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The effect of Emdogain® and 24% EDTA root conditioning on periodontal healing of replanted dog's teeth

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Abstract – Controversies still exist as for the regenerative role of enamel matrix derivatives and the need for removal of the periodontal ligament in replanted teeth. The purpose of this study was to evaluate the effect of Emdogain® and 24% ethylenediamine tetraacetic acid (EDTA) root conditioning on periodontal healing of replanted dog's teeth. Teeth were extracted, endodontically treated and preconditioned as follows: group 1, Emdogain®; group 2, Emdogain® + EDTA and group 3, EDTA. Teeth were replanted after 30 min extraoral time, splinted for 15 days and animals sacrificed after 8 weeks of observation. Histological evaluation was performed using hematoxylin/eosin and Masson trichrome and results scored based on previously reported criteria for histological evaluation. Replacement root resorption was histologically diagnosed in all groups except in the negative control. A parametric analysis showed no statistically significant differences between experimental groups. Root preconditioning with Emdogain® alone or in combination with 24% EDTA showed no evidence of regeneration of collagen fibers and consequently did not prevent the development of replacement root resorption on replanted dog's teeth.

The incidence of dental trauma due to falls, sports, automobile accidents and violence has increased significantly in recent decades, affecting children's and teenagers' anterior teeth (1). In 2003 Levin et al. (2) reported that 9% of young adults aged 18–19 years who have participated regularly in at least one sport had experienced dental injuries during sports participation at some point in their lifetimes. The first clinical and radiographic examination of the traumatized patient is crucial to determine the initial diagnosis, severity of the injury, treatment plan and to create a baseline for follow up. Success of tooth replantation following avulsion is affected by a number of factors including: extra oral time (3–5), storage medium until replantation (3, 5, 6), conditioning of the root before replantation (5–7), socket preparation (8), stage of root development (3, 5, 8), presence of necrotic periodontal ligament (PDL) (6, 9, 10), tooth splinting and masticatory stimulation (3, 5, 11), systemic antibiotic treatment (12–14), initiation time of endodontic treatment (3, 5), and intracanal medicaments (3, 5, 15).

Replacement root resorption is the most common complication following replantation of an avulsed tooth and lead to infraocclusion, poor esthetics, tilting of adjacent teeth, loss of function, and eventually loss of the tooth (1, 16). Treatment is often complex, time consuming, expensive and requires multidisciplinary approaches such as endodontic and periodontal treatments, surgery, orthodontic movements as well as esthetic coronal restoration (3).

Several treatment modalities were proposed in order to delay or prevent the associated root resorption and increase the long-term success rate of avulsed teeth (6–8, 10, 17). Among them, fluoride (18, 19), sodium alendronate (20), dexamethasone (21) enamel matrix derivatives (EMD) (22–24), and triamcinolone (15).

The enamel matrix proteins were used to commercially develop a product called Emdogain® (Biora, Malmo, Sweden) for regeneration of injured periodontal tissues (25–29). The clinical safety of Emdogain® for treatment of periodontal defects in humans has been confirmed and the US Food and Drug Administration, British Standard Institute, and the Scandinavian Institute of Dental Materials approved its use for periodontal therapy. Health authorities in the USA, Europe, and Japan as well as in several other countries already approved the use of Emdogain® in humans. In 2001 and 2003 Emdogain® was suggested by the International Association of Dental Traumatology (IADT) and the

American Association of Endodontists, respectively, for treatment of avulsed teeth replanted after 60 min in dry extraoral conditions (30, 31). The rational behind this recommendation was the ability of EMD to produce new PDL based on the socket- side cell population.

Controversies still exist as for the regenerative role of EMD in replanted teeth. While some studies have suggested its use for treating avulsed teeth prior to replantation in order to prevent or delay replacement osseous root resorption by regenerating a healthy periodontium (22–24, 32), others demonstrated that Emdogain® failed to prevent the onset and progression of replacement root resorption (33–38). The available data is even more controversial as to the removal of the necrotic PDL, despite the fact that current IADT guidelines recommend the removal of the necrotic PDL attached to the root surface of avulsed permanent teeth with more than 60 min extraoral dry time (5). Some studies recommended the use of 10% sodium hypochlorite (9, 10), citric acid (7, 9), pumice (38) to remove the PDL while others left the necrotic tissue attached to the root surface (23). Interestingly all the previously mentioned studies disregard the manufacturer recommendations of using ethylenediamine tetraacetic acid (EDTA), prior to the application of Emdogain. Perhaps due to the fact that the effect of EDTA alone or in combination with Emdogain for PDL regeneration of avulsed teeth was never investigated. The rational of using EDTA is aimed to remove the smear layer, demineralization of the acellular cementum and exposure of collagen (39, 40), all of which delay the onset of root resorption and perhaps allows more time for fibroblast regeneration. Tonetti et al. (29) conducted a very well designed prospective multicenter randomized controlled clinical trial to compare the clinical outcomes of papilla preservation flap surgery with or without the application of enamel matrix proteins (EMD). After debridement, roots were conditioned for 2 min with 24% EDTA gel. Emdogain® was applied in the test subjects and omitted in the controls. The results of this trial indicated that regenerative periodontal surgery with EMD offers an additional benefit in terms of clinical attachment levels and pocket probing depths. Yet again, the specific effect of EDTA on the outcome of the trial remains unclear.

The aim of our study was to evaluate the effect of Emdogain® and 24% EDTA gel, alone or in combination, applied to the root surface of teeth with dry extraoral time, in the regeneration of the PDL of replanted dog's teeth and prevention or delay of replacement root resorption.

Materials and methods

The study was approved by the Ethics Committee of the School of Dentistry in the University Autonomous of San Luis Potosí. Twenty-four single-rooted teeth (incisors and premolars) from four creoles dogs ranging between 1 and 2 years old age and weighing 4–5 kg were used for this study. Before any interventions, the teeth were radiographed with paralleling devices (Dentsply Rinn, Elgin, IL, USA) and only those dogs that had complete root formation were selected.

Dogs fasted for 12 h prior to the experimental procedure and preanesthetized with 8 mg of xilazina (Bayer, México SA De C.V. Mexico), intramuscular (IM). Immediately after, 0.5 mg of atropine was subcutaneously administered, the safena vein catheterized and 25 mg kg^{-1} of sodium pentobarbital (Aranda SA. Mexico DF, Mexico) administered intravenously (IV). The extractions were performed atraumatically as possible using elevators and forceps based on the following sequence: maxillary left first premolar, mandibular left first premolar, maxillary left incisor, mandibular left incisor, and finally the maxillary and mandibular right first premolars. Teeth were then randomized divided in three experimental and two controls groups.

Group 1. EMDOGAIN (n = 8)

Immediately after teeth were extracted, the PDL was removed mechanically using periodontal curettes. The root canal treatment was performed extraorally and the root surface coated with Emdogain® (0.175 ml) prior to replantation. The teeth were then replanted using gentle digital pressure (Fig. 1).

Group 2. EMDOGAIN + EDTA (n = 8)

Immediately after teeth were extracted, the PDL was removed mechanically using periodontal curettes. The root canal treatment was performed extraorally and the root surface preconditioned with EDTA (0.2 ml) for 1 min, washed with sterile saline solution and then coated with Emdogain® (0.175 ml) prior to replantation. The teeth were then replanted using gentle digital pressure.

Group 3. EDTA (n = 4)

Immediately after teeth were extracted, the PDL was removed mechanically using periodontal curettes. The root canal treatment was performed extraorally and the root surface preconditioned with EDTA (0.2 ml) for 1 min, washed with sterile saline solution and teeth replanted using gentle digital pressure.

Group 4. Positive control (n = 4)

Immediately after teeth were extracted, the PDL was removed mechanically using periodontal curettes. The root canal treatment was performed extarorally and teeth replanted using gentle digital pressure.

Group 5. Negative control (n = 4)

No treatment was performed.

Bio-mechanical instrumentation was accomplished with K-Flexofile files (Dentsply Maillefer, Tulsa, OK, USA) and 1% sodium hypochlorite (Clorox, Oakland, CA, USA). The canal was then dried with paper points (Dentsply Maillefer) and sealed with gutta-percha and zinc-oxide sealer (Silco; Productos endodónticos de México, SA De C.V, Mexico) using lateral condensation technique. Total extraoral time was 30 min in all groups except in the negative control. A flexible splint was

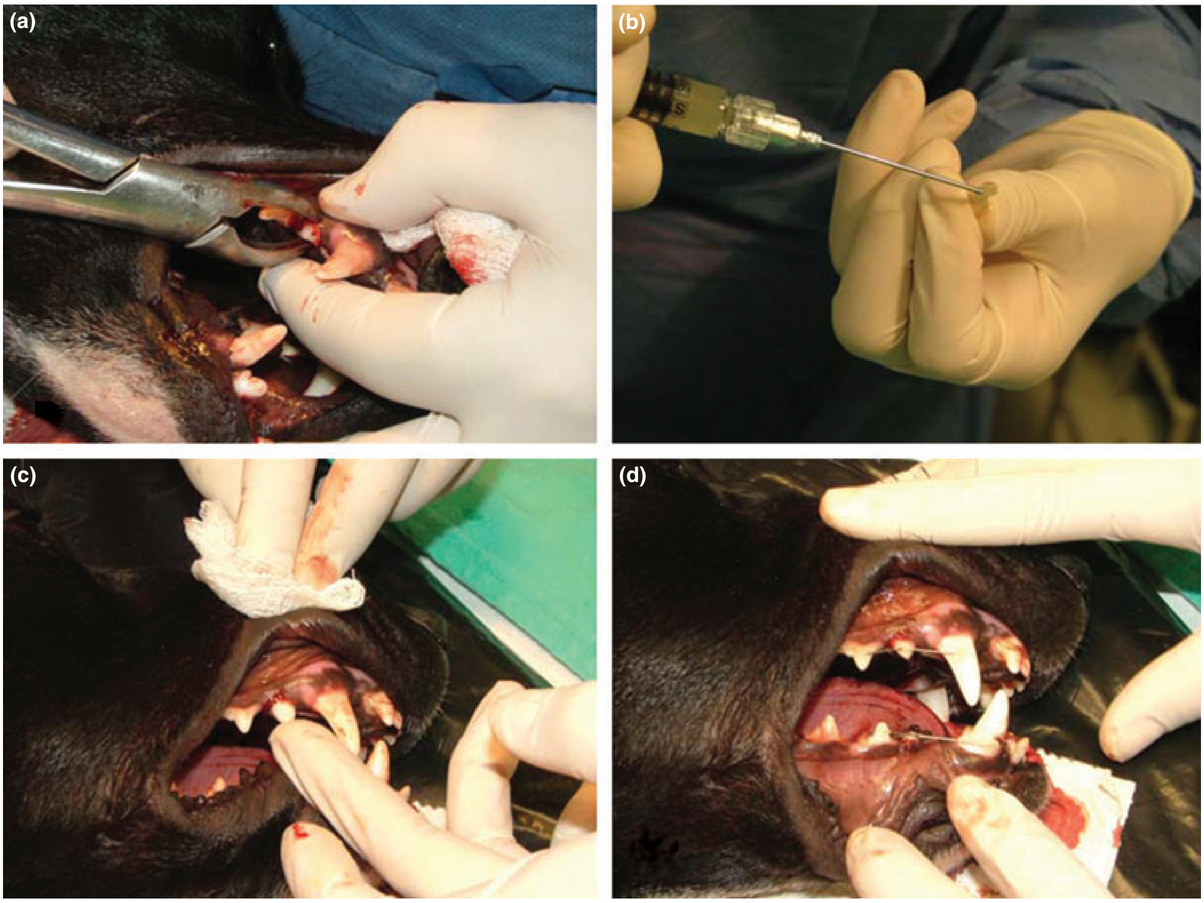

Fig. 1. (a) Extraction. (b) Emdogain application. (c) Replantation and (d) splinting.

applied for 2 weeks with orthodontic wire (0.01) and Ribbond-THM® (Ribbond, Seattle, WA, USA). Postoperative care included soft diet and systemic IM amoxicillin 500 mg day^{-1} for a week (Vetoquinol; Magny Vernois 7024, Lure Cedex, France). The animals were sacrificed at 8 weeks under general anesthesia induced with 30 mg kg^{-1} pentobarbital (Socumb; Butler Company, Columbus, OH, USA) intravenously. The carotid arteries were exposed and canulated and the animals were euthanized with additional pentobarbital (Socumb; Butler Company) at a dose of 90 mg kg^{-1} intravenously.

Sample processing

After removal of all soft tissue and excess hard tissue from the specimens, they were next submerged in 100% buffer formalin for 7 days. After this, specimens were decalcified in nitric acid for 48 days. The specimens were then dehydrated through ascending gradations of ethanol, embedded in paraffin and sectioned with a microtome at 7 μM longitudinal slices. Tissues were stained with hematoxylin and eosin Masson trichrome and evaluated under light microscopy at up to 10× magnification. The samples were histologically scored using the definitions described by Andreasen (41) (Table 1). A one-way univariable analysis was performed to find any differences between the experimental and control groups using the JMP 4.01 statistical software (SAS, Cary, NC, USA).

Results

Emdogain® (EMD)

Seven teeth demonstrated the presence of replacement root resorption (active replacement resorption). One tooth showed root resorption without bone deposition (active surface resorption) (Fig. 2a). All teeth demonstrated active resorption with multinucleated cells present along the root surface (Fig. 2b). Few and irregular collagen fibers were also observed.

Emdogain® + EDTA

Seven teeth exhibited replacement root resorption (active replacement resorption). One tooth showed root resorption without bone deposition (active surface resorption). All teeth demonstrated active resorption with multinucleated cells present along the root surface. Irregular and few collagen fibers were also observed (Fig. 3a,b).

EDTA

All four teeth in this group revealed the presence of replacement root resorption. Interestingly, teeth from this group showed evidence of larger areas of resorption comparing with teeth from the Emdogain® and Emdogain® + EDTA groups. However, the resorptive

Table 1. Criteria for histological assessment [Andreasen (42)]

1. Normal periodontium	Normal structured periodontal ligaments and an intact cementum layer
2. Active surface resorption	Resorption cavities are present on the root surface bordered by a normal structured periodontium. The resorption process takes place by uni- or multinucleated cells but without inflammatory changes in the soft tissue
3. Arrested surface resorption	The changes are similar to 2, apart from arrest of the resorption process
4. Repaired surface resorption	The changes differ from 2 by the repair of the resorption cavities with cellular or acellular cementum
5. Active inflammatory resorption	Active resorption is present on the root surface. The resorption takes place by uni- or multinucleated cells present in granulation tissue
6. Arrested inflammatory resorption	The changes are similar to 5, apart from an arrest of the resorption process
7. Repaired inflammatory resorption	The changes are similar to 5 apart from a repair of the resorption cavities with cellular or acellular cementum
8. Active replacement resorption	Resorption cavities are present on the root surface adjacent to bone deposition upon the root surface. The resorption process takes place by uni- or multinucleated cells in a loose structured connective tissue
9. Arrested replacement resorption	The changes are similar to 8 apart from an arrest of the resorption process
10. Repaired replacement resorption	Alveolar bone is deposited upon the root surface. The root surface may or may not have been resorbed before bone deposition
11. Inflammation	Inflammation on the periodontal ligament without root resorption
12 Downgrowth of pocket epithelium	Downgrowth of pocket epithelium below the dentino-enamel junction

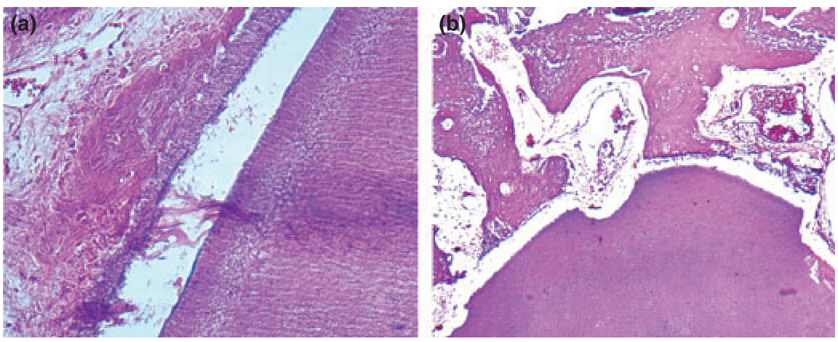

Fig. 2. Emdogain. (a) Hematoxylin & Eosin 40×. Few collagen fibers, representing the periodontal ligament, between the root surface and adjacent bone without resorptive defect is discernible. (b) Hematoxylin & Eosin 10×. Resorptive lesions are present on the root surface and formation of primary osteoid tissue is evident.

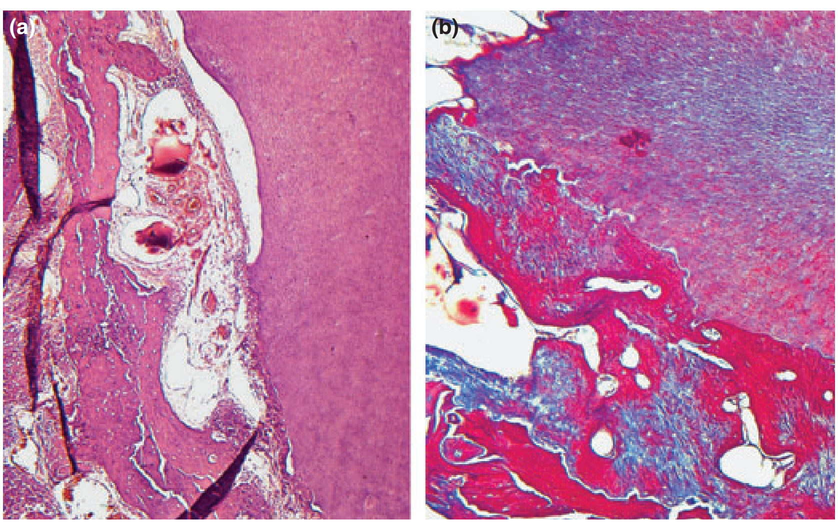

Fig. 3. Emdogain & EDTA. (a) Hematoxylin & Eosin 10×. Resorptive lacunae over the root surface in direct contact with multinucleated osteoclasts. (b) Masson trichrome 10×. Replacement resorption with mature bone (red) and ostoid tissue (blue).

surfaces were smaller than the ones observed in the positive control group. All samples presented multinucleated cells with resorption lacunae over the root surface adjacent to bone deposition (Fig. 4a,b).

Positive control

All teeth showed active replacement root resorption. Many bone lacunae with multinucleated cells were observed over the root surface. All samples were classified as active replacement resorption according to the classification employed. No significant statistical difference was observed between experimental groups and positive control (Fig. 5).

Negative control

All teeth in this group demonstrated a normal periodontium without histological evidence of root resorption (Fig. 6). Root resorption was observed in all samples

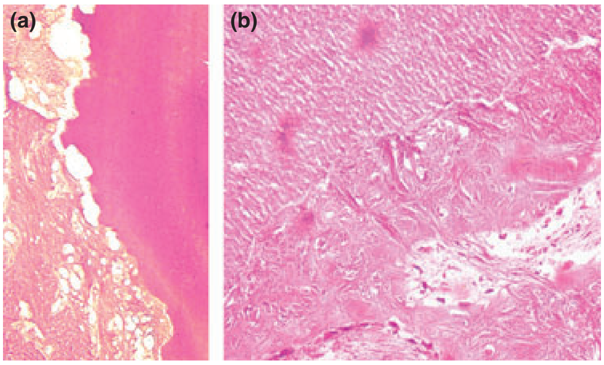

Fig. 4. EDTA. (a) Hematoxylin & Eosin 10×. Resorptive lacunaes on the root surface with primary osteoid tissue. (b) Hematoxylin & Eosin 40×. Replacement resorption is evident with direct apposition of bone and dentin.

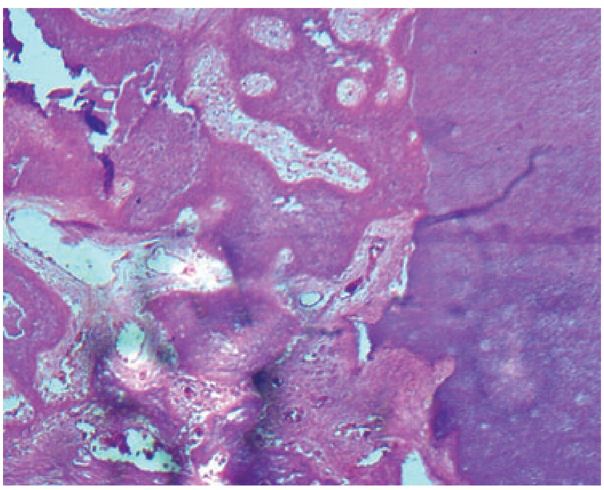

Fig. 5. Positive control. Hematoxylin & Eosin 10×. Replacement resorption with direct apposition of bone and dentin.

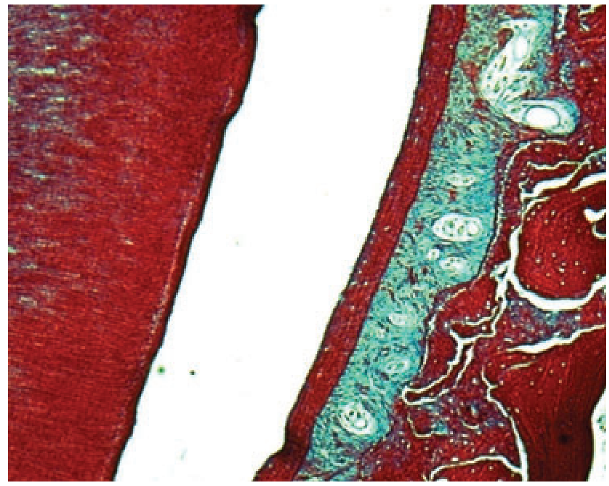

Fig. 6. Negative control. Masson trichrome 10×. Normal periodontium without evidence of root resorption.

Table 2. Contingency table. Results by group

Count Total % Col % Row %	1	2	8	Total
EMD	0	1	7	8
	0.00	3.57	25.00	28.57
	0.00	50.00	31.82	
	0.00	12.50	87.50	
EMD + EDTA	0	1	7	8
	0.00	3.57	25.00	28.57
	0.00	50.00	31.82	
	0.00	12.50	87.50	
EDTA	0	0	4	4
	0.00	0.00	14.29	14.29
	0.00	0.00	18.18	
	0.00	0.00	100.00	
C+	0	0	4	4
	0.00	0.00	14.29	14.29
	0.00	0.00	18.18	
	0.00	0.00	100.00	
C–	4	0	0	4
	14.29	0.00	0.00	14.29
	100.00	0.00	0.00	
	100.00	0.00	0.00	
Total	4	2	22	28
	14.29	7.14	78.57	

EMD, enamel matrix derivatives; EDTA, ethylenediamine tetraacetic acid.

except for the negative control (Table 2). No statistical differences were found between the experimental groups and the positive control (Fig. 7).

Discussion

Prognosis of avulsed teeth is poor due to the irreversible damage of PDL and the subsequent development of replacement root resorption. The design of this study aimed to evaluate the effect of Emdogain® and 24% EDTA gel, alone or in combination, in the prevention or delay of replacement root resorption of replanted teeth with long extraoral dry time. In order to test this hypothesis, we mechanically removed PDL from the root surface only. In such cases, Emdogain® gel has been proposed as an alternative biological approach to induce the regeneration of the alveolar-based periodontium and specifically the collagen fibers (42, 43). It is known now that developing enamel is mainly composed of hydrophobic proteins known as amelogenins and other non-amelogenin proteins like ameloblastin, enamelin, tuftelin, tuft proteins, sulfated proteins, and enamel proteases such as enamelysin and EMSP1 (26). Some studies have suggested that the active component in Emdogain® is amelogenin (44) or enamel matrix proteases (45) while others suggest that it is the presence of growth factors like TGF-β1 (46) or BMPs (47).

For avulsed teeth with long extraoral dry time, Emdogain® may have the ability to stimulate cells with the potential to form cementum and PDL from the socket environment. Hypothetically, this regeneration might occur based on viable PDL cells that remained

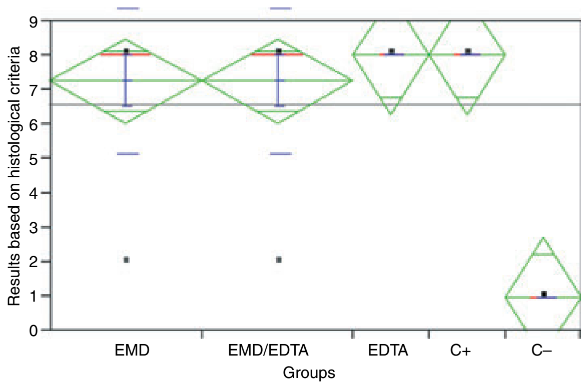

Fig. 7. One-way analysis. Results by group.

attached to the socket (8, 22, 23, 38). A vital PDL present adjacent to the pathologically compromised cell is critical for tissue regeneration using Emdogain. Regeneration is based on migration of these adjacent cells. In the present study, we only addressed the capability of Emdogain with and without EDTA to regenerate the periodontium based on viable PDL cells that remained attached to the socket. As the PDL attached to the root surface was removed, no regeneration of these cells was expected. As a result, all experimental samples showed a scarce presence of collagen fibers in direct apposition with the root and development of replacement resorption. Araujo et al. (33) reported similar results in teeth of dogs treated with EMD prior to replantation with no significant presence of collagen fibers and progressive replacement resorption.

In an *in vitro* and *in vivo* study, Hocino (43) applied EMD over roots stored in a culture media. A fibroblasts layer was diagnosed on samples after 14 days of incubation. These results were in accordance with their *in vivo* study using the canine model in which roots treated and replanted with EMD showed less ankylosis than the control group after 4 weeks. The findings of Hocino are not in accordance with results of the current study. Two reasons could explain these circumstances: first, conditions in a culture are ideals for cell growth and probably EMD effect might be dissimilar at *in vivo* models. Second, histologically, the development of replacement resorption is time related and could be different at 8 weeks. Thus, the early histological evaluation could lead to a misinterpretation of the results.

Iqbal & Bamaas (23) evaluated the effect of EMD to avoid replacement resorption on replanted teeth of beagle dogs. EMD was applied over root surfaces and remnants of the necrotic PDL. Their result showed less resorption in the experimental group treated with Emdogain. Recently, Poi et al. (37) compared EMD against 2% acidulated-phosphate sodium fluoride after 6 h in dry extraoral time in a rat model and clearly demonstrated that delayed tooth replantation harms cellular viability of fibroblasts thus decreasing the capacity to produce collagen fibers. Although it has been shown that EMD has the potential to form cementum and PDL from the tooth and socket environment, apparently it is incapable to stimulate regeneration of collagen fibers covering the root surface.

Clinical trials demonstrated similar results. Schjott & Andreasen (35) reported a clinic and radiographic evaluation of 16 avulsed teeth with extra-alveolar time ranging from 20 to 270 min. Emdogain® was applied in the root surface and inside the socket prior to replantation of teeth. At the 6 months follow up, all teeth showed ankylosis. Barrett et al. (38) evaluated 25 cases of avulsed incisors treated with EMD. Extra-alveolar time and follow-up averages were 185 min and 20.6 months, respectively. All cases showed the presence of replacement resorption and ankylosis.

Andreasen & Kristerson (48) and Lindskog et al. (10) proposed the removal of the necrotic PDL and conditioning of the root surface with chemical substances to induce regeneration of collagen fibers. Among the agents used to condition root surfaces are included 36% ortho-phosphoric acid (49), 3% citric acid (50), enzymes (51), sodium fluoride (19, 52), sodium hypochlorite, and 24% EDTA (53). Application of Pref-gel® (Biora, Malmo, Sweden) (EDTA 24% with neutral pH) over the root surfaces prior to EMD has been proposed (54). The biological rational behind this recommendation is based on the use of a solution with a neutral pH to remove the smear layer and to expose collagen on the root surface to stimulate regeneration (39, 55). The results of this study do not support the use of EDTA for removal of the necrotic PDL and coincide with results of Lam et al. (34). Moreover, Molina & Brentegani (36) demonstrated that roots in which the PDL was not scrapped showed higher PDL regeneration than the scrapped roots covered by EMD. The fact that the necrotic PDL was not removed in some of the previously mentioned studies in addition to the fact that the cementum was not modified chemically or mechanically could be an important factor to be considered to explain their positive result (23, 38).

The methodology used in the present study focused on analyzing the histological findings based on a very strict criteria and classification described by Andreasen (41). This presents an important limitation to our study regarding the assessment of amount and progression of root resorption. Other authors described a different methodology to evaluate the degree of root resorption using eight predetermined circumferential recording points in at least eight sections of the tooth (56, 57). This 8-point grid methodology allows for a better quantitative analysis of root resorption.

To conclude, replantation of teeth with Emdogain® alone or in combination with 24% EDTA showed no evidence of regeneration of collagen fibers and consequently did not prevent the development of replacement root resorption of replanted dog teeth.

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