

## Effects of diode laser irradiation and fibroblast growth factor on periodontal healing of replanted teeth after extended extra-oral dry time

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**Key words:** avulsion; fibroblasts growth factor; laser; periodontal healing; root resorption; tooth replantation

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Accepted 11 October, 2016

**Abstract – Aim:** The search for effective protocols to reduce the incidence of root resorption and allow periodontal ligament repair is still challenging, given the unpredictable outcome of late tooth replantation. The aim of this study was to assess the effects of both high-power diode laser irradiation (DL) and basic fibroblast growth factor (FGF) on the periodontal healing of replanted teeth after extended extra-oral dry time. **Methods:** Maxillary incisors of 50 male rats were extracted and assigned to three experimental and two control groups ( $n = 10$ ). DL: root surfaces treated with DL (810 nm, continuous mode, 1.0 W, 30 s), FGF: topical application of FGF gel to the root surface and in the alveolar wound, DL + FGF: DL and topical application of FGF gel, C+: no treatment after extraction and immediate replantation and C–: no treatment after extraction and replantation after 60 min. In the experimental groups, the specimens were kept dry for 60 min, the pulps were removed and the canals were filled with calcium hydroxide paste prior to tooth replantation. The animals were euthanized after 60 days. The specimens were processed for radiographic, histological and immunohistochemical analyses. **Results:** The radiographic analysis showed fewer resorptive areas in DL + FGF ( $P < 0.05$ ). The histological and immunohistochemical analyses showed that the DL group had lower mean values of ankylosis, replacement and inflammatory resorption when compared to C–, not differing statistically from C+. DL + FGF produced significantly more collagen fibers (type I and type III) than C–, not differing from C+ in the case of type I fibers ( $P < 0.05$ ). **Conclusions:** DL, with or without FGF, reduced the occurrence of external root resorption and ankylosis. Periodontal healing was favored and some fiber reinsertion occurred only when FGF was used.

Epidemiological studies show that dental trauma represents a serious public health problem in children and teenagers due to its high prevalence and high social and psychological impact (1). Tooth avulsion is one of the most serious dental injuries due to the damage caused to the pulp and periodontal tissues. Replantation of the permanent tooth is indicated for promoting the recovery of function and esthetics, especially in children whose bone growth is still in progress, and who are therefore unable to receive definitive prostheses or implants (2, 3).

Avulsed teeth that are replanted after extended extra-oral dry time (i.e. more than 30 min) usually present with extensive necrosis of the periodontal ligament (PDL) cells (4). The necrotic PDL cells prompt an inflammatory process along the root surface that culminates in activation of cementoclasts and initiation of

ankylosis and replacement root resorption (5, 6). Despite various studies utilizing different approaches, the success rate for complete healing of delayed replanted teeth is still quite low. Consequently, there is demand for effective clinical protocols to minimize, and ideally to prevent external root resorption and favor periodontal healing (7–10).

Evidence shows that high-power lasers have antimicrobial activity when applied in the root canal system (11, 12). Furthermore, when used on the root surface, lasers can promote fusion and melting of the dental structure, thus making it more homogeneous and favoring the adhesion of connective tissue fibers and cells, and new cementum formation. This renders the root surface more resistant to microbial and clastic cell action (13–15). Root surface treatment with high-power diode laser irradiation prior to delayed replantation

has shown potential to reduce the occurrence of external root resorption and to inhibit ankylosis. Nevertheless, histologically the connective tissue produced was thin and disorganized with the fibers arranged parallel to the root surface, indicating that there was no periodontal healing (4).

Replantation of avulsed teeth must include strategies for promotion of PDL regeneration, which can potentially minimize, or ideally prevent, external replacement root resorption. Progress in tissue engineering and periodontal regeneration therapy has opened the field for new research to improve the prognosis of delayed replanted teeth (16, 17).

Periodontal regeneration mechanisms are stimulated by cytokines such as fibroblast growth factor (FGF). Basic fibroblast growth factor (bFGF or FGF-2) is a single-chain polypeptide that induces various biological responses including enhancement of angiogenesis, migration and proliferation of fibroblasts, and is one of the most potent mitogens for periodontal cells (18, 19). Topical local bFGF application has been shown to be a promising therapy for the regeneration of periodontal tissues damaged by progression of periodontitis (20–23). The use of bFGF in stimulating periodontal healing of delayed replanted teeth has also obtained positive results (24, 25).

As the literature presents promising results with the use of lasers and biostimulating factors separately, there may be benefits in using both root treatments in combination to promote healing and minimize the chances of catastrophic root resorption after replantation of avulsed teeth.

The aim of this study was to assess the effects of diode laser irradiation (DL) and FGF, separately and in combination, in the treatment of root surfaces of replanted teeth with extended extra-oral dry time.

## Materials and methods

This research was approved by the Animal Experimentation Ethics Committee of the University of Taubate (Process 004/11).

### Surgical intervention

Fifty male rats (*Rattus norvegicus albinus*, Wistar, 250–300 g) were randomly assigned to cages identified according to the group and experimental periods. They received grained solid food before and during the study plus water *ad libitum*.

Before the procedures, the animals received an intraperitoneal injection of anesthesia with a mixture of ketamine (Dopalen; AgribRANDS do Brasil Ltda, Paulinia, Brazil) and xylazine (Anasedan; AgribRANDS do Brasil Ltda, Paulinia, Brazil) at 0.7:0.5 ml ratio, and in a dose of 0.1 ml per 100 g of weight. Asepsis of the anterior portion of the maxilla was performed with 2% chlorhexidine solution. The maxillary right incisors were carefully extracted to simulate tooth avulsion. They were fixed by the crown with wax and then left to dry on a benchtop for 60 min. The dental papilla was excised with a #11 scalpel blade. In order to potentiate the anesthesia effect and reduce pain, the rats

were medicated with 0.02 ml tramadol hydrochloride diluted in water, 24 h before and after the experiment.

### Group assignment

The rats were assigned to three experimental and two control groups ( $n = 10$ ) according to the root surface treatment applied, as follows:

- 1 Group DL: Root surfaces of specimens were irradiated with high-power diode laser (Tera Surgery; DMC equipamentos Ltda, Sao Carlos, Brazil) with a wavelength of  $808 \pm 10$  nm. Laser light was delivered through a 600- $\mu$ m quartz optic fiber using a 650-nm red light diode guide beam in contact with the root surface with a 45° incidence angle and with scanning movements. Power output was measured using a power meter device. Laser parameters were set at 1.2 and 1.0 W at the end of the fiber (output) in a continuous wave mode. The power density was  $7.14 \text{ W cm}^{-2}$ , the energy density was  $214.3 \text{ J cm}^{-2}$  and the tip of the laser fiber was cleaved with a scribe pen cleaving tool (DMC equipamentos Ltda) prior to being used. The total exposure time was 30:5 s for the buccal face, 5 s for the lingual face and 10 s for each proximal face (mesial/distal), totaling 45 J of energy (4).
- 2 Group FGF: Topical application of 50  $\mu$ g of 0.2% basic fibroblast growth gel (PeproTech Inc., Rocky Hill, NJ, USA) in 3% hydroxypropyl methylcellulose gel (Farmacia de Manipulação Terapêutica, Sao Jose dos Campos, Brazil) to the palatal root surface and in the alveolar wound.
- 3 Group DL + FGF: Same procedures as in groups DL and FGF applied sequentially.
- 4 Positive control (C+): No treatment after extraction and immediate replantation.
- 5 Negative control (C-): No treatment after extraction and replantation after 60 min of extraction.

In all specimens of the experimental groups, the pulps were removed via the apical foramen with a #15 K-Flexofile (Dentsply Maillefer, Ballaigues, Switzerland). The root canals were instrumented with #20 and #25 K-Flexofiles (Dentsply Maillefer) and were copiously irrigated with 1% sodium hypochlorite (NaOCl) followed by a rinse with 17% EDTA-T (Farmacia Formula & Acao, Sao Paulo, Brazil) for 3 min. The root canals were then dried with sterile paper points and filled with calcium hydroxide paste (Calen®; S.S. White Artigos Dentarios Ltda, Rio de Janeiro, Brazil).

Prior to the root surface treatment, the root surface was rubbed with gauze moistened in 1% NaOCl to remove the PDL fibers that were still attached and then they were rinsed with saline. The sockets were gently irrigated with saline and the teeth were replanted. No splint was used. The animals received a single intramuscular injection of 24 000 IU antibiotic (benzathine benzylpenicillin – 12 000 IU, procaine benzylpenicillin – 6000 IU, potassium benzylpenicillin – 6000 IU, dyhydrostreptomycin sulfate – 5 mg, streptomycin sulfate – 5 mg; Fort Dodge; Animal Health Ltda, Campinas, Brazil) and were fed only water 12 h before and after surgical procedures.

The animals were euthanized at 60 days after tooth replantation with an overdose intraperitoneal injection of sodium thiopental followed by cervical dislocation to assure death. The right and left sides of the maxilla were separated at the median line with a #15 surgical blade, and the specimens containing the replanted and homologous teeth were fixed in 10% neutral-buffered formalin for 7 days.

#### Radiographic analysis

Radiographs were obtained with a perpendicular incidence to the film–object plane. The exposure time was 0.25 s, as determined by a previous pilot study. The two hemimaxillas from each animal were positioned on the optical plate.

For image standardization, a device was developed to maintain the sensor in the same position, controlling the distance and the X-ray incidence angle. A single examiner reviewed all images. The image of each replanted tooth was analyzed for the presence of root resorption and compared to the image of the homologous tooth. The number of sites exhibiting resorption and the percentage of these resorptive areas in relation to the total tooth area were calculated using the software IMAGE-PRO PLUS version 7.0 (Media Cybernetics, Silver Spring, MD, USA). The mean percentage resorptive area was compared between groups using Kruskal–Wallis with Dunn's *post hoc* tests ( $P < 0.05$ ). The mean number of root resorptive sites was compared between groups using ANOVA ( $P < 0.05$ ).

#### Histological analyses

The anatomic pieces were decalcified in 10% EDTA solution (pH 7), dehydrated, clarified and embedded in paraffin. Semi-serial longitudinal 6-mm-dense sections were obtained; half of the sections were stained with hematoxylin and eosin for qualitative histological analysis under optical microscopy and the other half were stained with picosirius red (Direct Red 80; Sigma-Aldrich, Brasil Ltda, Sao Paulo, Brazil) for quantitative analysis of type I collagen fibers (picrosirius red method) and type III collagen fibers (immunohistochemical method). Images of the longitudinal sections were captured with a digital camera (Canon Powershot A640- Japan Canon USA Inc., Newport News, VA, USA.) attached to a microscope (Carl Zeiss, Axiolab, Jena, Germany) at 50× magnification, which provided a panoramic view of the tooth. The images were saved in JPEG format.

#### *Descriptive and semi-quantitative analyses of histological characteristics*

The palatal root surface was analyzed and characteristics of the PDL, cementum, dentin, alveolar bone, epithelial reattachment and the subjacent connective tissue were observed.

Scores of 0–3 were attributed to inflammatory and replacement resorption and ankylosis. Areas of inflammatory and replacement root resorption were measured in representative slides using the following scoring system: 0 – absence of resorption; 1 – less than 1/3 of the area with resorption; 2 – between 1/3 and 2/3 of the

area with resorption; 3 – more than 2/3 of the area with resorption.

For analysis of periodontal healing, the characteristics of the connective tissue and the reinsertion of PDL fibers were evaluated using the following scoring system:

- 1 Connective tissue: 0 – absence of connective tissue; 1 – thin/disorganized; 2 – dense/organized.
- 2 Reinsertion of PDL fibers: 0 – no PDL fibers; 1 – PDL fibers not attached to cementum; 2 – PDL fibers attached to cementum.

All of these analyses were performed by a single calibrated examiner who was blinded to group allocation.

#### Quantitative analysis of the type I collagen fibers (picrosirius red)

Three histological sections of each specimen were analyzed by polarized microscopy (400× magnification) in areas representing the cervical, middle and apical thirds of the root. Two representative images from each area of the root were selected. The images were saved in JPEG format and IMAGE-PRO PLUS 7.0 software (Media Cybernetics) was used to measure the selected area by colorimetric differential. The examiner marked the color to be analyzed and the system identified the color, filled the selected area and processed the measurement. The percentage of type I collagen fibers in that fixed area was calculated (Fig. 1a). The mean percentage area of type I collagen fibers was calculated for each group and compared (Kruskal–Wallis and Dunn's *post hoc* tests,  $P < 0.05$ ).

#### Immunohistochemical analysis

Collagen III primary antibody (mouse monoclonal, ab6310 – lot GR21079-3; Abcam, Cambridge, MA, USA) was used to evaluate the percentage of thin type III collagen fibers. For immunohistochemical analysis of type III collagen expression, sections were immersed in 0.3% hydrogen peroxide to block endogenous peroxidase activity and then incubated with primary antibodies diluted in phosphate-buffered saline (1:200). N-Histofine Simple Stain Rat Max PO (Nichirei Co., Tokyo, Japan) was used to detect the antibodies according to the manufacturer's protocol. Signals for immunoreactions were detected using diaminobenzidine (DAB) substrate–chromogen mixture (Spring Bioscience, Pleasanton, CA, USA) and counterstained with hematoxylin (Renylab Química e Farmacêutica, Barbacena, Brazil).

Three histological sections were examined as in the picosirius analysis (Fig. 1b). The mean percentage area of type III collagen fibers was calculated for each group and compared (Kruskal–Wallis and Dunn's *post hoc* tests,  $P < 0.05$ ).

#### Results

##### Radiographic analysis

The results of the number of sites and percentage area of root resorption in each specimen are presented in Table 1.

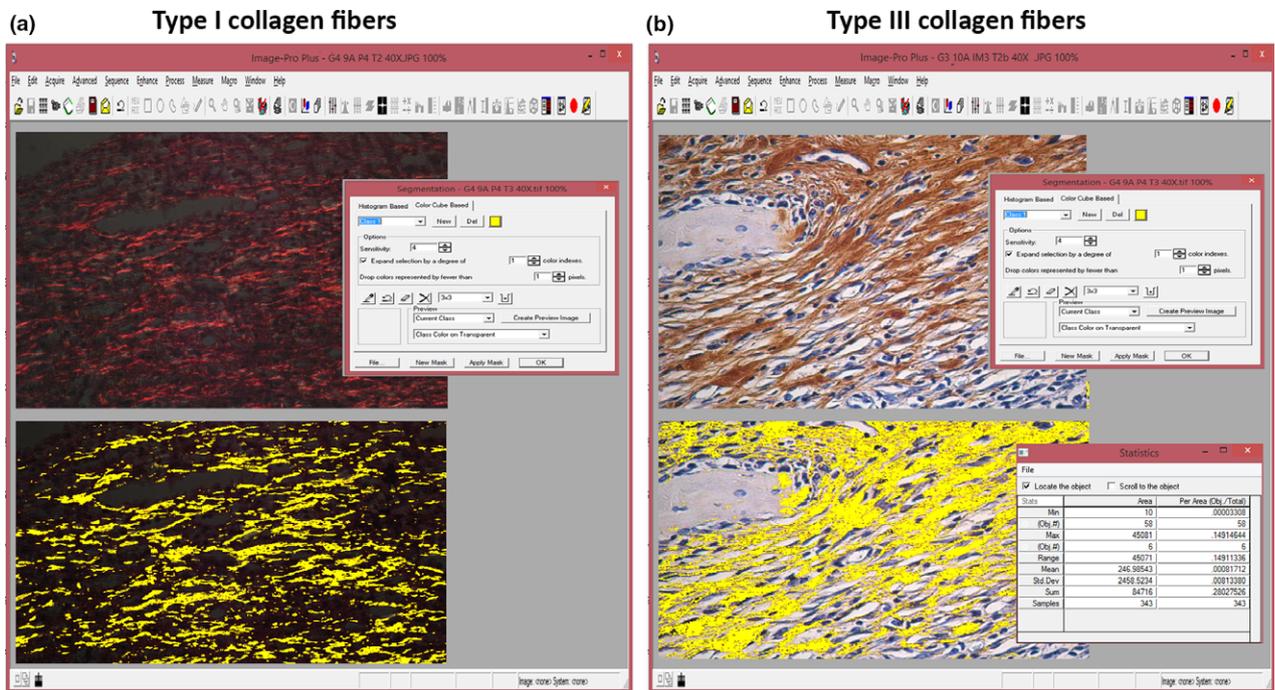


Fig. 1. Representative images of areas containing (a) type I and (b) type III collagen fibers (in yellow), quantified by IMAGE-PRO PLUS 7.0 software.

The percent area of root resorption showed the following trend: C- > FGF > DL > C+ > DL + FGF. The only experimental group that had a statistically significant lower percent area of resorption than the negative control (C-) was DL + FGF ( $P < 0.05$ ).

Regarding the number of sites exhibiting resorption, the results in decreasing order were as follows: C- > FGF > C+ > DL > DL + FGF. All of the experimental groups and C+ had significantly fewer resorptive sites than C- ( $P < 0.05$ ). There were no statistically significant differences between the experimental groups.

#### Descriptive analysis of histological events

During the histological processing 13 specimens were lost, which reduced the total sample size to 37 (DL = 7, FGF = 8, DL + FGF = 7, C+ = 7, C- = 8). The results are described after qualitative analysis of the root thirds under light microscopy 60 days after tooth replantation.

##### Group C+

In most specimens, the root surface showed no inflammatory or replacement resorption and no ankylosis. The subjacent connective tissue was dense and organized and no inflammatory cells were observed. Perpendicular PDL fibers were seen attached to the root surface (Fig. 2).

##### Group C-

In most specimens, cementum and dentin were either resorbed with the presence of inflammatory cells or replaced by bone tissue. Extensive areas of inflammatory or replacement resorption and ankylosis were observed

Table 1. Number of sites per specimen (mean  $\pm$  SD) and percent of total tooth area (mean  $\pm$  SD) exhibiting external root resorption as detected by radiographic analysis at 60 days after tooth replantation

Groups	Areas of external root resorption	
	Percent area of resorption areas	Number of resorption areas
C+	1.03 $\pm$ 1.03 <sup>a</sup>	1.89 $\pm$ 0.99 <sup>c</sup>
C-	3.83 $\pm$ 2.68 <sup>b</sup>	4.0 $\pm$ 1.25 <sup>d</sup>
DL	1.16 $\pm$ 0.93 <sup>ab</sup>	1.70 $\pm$ 1.25 <sup>c</sup>
FGF	3.09 $\pm$ 1.89 <sup>ab</sup>	2.6 $\pm$ 0.84 <sup>c</sup>
DL + FGF	0.99 $\pm$ 0.35 <sup>a</sup>	1.5 $\pm$ 0.53 <sup>c</sup>

<sup>a</sup>Significant statistical difference from<sup>b</sup> (Kruskal-Wallis and Dunn's *post hoc* tests,  $P < 0.05$ ). <sup>c</sup>Significant statistical difference from<sup>d</sup> (ANOVA and Tukey's tests,  $P < 0.05$ ).

along the entire root (Fig. 3). The subjacent connective tissue was thin and disorganized and there were no PDL fibers attached to the root surface.

##### Group DL

Only one specimen showed areas of replacement resorption and ankylosis. Cementum and dentin were resorbed with the presence of inflammatory cells in two specimens. The subjacent connective tissue was dense and organized in most specimens and the PDL fibers were thin and not attached to the root surface (Fig. 4).

##### Group FGF

In most specimens, cementum and dentin presented some areas of inflammatory and replacement resorption. Few areas of ankylosis were observed. Most of

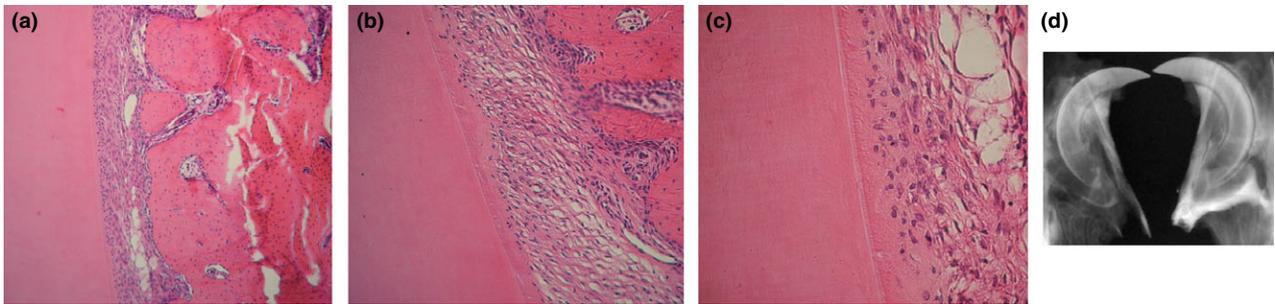


Fig. 2. Representative histological sections of group C+: absence of inflammatory resorption, replacement resorption or ankylosis. Perpendicular reinsertion of fibers to the cementum layer and presence of dense and organized connective tissue. HE staining, original magnification (a) 50×, (b) 100× and (c) 400×; (d) representative radiographic image.

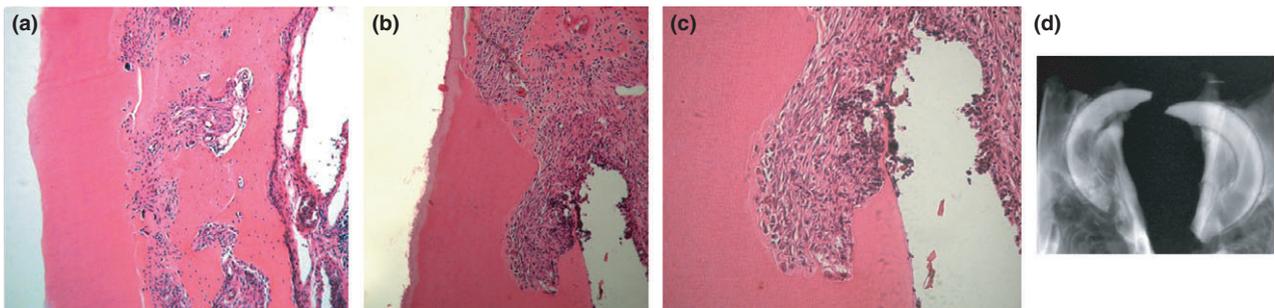


Fig. 3. Representative histological sections of group C-: (a) replacement resorption and root surface in direct contact with bone tissue adjacent (ankylosis). Original magnification at 50× (HE staining); (b) and (c) wide areas of inflammatory resorption and magnification at 100× and 400× respectively; (d) representative radiographic image.

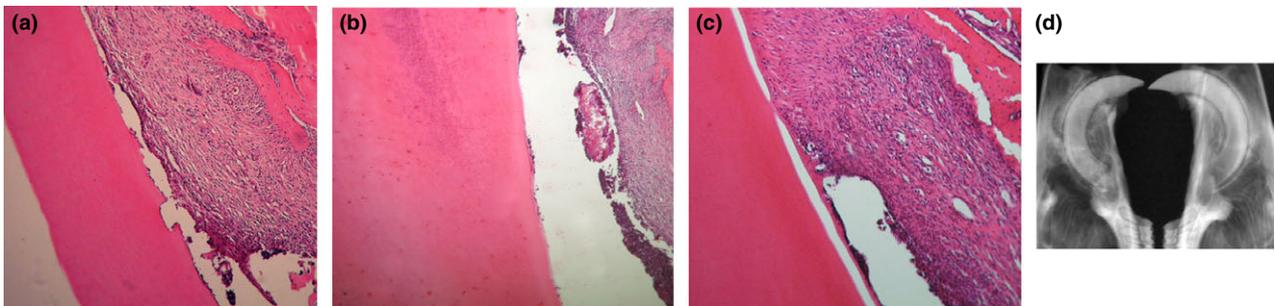


Fig. 4. Representative histological sections of group DL: (a) superficial inflammatory resorption, connective tissue organized with fibers not attached to the root surface. Original magnification 50× (HE staining); (b) and (c) absence of inflammatory and replacement resorption and ankylosis, connective tissue organized with PDL fibers distant from the root surface (100× and 200×); (d) representative radiographic image.

the connective tissue was thin and disorganized and the PDL fibers were absent or not attached to the root surface, when present (Fig. 5).

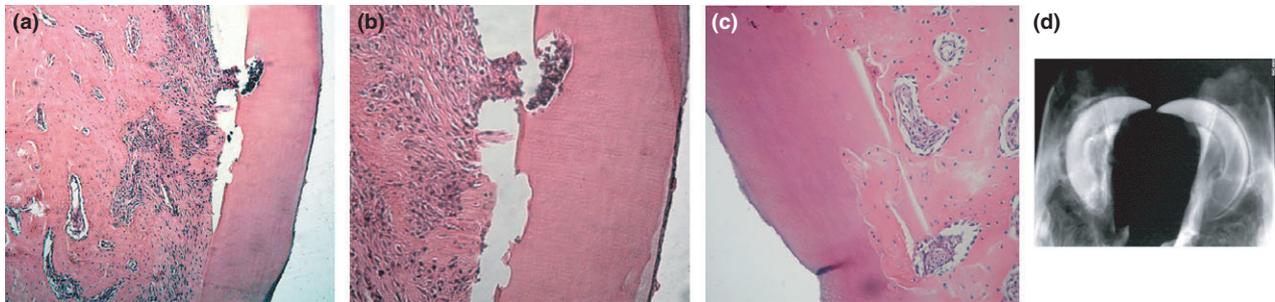
**Group DL + FGF**

In a few specimens, cementum and dentin were resorbed with the presence of inflammatory cells or replaced by bone tissue and the root surface was juxtaposed to the bone tissue in some areas along the root surface (ankylosis). In most specimens, the subjacent connective tissue was dense and organized. PDL fibers were seen attaching perpendicularly to the root surface of two specimens (Fig. 6).

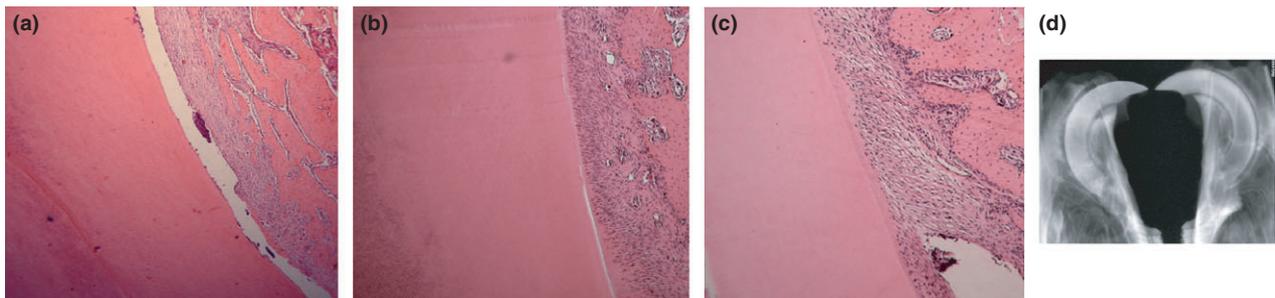
**Semi-quantitative analysis of histological events**

Semi-quantitative analysis (scores) of histological features of both the root surface and the PDL are described in Table 2.

Group DL had significantly lower inflammatory and replacement resorption scores than group C-, and was similar to group C+ ( $P < 0.05$ ). Most of the specimens in the DL group had resorption scores of 0 (absence of resorption) with only one specimen exhibiting small areas of replacement resorption. Groups DL, FGF and DL + FGF showed less ankylosis than group C-, similar to group C+ ( $P > 0.05$ ).



**Fig. 5.** Representative histological sections of group FGF: (a) and (b) presence of inflammatory and replacement resorption and ankylosis. Dense connective tissue with no reinsertion of PDL fibers (magnification 50 $\times$  and 100 $\times$ ); (c) replacement resorption areas and ankylosis (50 $\times$ ); (d) representative radiographic image.



**Fig. 6.** Representative histological sections of group DL + FGF: (a) absence of inflammatory and replacement resorption and ankylosis. Original magnification 50 $\times$  (HE staining); (b) and (c) connective tissue dense and organized PDL fibers reinserted to the cementum (50 $\times$  and 100 $\times$ ); (d) representative radiographic image.

**Table 2.** Histological events on root surface and periodontal healing – scores at 60 days after tooth replantation

Groups	Root surface changes (scores) 0 – absence, 1 – less than 1/3, 2 – between 1/3 and 2/3, 3 – more than 2/3			Periodontal healing (scores)	
	Inflammatory resorption	Replacement resorption	Ankylosis	Connective tissue 0 – absence, 1 – thin/disorganized, 2 – dense/organized	PDL fibers reinsertion 0 – no PDL fibers, 1 – PDL fibers not attached to cementum, 2 – PDL fibers attached to cementum
C+	0.19 <sup>a</sup>	0.19 <sup>c</sup>	0.14 <sup>e</sup>	1.68 <sup>a</sup>	1.58 <sup>c</sup>
C–	1.10 <sup>b</sup>	1.57 <sup>d</sup>	1.57 <sup>f</sup>	0.45 <sup>b</sup>	0.40 <sup>d</sup>
DL	0.30 <sup>a</sup>	0.29 <sup>c</sup>	0.29 <sup>e</sup>	1.00 <sup>b</sup>	0.79 <sup>cd</sup>
FGF	0.50 <sup>ab</sup>	0.75 <sup>cd</sup>	0.60 <sup>e</sup>	0.86 <sup>b</sup>	0.64 <sup>d</sup>
DL + FGL	0.38 <sup>ab</sup>	0.50 <sup>cd</sup>	0.50 <sup>e</sup>	1.11 <sup>ab</sup>	0.95 <sup>cd</sup>

<sup>a</sup>Significant statistical difference from<sup>b</sup>. <sup>c</sup>Significant statistical difference from<sup>d</sup>. <sup>e</sup>Significant statistical difference from<sup>f</sup> (Kruskal–Wallis and Dunn's *post hoc* tests,  $P < 0.05$ ).

Concerning the analysis of periodontal healing, the presence of dense and organized connective tissue was observed frequently in groups C+ and DL + FGF. The only group which showed PDL fibers reinserted to the cementum was the DL + FGF group (three specimens).

#### Quantitative analysis of collagen fibers

Table 3 presents the results of the quantitative analysis of type I collagen fibers by the picosirius red method and type III collagen by immunohistochemical analysis.

Groups C+ and DL + FGF exhibited larger amounts of type I collagen fibers than the other experimental groups and group C–. Group DL had a few type I collagen fibers, similar to group C–. Groups FGF and DL + FGF had more type III collagen fibers than group C–, but not as many as group C+ ( $P < 0.05$ ).

#### Discussion

Limiting the extra-oral dry time of avulsed teeth before replantation is paramount in the prognosis of such teeth. Extended extra-oral dry time will cause extensive

Table 3. Percentage of type I and type III collagen fibers (mean  $\pm$  SD) at 60 days after replantation

Groups	Type I collagen	Type III collagen
C+	13.87 $\pm$ 6.03 <sup>a</sup>	22.89 $\pm$ 9.18 <sup>c</sup>
C-	4.26 $\pm$ 2.25 <sup>b</sup>	4.08 $\pm$ 4.59 <sup>d</sup>
DL	4.85 $\pm$ 2.56 <sup>b</sup>	8.88 $\pm$ 7.00 <sup>de</sup>
FGF	7.57 $\pm$ 5.95 <sup>b</sup>	13.29 $\pm$ 9.58 <sup>e</sup>
DL + FGL	15.22 $\pm$ 10.46 <sup>a</sup>	15.41 $\pm$ 10.35 <sup>e</sup>

<sup>a</sup>Significant statistical difference from <sup>b</sup>(Kruskal–Wallis and Dunn post hoc tests,  $P < .05$ ); <sup>c</sup>Significant statistical difference from; <sup>d</sup>Significant statistical difference from <sup>e</sup>(ANOVA and Tukey tests,  $P < .05$ ).

damage to the PDL cells leading to a dramatic inflammatory reaction in the connective tissue around the root surface (3, 10). When avulsed teeth are kept dry or are not stored in proper storage media, the PDL cells attached to the root surface will progressively degenerate, exposing the cementum layer. Upon replantation of the tooth, the nude cementum will attract osteoclasts, which will start the resorptive process (2, 7). This study confirms these previous findings as specimens in group C– presented high occurrence of ankylosis, inflammatory and replacement resorption and absence of periodontal healing.

This study also confirmed that immediate replantation plays an important role in the prognosis of avulsed teeth since PDL cells in the specimens in group C+ were still viable, resulting in complete periodontal regeneration with perpendicular reintegration of PDL fibers to the cementum, as demonstrated in the radiographic, histological and immunohistochemical analyses. Others studies also used radiographic and histological analyses to assess healing after tooth replantation (7, 10, 24, 26, 27). PDL is a group of specialized connective tissue fibers composed mostly of collagen bundles. Type I and III collagen fibers are the major components of the PDL fibers (28). Most of the organic matrix of cementum is composed by type I (90%) and type III (5%) collagen fibers. For this reason, quantitative analysis of type I and type III collagen fibers by picrosirius red and immunoperoxidase detection, respectively, were included in this study.

Since the remaining PDL cells on the root surface of avulsed teeth with extended extra-oral dry time will contribute to initiation of the resorptive process, treatment of the root surface through either chemical (acidic solutions, sodium hypochlorite, antibiotics, alendronate, vitamin C, tooth enamel protein) or mechanical (lasers, curettage with instruments) procedures for removal of PDL cells have been indicated prior to replantation, in an attempt to improve repair and attachment of PDL cells to the root surface and reduce the occurrence and severity of ankylosis and root resorption (4, 8).

In this study, treatment of the root surface with DL displayed positive results toward reduction of resorption on the root surface, similar to those reported by Carvalho et al. (4). The occurrence of replacement and inflammatory resorption and ankylosis in groups DL and DL + FGF was low (<10%). The changes in the cementum structure promoted by the DL due to fusion

and melting of the irradiated surface (14, 15), making it more resistant against the clastic cells' action, also contribute to healing (6). In addition, the physical contact of the optical fiber at 45° with the root surface in a scanning movement, promotes the removal of necrotic PDL fibers adhered to it. This confirms the primary importance of total removal of the necrotic fibers for healing (4, 8, 25).

Since FGF stimulates migration and proliferation of fibroblasts, it was theorized that it would favor PDL regeneration. However, the isolated use of FGF showed, in some of the specimens, a thin and disorganized connective tissue and, when PDL fibers were present, they were not reintegrated to the root surface. The picrosirius method also revealed fewer type I collagen fibers than expected. Sae-Lim et al. (26) did not achieve favorable results when filling the alveolus with recombinant FGF solution as well as applying it to the root surface prior to tooth replantation. One of the speculated possible causes is that the vehicle used for delivery (fibrin glue) did not allow ideal release of FGF. Zhou et al. (29) suggest that the vehicle must be efficient in covering the root surface, since the absence of FGF in some areas can cause ankylosis and replacement resorption. In the present study, hydroxypropyl methylcellulose (HPMC) was used as vehicle for the FGF. HPMC is a hydrophilic polymer able to adhere to water and form a gel layer that serves as an essential carrier for controlling FGF delivery (24). Conversely, Tuna et al. (25) showed that the topical application of FGF promoted healing of the periodontal tissues after delayed replantation without ankylosis or root surface resorption, and with new cementum formation. However, in their study, the avulsed teeth were kept in milk, which may have maintained the viability of the PDL cells and favoring periodontal healing.

When DL was associated with FGF (DL + FGF), fewer areas of root resorption were exhibited on both histological and radiographic analyses, corroborating one more time the effectiveness of DL. DL + FGF was the experimental group which exhibited more prominent type I collagen fibers, similar to group C+. Type III collagen fibers were also more prominent in this group, but not quite comparable to group C+. Such results can be explained by the combination of DL irradiation, which eliminates microorganisms, degenerating and necrotic cells (11, 12) and changes the cementum structure (4, 13–15), and FGF, which aids in migration and proliferation of fibroblasts, precursor cells of PDL (18, 23, 30, 31). Furthermore, reintegration of PDL fibers to the cementum was observed in three specimens in group DL + FGF, which could have been influenced by the laser action, since DL (in the same parameters used in this study) has been shown to further cell adhesion to the cementum surface (15).

The choice of the 60-day interval for the rats' euthanasia was based on previous studies. After 8-weeks, mineralized cementum and mature PDL with organized fibers can be seen (10, 29).

In light of the favorable results found with the protocols used in this study, it is clear that root surface treatment associated with FGF plays an important role

in periodontal healing of avulsed teeth with extended extra-oral dry time. While this study has its limitations, the results are promising and open the opportunity for future research with other protocols with associated regenerative procedures to improve the prognosis of such teeth (i.e. guided tissue regeneration, bone grafts, application of enamel matrix-derived proteins, transplantation of bone marrow and PDL stem cells). Biosimulation with FGF does not require either specific laboratories or complicated tissue engineering techniques; however, improvement in the methodology and further scientific validation of these findings for the treatment of dental replantation are needed.

### Conclusions

Irradiation of the root surface of replanted teeth with extended extra-oral dry time with high-power DL with or without FGF reduced the occurrence of external root resorption and ankylosis. Some PDL fiber reinsertion occurred only when FGF was used.

### Acknowledgements

This study was fully supported by a grant-in-aid of research from the FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo). The authors would like to thank Dr. Martha Brillant for her assistance in reviewing this manuscript and the staff members of the Laboratories of Physiology and Histology of the University of Taubaté for their technical assistance.

### Conflict of interest

The authors deny any conflict of interest related to this study.

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