeth independent of their varying root lengths.

Results

Clinical and radiographic observations

Nine participants completed the study, while in one patient (No. 8), the teeth had to be removed prematurely because of extensive radioluscency in adjacent bone. Four patients experienced discomfort in the maxillary teeth because of ill-fitting Essix retainers. In the first of these patients (No.5), the retainer was removed on the second day and replaced with a new retainer. For the second patient (No. 7) on day two, the retainer was altered to a semi-rigid resin orthodontic wire splint for another 5 days. In the other two cases, the retainer fitted in the beginning but because of increasing discomfort it was removed on day three (No. 9) and day four (No. 10), respectively, without being replaced by any other fixation. All four patients were immediately relieved of the discomfort after removal of the poorly fitting retainers.

Table 1. Clinical and radiological findings

Clinical observations							Radiological observations	
Patient no.		Day 8	Day 29	Day 50	Day 71	Day 92	Day 29	Day 92
1	E		♪	♪	♪	♪		
	C		♪	♪	♪	♪		
2	E						♦	
	C		♪	♪	♪	♪		
3	E							
	C		♪	♪	♪	♪		
4	E						♦	■
	C			♪	♪	♪		
5	E	π						
	C	π						
6	E							
	C							
7	E	π				M	♦	•
	C	π						
8	E	π	X				•	
	C	π	X				•	
9	E	π		♪	♪	♪		
	C	π		♪	♪	♪		
10	E	π						■
	C	π	π		♪	♪	♦	■

E, experimental tooth covered with Emdogain®; C, control tooth replanted without any prior treatment; M, mobility; ♪, percussion sound indicating ankylosis; π, sensitivity to percussion; X, extracted; ♦, widened PDL; •, infection-related resorption of adjacent bone; ■, root resorption.

The results of the clinical and radiological observations are presented in detail in Table 1. At the end of the study period (day 92), the depths of the gingival pockets were less than 3 mm for all the 18 teeth involved, and increased mobility was registered for one tooth only. Percussion sound indicating ankylosis was registered for two Emdogain®-treated teeth and six control teeth and in both cases when the experimental tooth was involved, so was its control tooth. Radiographically 14 of the 18 teeth that were followed up for the 92 days showed normal periodontal conditions at the final extraction. Six of these teeth had been treated with Emdogain® and eight were controls. The remaining four teeth (in three patients) showed resorption of the root surface or radioluscency in the adjacent bone. Two of the patients who showed radiological complications on day 92 also had problems with the Essix retainer (No. 7 and 10).

Histological observations

All the teeth showed varying degrees of pathological changes and the relative areas covered with an attached PDL varied between 14% and 83% for the Emdogain®-treated teeth and between 20% and 57% for the controls (Fig. 2). Comparison of the results within each patient showed that six Emdogain®-treated teeth had a higher

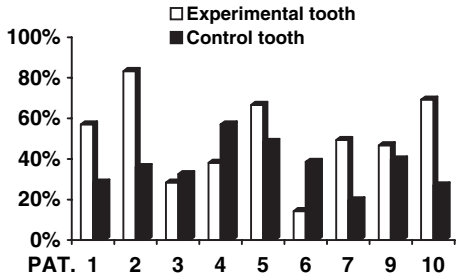

Fig. 2. Percentage of the surface area showing cementum with attached periodontal ligament.

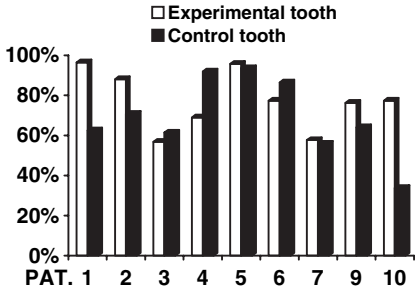

Fig. 3. Percentage of the surface area covered by cementum.

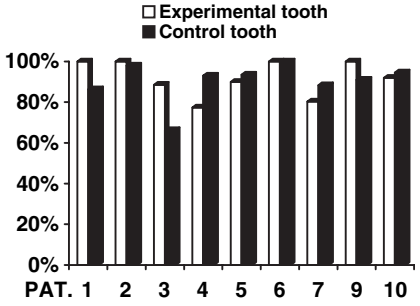

Fig. 4. Percentage of the surface showing no or only superficial root resorption.

degree of root coverage with attached PDL than the control teeth, while the situation was the opposite in three cases (Fig. 2). The relative areas covered with cementum also showed a slightly higher average percentage for the Emdogain®-treated teeth (Fig. 3). No resorption or only superficial resorption was registered rather equally for the experimental tooth and its control (Fig. 4), while no deep penetrating root resorption could be registered in four Emdogain®-treated teeth compared with one control tooth (Fig. 5). To create an index for the health condition of the periodontium, the percentages of attached PDL, presence of cementum, and no root resorption were combined. This index is presented in Fig. 6 and showed that the periodontal condition was healthier for the Emdogain®-treated tooth in six of the nine cases subjected to histological evaluation. For two cases, the control tooth showed better conditions, while in one patient (No. 3), the results of the experimental and control tooth were very similar.

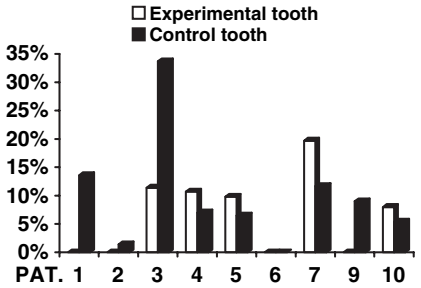

Fig. 5. Percentage of the surface showing deep penetrating root resorption.

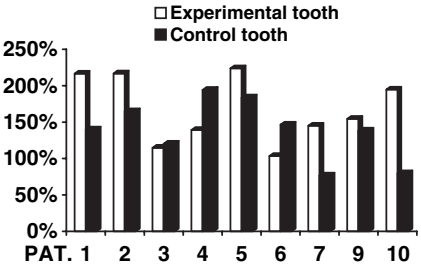

Fig. 6. Quantitative histological results based on three parameters: 1, cementum present; 2, periodontal ligament (PDL) with collagen fibers attached to the cementum, and 3, no root resorption.

Discussion

Analyses of different treatment procedures for traumatically injured teeth are based on clinical experience and knowledge from case reports, or from experimental studies in animals. To our knowledge, this study seems to be the first time healthy human teeth designated for extraction were used to investigate healing after replantation. The advantages with the present study design were obvious. The study was performed on humans. The experimental tooth and its control were from the same individual, and with the exception of Emdogain®, they were treated equally allowing an intra-individual comparison as well. On the other hand, a disadvantage with this approach might be that only parts of the PDL were available for histological examination, which limited the obtainable information regarding bone-forming cells and ankylosis. In animal studies, blocks containing the root and surrounding tissues are available for examination making it possible to investigate the entire PDL and adjacent hard tissues (17–19).

A limited number of studies have been performed to evaluate the effect of Emdogain® treatment on periodontal healing following replantation of avulsed teeth. An animal study (19) reports a lower incidence of replacement resorption in dog teeth when Emdogain® is applied to the root surface prior to replantation compared with teeth without any pretreatment before replantation. In a clinical study on previously ankylosed human teeth which were gently removed and treated with Emdogain® before replantation, 11 out of 16 teeth show no signs of ankylosis after an average observation time of 15 months (6). In contrast to these studies, there are

also reports concluding that Emdogain® neither is able to cure nor probably not even prevent ankylosis nor able to significantly reduce replacement resorption in teeth which underwent delayed replantation (20–22). The percussion sound of ankylosis, the occurrence of deep penetrating resorption, and the periodontal health index used in the present study indicated that the teeth treated with Emdogain® prior to replantation managed better than their controls. On the other hand, the material as well as the differences were considered to be too small to make a meaningful statistical evaluation, and looking at the overall clinical and radiological observations, it seemed questionable if the registered differences could be of any obvious practical clinical importance.

Several studies have emphasized that minimizing the extra oral time and the use of a physiologic storage medium are the most important factors for survival of the PDL cells of an avulsed tooth. A recent report including 45 avulsed teeth shows that after observing for a year, the occurrence of replacement resorption is directly correlated to the time period of extra-oral dry storage, and after 60 min the replacement resorption is 100% regardless of pretreatment with Emdogain® (12). Furthermore, it is recommended to improve the prognosis by not keeping the tooth dry during the extra oral time, but in a physiologic storage medium like milk, saline, saliva, or special cell culture media (3, 11). In addition, local and systemic administration of antibiotics is shown to have a favorable impact on periodontal healing and is recommended as a standard procedure following replantation (23–25). The Ethics Committee of Örebro County Council did not, however, approve the use of systemic antibiotics in the present study.

Badly fitting Essix retainers caused discomfort in four patients. Two of them showed radiological complications while the other two did not. This fact, together with that all four patients experienced immediate pain relief after removal of the retainer and that the discomfort was not localized in the two teeth involved in present study but to all the teeth in the maxilla, suggested that the radiological findings most probably were unrelated to the retainer problem.

The intention of this study was to develop a more scientific study design for investigation of different treatment procedures on human teeth after dental trauma and to test this design on Emdogain® after replantation with a prolonged dry extra oral time. In a normal clinical situation, it is not possible to create a study allowing intra-individual comparison of experimental and control teeth, and consequently, this kind of studies have always been previously performed on animals (17–19). In hindsight and in accordance with another recent study (12), maybe a 60-min dry time was too long, especially as systemic antibiotics were not allowed. The overall difference between the Emdogain®-treated tooth and its control was rather small, and after such a long dry extra oral time the outcome seemed to be more correlated to the individual than to the treatment.

Administration of systemic antibiotics must be considered as a routine standard when teeth are subjected to replantation. Even in the rather aseptic environment of a dental operatory and with teeth very carefully handled for research purpose, there were several failures because of infection. The histological results were in favor of Emdogain® and given more favorable conditions regarding extra oral time, storage medium, and systemic antibiotics (or aseptic handling), Emdogain® might still be of value for an uncomplicated healing after replantation. However, this has to be proven in additional studies. For this purpose, the present study design is highly recommended, preferably with the use of a fixation causing less discomfort for the participants.

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