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Replantation of permanent incisors in children using Emdogain®

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Abstract – The aim of this study was to determine whether application of an enamel matrix protein derivative, Emdogain® (Biora AB Malmo, Sweden) to the root surface of avulsed permanent incisors would improve postreplantation outcomes in a pediatric population. Between June 1999 and May 2002, 25 avulsed permanent maxillary incisors (22 centrals and three laterals) were treated with Emdogain® and followed for up to 32 months, mean duration 20.6 months (range: 6.9–32.5 months). Mean patient age at the time of treatment was 12.0 years (range: 7.7–17.6 years) and mean extra-alveolar duration was 185 min (range: 100–300 min). At the end of their follow-up each of the replanted incisors demonstrated radiographic evidence of replacement root resorption and clinical evidence of ankylosis. None of the replanted teeth were affected by inflammatory root resorption and there was no evidence of infection. When compared with the control samples from Barrett and Kenny (*Endod Dent Traumatol* 1997;15:269–72.) and Andersson et al. (*Endod Dent Traumatol* 1989;5:38–47.) this sample treated with the Emdogain® protocol demonstrated significantly less root resorption than either of the control samples (ANOVA, $P < 0.0001$). Although the Emdogain® protocol did not produce periodontal regeneration, it did eliminate inflammatory resorption and infection and led to significantly less root resorption compared with the two historical controls.

Key words: dental trauma; replantation; Emdogain; amelogenin

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Despite the 'advances' in treatment for avulsed teeth reported over the past decade, replantation outcomes have not improved (1–3). Unfortunately, storage media, root surface treatments and endodontic protocols have not delivered on the quest for periodontal ligament (PDL) regeneration and a return to preinjury state following replantation. Consequently, clinicians, parents and patients are left to deal with the complications associated with the replantation decision (4, 5). An ever-increasing body of evidence has demonstrated that replantation delayed beyond 5 min is associated with a decreased likelihood of PDL regeneration (1, 6). Similarly, desiccation beyond 15 min renders root-surface PDL cells incapable of normal function and affects their ability to reproduce (7–9). Despite this evidence, immediate replantation is rarely performed (2) because of disruption associated with the injury or the reticence of bystanders to become involved (10, 11).

As a consequence, most incisors are replanted after protracted extra-alveolar storage and sustain catastrophic damage to their PDL, develop pulpal necrosis, root resorption, ankylosis and subsequent infraocclusion during adolescent growth. (1, 12). Andreasen et al. (1) reported that 30% of all replanted permanent maxillary incisors were extracted over the course of a large clinical study that involved both children and adults. Barrett and Kenny (2) reported the 5-year survival of incisors replanted in children and adolescents to be 0.57. The long-term prognosis for permanent incisors replanted beyond 5 min extraoral duration remains poor, especially in the preadolescent population. Clinicians are at a loss to improve outcomes without the ability to stimulate PDL regeneration across damaged root surfaces to protect the tooth from external resorption.

Wounding causes cells from root-side PDL remnants or paravascular endosteal spaces with the characteristics of early PDL progenitor cells to proliferate, migrate and produce new cells that can synthesize alveolar bone, cementum and PDL (7). A reduced or altered population of progenitor cells will significantly reduce regeneration and this will adversely affect the survival of replanted teeth. Factors capable of promoting PDL regeneration and root-surface repopulation could provide a means to protect the replanted root from resorption and ankylosis (8, 9).

The enamel matrix derivative Emdogain® (Biora AB, Malmo, Sweden) is a therapeutic agent that has been proposed to promote PDL regeneration after periodontal surgery. Emdogain® (EMD) is a sterilized aqueous solution of propylene glycol alginate that contains proteins of the amelogenin family that have been extracted from developing porcine embryonal enamel (13–15).

Animal studies have demonstrated that EMD might enhance the migration, attachment, proliferative capacity and biosynthetic activity of PDL cells (16, 17) but the precise mechanism of action has not been completely elucidated. For example, Chano et al. (18) reported that induction of cellular differentiation might not be part of the mechanism of action of EMD when tested on a rat PDL wounding model. Despite the lack of a consistent explanation of the mechanism of action of EMD in periodontal regeneration, the possibility that it might bolster PDL regeneration is a compelling stimulus to investigate these properties in the light of the predictable failure of most cases following delayed replantation (1–3).

The purpose of this study was to determine if incisors replanted according to a novel EMD-based protocol would demonstrate different clinical or radiographic outcomes than two separate, previously reported samples (2, 6).

Materials and methods

Sample

Patients who attended the Department of Dentistry of The Hospital for Sick Children (HSC) for treatment of avulsed permanent maxillary incisors between July 1999 and September 2001 were considered for entry into the study. Inclusion criteria allowed any patient younger than 18 years with avulsed permanent maxillary incisor(s) that had been subjected to prolonged extra-alveolar duration (dry period in excess of 15 min) and root maturity equal to or greater than Moorrees et al. Stage 4 (19). Stage 4 was defined as immature while stages 5 and 6 were considered mature. Avulsed teeth with associated crown or root fractures, periodontal disease or alveolar fractures were excluded, as were patients with significant health problems.

Technique

After obtaining informed consent to participate in the study, avulsed incisors were radiographed extra-orally to classify root maturity. Next, while holding the avulsed tooth in a moistened gauze sponge lingual access was obtained and the dental pulp extirpated. If root apices were not sufficiently constricted to allow for conventional gutta percha/sealer obturation, the apical foramen was sealed with fortified zinc oxide-eugenol (IRM, Caulk Dentsply, Milford, DE, USA) following gutta percha/sealer obturation. In accordance with directives from the manufacturer of EMD, no calcium hydroxide products were placed in the root canals and the root surfaces were prepared by removal of the PDL with a rubber prophylaxis cup charged with flour of pumice and water and the root surface rinsed with water. Root 'conditioning' with citric acid was not recommended by the manufacturer and was not part of this protocol. EMD was prepared according to the manufacturer's specifications from a liquid vehicle and enamel matrix derivative pellet. Alveolar sockets were rinsed free of blood clots without curettage and hemostasis was achieved with wet gauze pressure packs. The fully mixed solution of EMD was delivered to the root surface and into the alveolar socket with the blunt cannula provided. The incisors were replanted and splinted with flexible wire secured with composite resin for 6–8 weeks. All patients received 7-day courses of penicillin or clindamycin if sensitive to penicillin products and reappointed for follow-up within 14 days.

Clinical and radiographic follow-up

Standard clinical and radiographic examinations were available for all patients. A single clinician (EJB) performed all follow-up examinations and took all radiographs. Maxillary periapical radiographs of replanted incisors were available for each patient. Exposures were made at 55 kVp and 15 mA. All films were processed and examined before the patient was dismissed. Periapical radiographs were taken by a paralleling technique with a Rinn XCP® kit (Rinn Corp., IL, USA) and small film (Kodak® Ultra-speed, size 0, Eastman Kodak Co., Rochester, NY, USA). The orientation of the central beam for periapical radiographs depended upon which teeth were replanted. Standardized periapical radiographs taken at the time of the trauma and the last follow-up were compared.

Radiographic assessment

Radiographs obtained at follow-up during the course of the current study as well as those used in Barrett and Kenny (2) (current control sample) were assessed for quantity and type of external root resorption using the scale developed and validated by Andersson et al. (6). Radiographs taken at the time of initial presentation were used to determine stage of apical development using the stages defined by Moorrees et al. (19).

Two pediatric dentists independently evaluated all of the radiographs from both studies. Prior to the first evaluation the raters took part in an exercise to familiarize themselves with the indices and diagnosis of types of root resorption. They were then asked to apply the criteria to a series of radiographs drawn from a small test sample. Raters were encouraged to ask questions and discuss their application of the indices with each other. When the raters were comfortable with the use of the root resorption and apical development indices they were each taken to a separate room to view and score radiographs.

An assistant presented the raters with a series of radiographs for each patient in random order. Each series was labeled with a number so that the raters and assistants were blinded to the identity of each patient. The first radiograph in the series was a periapical film taken at the time of initial presentation. This was used to score apical development and to serve as a baseline reference when scoring external resorption. The next films were periapical and occlusal views obtained at the last follow-up visit. Raters were asked to use both films to score and classify external root resorption. Radiographs were viewed in darkened rooms equipped with a lighted viewbox. A hand held magnifying lens (2.5×) was provided for each rater and they were encouraged to use it.

Statistical methods

As some patients had more than one replanted tooth individual observations were not independent. Therefore, in cases where more than one tooth had been replanted a single tooth was chosen by random draw to be included in the analysis thereby assuring independence of observations.

The quantity and type of root resorption demonstrated radiographically from the current sample as well as the two control samples (2, 6) were recorded in a computer database. One-way ANOVA was used to test for significant differences in the quantity of root resorption between samples. When necessary, the Tukey-Kramer multiple comparison test was employed to test for significant differences between means. The distribution of resorption types was investigated by generating stacked bar charts and chi-square statistics were used to test for differences in the distribution of resorption types between groups. All statistical tests were performed using JMP 3.1 (SAS institute, Cary, NC, USA) with a critical value of 0.05.

Inter-rater agreement values were estimated for the stage of apical development and inflammatory resorption using the kappa statistic (20). To calculate intra-rater reliability, a random sample of fifteen patients was drawn for each rater to score. A second session was conducted under identical circumstances 7 days after the first session. In cases where disagreement existed between raters an 'expert panel' was convened to produce a consensus decision. Data for the Andersson root resorption index was evaluated according to Andersson (6).

Results

Sample

Demographic information for the sample of patients and teeth is presented in Table 1. For the purpose of statistical analysis, only a single tooth from each patient was considered and labeled as the 'final' sample in this table.

Radiographic assessment of root resorption

Results of the intra and inter-rater reliability study for the Andersson root resorption study for the current sample are presented in Table 2.

Table 1. Demographic information for all patients and replanted teeth

Number of patients	25
Males:females	16:9
Mean age at trauma	12.0
Age range	7.7–17.6 years
Number of replanted teeth	28
Central:lateral incisors (total)	24:4
Centrals:lateral incisors (final)	22:3
Mean extra-alveolar duration	185 min
Extra-alveolar duration range	100–300 min
Mean extra-alveolar dry duration	64.8 min
Mean extra-alveolar wet duration	120.2 min
Mean follow-up	20.6 months
Follow-up range	6.9–32.5 months

Table 2. Results of the intra and inter-rater agreement study for Andersson root resorption index [after Andersson et al. (6)]

Session	Rater 1	Rater 2	Rater 1 – Rater 2
1	–	–	MD = 0.2; SD = 0.3
2	–	–	MD = 0.3; SD = 0.3
	MD = 0.2; SD = 0.3	MD = 0.1; SD = 0.2	

MD, mean difference; SD, standard deviation

Post-replantation root resorption

The ANOVA results that compare the current sample with the results of Andersson et al. (6) and Barrett and Kenny (2) are presented in Figs 1–3. The three samples did not differ significantly from each other based on the number of sites affected by total amount of root resorption or replacement resorption (Figs 1 and 2) but did differ based on the total amount of inflammatory resorption (Fig. 3). The Tukey-Kramer test demonstrated that the EMD protocol subset differed significantly from both the Barrett and Kenny (2) and Andersson et al. (6) control samples ($Q = 3.07$, $P < 0.05$) yet the two control samples did not vary significantly from each other.

The distribution of resorption types over time and between samples are presented in Figs 4–6. Results of ANOVA demonstrated that there was no significant difference between the percentages of sites affected by root resorption regardless of treatment group (ANOVA, $P = 0.98$). The proportion of sites that exhibited inflammatory root resorption was not significantly different between the two control samples. (χ^2, $P = 0.006$). However, comparison of the EMD sample with the two control samples suggests that at 3 years the total amount resorption may be less under the experimental protocol.

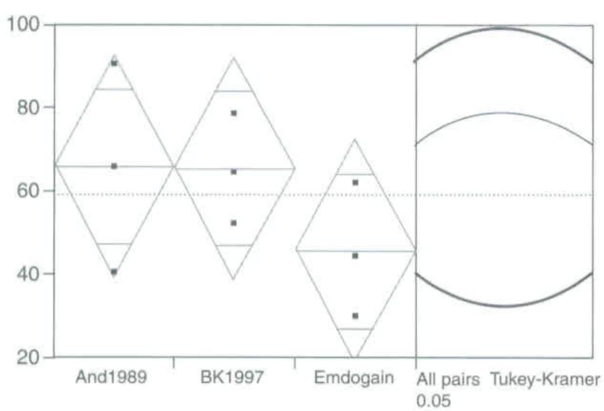

Fig. 1. Graphical ANOVA presentation comparing the total amount of root resorption between the two control samples, And1989 (Andersson et al. 1989), BK1997 (Barrett and Kenny 1997) and the Emdogain® sample.

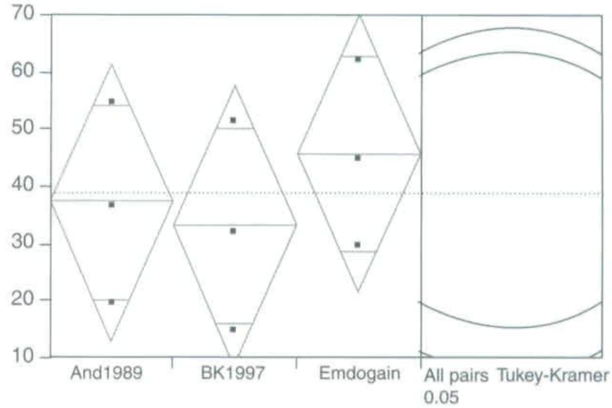

Fig. 2. Graphical ANOVA presentation comparing the quantity of replacement resorption between the two control samples, And1989, BK1997 and the Emdogain® sample.

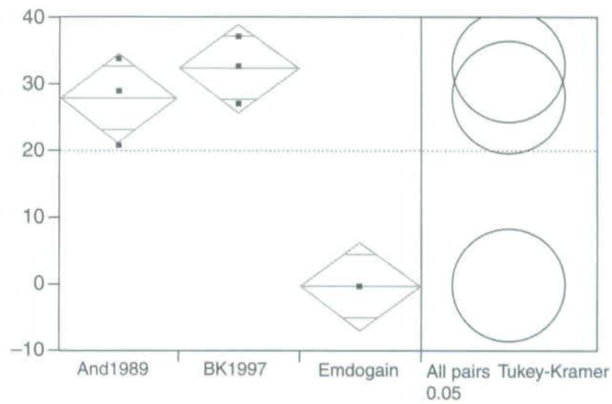

Fig. 3. Graphical ANOVA presentation comparing the quantity of inflammatory resorption between the two control samples, And1989, BK1997 and the Emdogain® sample.

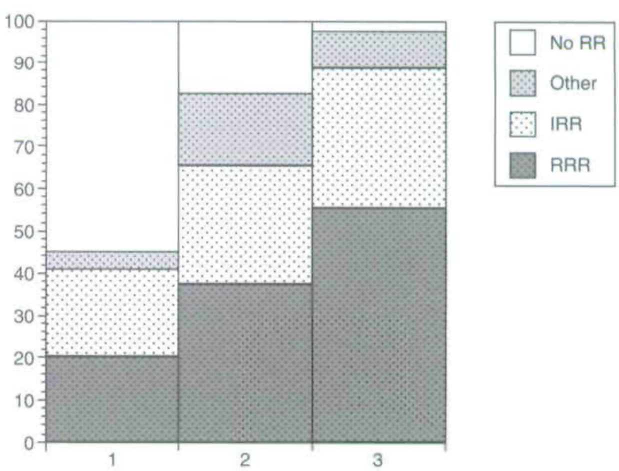

Fig. 4. Distribution of root resorption types over time (years) for the sample reported by Andersson et al. (6) root canal treatment <3 weeks. Inflammatory root resorption (IRR) and replacement root resorption (RRR).

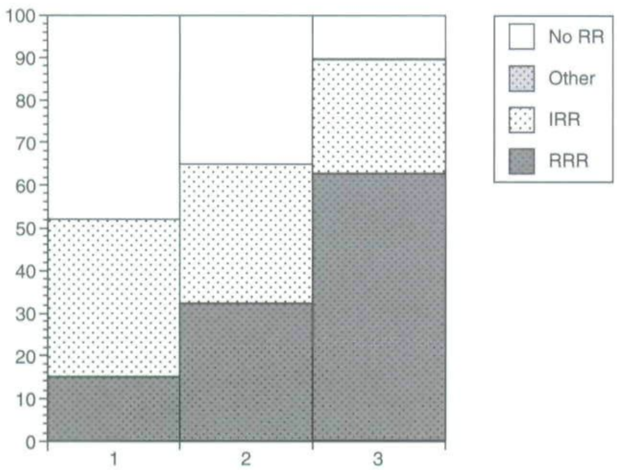

Fig. 5. Distribution of root resorption types over time (years) for the sample reported by Barrett and Kenny (2) root canal treatment <3 weeks. Inflammatory root resorption (IRR) and replacement root resorption (RRR).

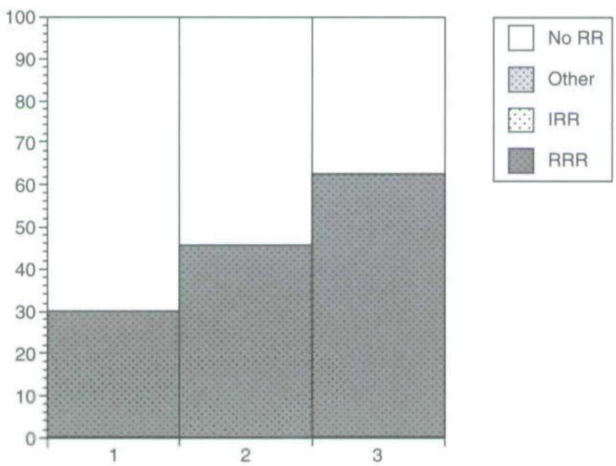

Fig. 6. Distribution of root resorption types over time (years) for the Emdogain® sample, root canal treatment prereplantation. Inflammatory root resorption (IRR) and replacement root resorption (RRR).

Discussion

To date, most replantation studies have focused on associations between injury-related (extraoral period, storage media, etc.) and treatment variables (timing of endodontic treatment, duration of root canal medication, etc.) with the development of healing complications. Unfortunately, there has not been a corresponding improvement in clinical outcomes. Consequently we know for certain that teeth that are not replanted immediately all suffer the same fate: eventual loss because of root resorption or infraocclusion.

In 1995 Andreasen reported that the decisive factor for PDL healing was immediate replantation (1). Unfortunately, immediate replantation is not the norm (1, 2, 6) so clinicians continue to be confronted with avulsed teeth that have a guarded prognosis at best. Despite a growing literature that describes the irreversible cellular damage that usually accompanies an avulsion injury (7–9), current treatment guidelines continue to advocate prereplantation immersion of avulsed teeth in various solutions (balanced salts, tissue culture media, stannous fluoride) in an effort to 'optimize' postreplantation outcomes. These recommendations are at odds with the results of in vitro research and unsubstantiated by clinical trials yet they continue to be advocated in clinical guidelines.

Replanted teeth usually fail because of progressive external root resorption of either the replacement or inflammatory type (2, 3, 6). While inflammatory root resorption may be prevented by early (<3 weeks) endodontic treatment, replacement root resorption is not treatable and arises from a failure of PDL regeneration. Thus, while we have effective endodontic techniques at our disposal, there is no treatment available to direct PDL regeneration following replantation. Until a predictable, safe and cost-effective means of directing regeneration of the PDL is found, the long-term prognosis for avulsed teeth that are not immediately replanted will continue to be grave. This is the first clinical trial to test a pharmacotherapeutic modality aimed at the regeneration of PDL in replanted permanent incisor teeth. When this study began EMD was being used as a potential treatment for periodontally diseased teeth. The use of EMD to treat avulsed permanent incisors was a logical extension given that the defining feature of replanted teeth is the failure of PDL regeneration.

The root resorption index developed by Andersson was chosen for this study. In addition to being a robust measure of root resorption activity it is both qualitative and quantitative and has been validated and shown to be reliable and reproducible in subsequent clinical studies (2, 6). The results of the current reliability study of the index showed that raters were consistent in their individual scoring and demonstrated a high degree of agreement with each other.

The primary objective of this study was to determine whether the EMD protocol would improve healing outcomes for replanted permanent incisor teeth. The primary causes of extraction, ankylosis in growing children and severe external root resorption at any age, are discernible by 6 months so it was decided that this longitudinal outcome study of EMD should be 6 months to approximately 2 years in duration to ensure both the short and long-term effects of wound healing would be identified.

This investigation failed to demonstrate that EMD itself prevented the development of inflammatory root resorption or promoted PDL regeneration. However, the EMD protocol was associated with significantly better postreplantation healing when compared with the historical control samples. As this model and others demonstrate, under the best circumstances delayed replantation inevitably results in failure because of the effects of root resorption. Whereas the samples of Barrett and Kenny (2) and Andersson et al. (6) do not differ significantly with respect to the relative quantities of replacement or inflammatory root resorption, the EMD protocol sample exclusively developed replacement resorption.

While this complete absence of inflammatory root resorption in the EMD protocol sample is notable, similar results have been reported previously. In a landmark primate study, Andreasen (21) reported that teeth that were endodontically treated prior to replantation exhibited significantly more replacement root resorption than those replanted without having conventional endodontic treatment. In a clinical study, Andersson (6) reported on the amount of root resorption affecting teeth replanted with or without the completion of root canal treatment. The results of this study are consistent with those of Andreasen (21) in that teeth that had endodontic treatment completed in a timely manner predominantly exhibited replacement resorption while those for which endodontic treatment was delayed were affected mainly with inflammatory resorption. The results of these two studies mirror so precisely the current investigation that it seems that the timely performance of endodontic treatment alone could explain the observations and the application of EMD was of limited utility.

The decision to gently remove the PDL from the avulsed tooth prior to replantation was taken during protocol development and following several discussions with Biora scientists. As EMD was a relatively new material in Canada at the initiation of this study scientific and ethical approval was particularly rigorous as this was a novel use of a therapeutic agent in children. Approval was granted but in order to be consistent with the manufacturer's instructions all residual PDL was removed from the avulsed teeth with a rubber cup and pumice prior to coating with EMD and replantation. This was a compromise given that the product monograph originally written for periodontally involved teeth called for root planning prior to the application of EMD.

There is evidence that suggests the results of this study might have been different if the PDL had been left on the surface of the tooth. In a recent dog study (22) it was found that teeth coated with EMD prior to replantation exhibited significantly more healed PDL than control teeth that became ankylosed. The findings of this study are consistent with the current study and suggest that 'root-side' PDL may play a role in the action of EMD in humans as well. This may become the focus of a future study.

As this study and others demonstrate, under the best circumstances delayed replantation inevitably fails because of the effects of root resorption. The primary advantage of eliminating inflammatory resorption is that replanted teeth might be retained *in situ* for longer if unaffected by inflammatory resorption (6). This might provide an advantage for physiologically mature patients but the utility of replanting teeth in preadolescents remains unclear as the infraocclusion that occurs with adolescent growth often leads to elective extractions.

This investigation represents an evolution in dental traumatology in that a bioactive substance has been tested in a prospective case series under well-controlled conditions. Two major questions have developed. Would leaving the 'root-side' PDL on avulsed teeth lead to more favorable results? Was the absence of inflammatory root resorption solely because of endodontic treatment prior to replantation or the effect of EMD? Irrespective of the answers, clinicians faced with the prospect of delayed replantation of avulsed teeth will continue to be confronted with patients/parents whose expectations exceed their ability to regenerate PDL and protect the root from resorption (5).

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