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Root surface treatment using diode laser in delayed tooth replantation: radiographic and histomorphometric analyses in rats

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Abstract – *Background aim*: The aim of this study was to evaluate, by radiographic and histomorphometric analyses, the effects of high-power diode laser irradiation on the root surfaces of delayed replanted rat teeth. *Material and methods*: Maxillary right incisors were extracted from 60 Wistar rats and kept dry for 60 min. Subsequently, the root canals were prepared and filled with calcium hydroxide paste. According to the root surface treatment before the replantation, the teeth were assigned to four groups ($n = 15$): G1 (negative control) – no root surface treatment; G2 (positive control) – treated with 2% sodium fluoride solution; G3 – irradiated with a high-power diode laser (810 nm, continuous mode, 1.0 W, 30 s); and G4 – irradiated with a diode laser using the same parameters as those used for G3 but in pulsed mode. The rats were euthanized after 15, 30, and 60 days of replantation. The specimens were digitally radiographed and processed for histomorphometric analysis to determine the average root resorption areas and to evaluate the histological events. *Results*: The percentage of root resorption was in the following order: G1 > G2 > G4 > G3. Both histomorphometric and radiographic analyses showed significantly lower means ($P < 0.05$) of the occurrence of root resorption in the irradiated groups (G3 and G4) when compared to the control groups (G1 and G2). Replacement resorption and ankylosis were observed in histological sections only after 30 and 60 days; however, such events were not observed in G3. *Conclusion*: Root surface treatments with high-powered diode laser irradiation prior to delayed replantation reduced the occurrence of external root resorption compared to no treatment or sodium fluoride treatment at up to 60 days.

The reported incidence of tooth avulsion ranges from 1% to 16% of all traumatic injuries of permanent dentition. Tooth replantation is an intervention that will provide a transitory return to normalcy and time to determine and provide definitive oral rehabilitation (1, 2).

External root resorption is an unfortunate outcome of replanted avulsed teeth. Its development and severity depend on factors such as the extra-alveolar dry period, the storage media for the avulsed tooth and the presence of a microbial infection (3, 4). After an extra-alveolar dry period of 5 min, the replantation process is considered delayed, with severe attachment injury on the root surfaces, resulting in extensive necrosis of the periodontal ligament (PDL). In these cases, endodontic therapy should be performed to prevent the inflammatory root resorption often associated with pulp infection (5). In addition, necrotic remnants stimulate the occurrence of external root resorption and ankylosis, which is the major cause of replanted tooth loss (3); treatment of the root surface is necessary to remove the necrotic PDL (2, 4).

Several protocols have been used for this purpose, such as sodium fluoride (6), propolis (7) and antiresorptive substances, such as alendronate (8), gallium nitrate (9) and tooth enamel protein (Emdogain) (10). However, the results of these studies have not shown an effective protocol to prevent or control the development of external root resorption, especially the replacement resorptions (10, 11).

Currently, several studies have investigated the irradiation of periodontally diseased root surfaces with high-power lasers such as Er:YAG, diode and Nd:YAG lasers (12–15). Besides the antimicrobial effects (16–18), those lasers also are able to cause morphological changes on root surfaces, which improve the cell's adhesion and proliferation and subsequent attachment of periodontal tissues (13, 19, 20). Such events are considered an important step in periodontal healing (20). Therefore, there are few studies evaluating the effects of high-power lasers on the root surfaces of replanted teeth. Particularly, the study by Hamaoka et al. (21) verified that Nd:YAG laser irradiation improves the biocompatibility of dental roots. According to the authors, it could be an alternative treatment for the dental root prior to replantation. In a previous *in vitro* study, our group verified that irradiation with Er:YAG and diode high-power lasers promoted morphological changes on the surfaces of simulated external resorption that favored the cells' adhesion (22). These results led the hypothesis that irradiation of root surfaces with the lasers described before replantation could reduce the incidence of external root resorption and improve the prognosis of delayed replanted teeth.

The aim of this study was to evaluate the effects of irradiation with a high-power diode laser on root surface of rats teeth in delayed replantation by radiographic and histomorphometric analyses.

Materials and methods

Pretreatment of experimental rats and surgical intervention

Sixty male rats, weighing approximately 300–350 g, were used in this study (*Ratus norvegicus*, albinus). The research protocol was approved by the Internal Animal Research and Ethics Committee (Process 011/08). The animals were kept in cages identified according to treatment group and experimental periods. They received grained solid food before and during the study and water *ad libitum*.

The maxillary right incisors were extracted, simulating a case of tooth avulsion, under intraperitoneal injection anesthesia with a mixture of ketamine (Dopalena-Agribrands do Brazil Ltda, Paulínia, SP, Brazil) and xylazine (Anasedan-Agribrands do Brazil Ltda, Paulínia, SP, Brazil) in the proportion of 0.7/0.5 ml and in a dose of 0.1 ml per 100 g of weight. Anesthesia was administered with disposable insulin syringes. Asepsis of the anterior portion of the maxilla was performed with Chlorohex [2% chlorexidine solution (Ceras Johnson Ltda, Jacarepaguá, RJ, Brazil)].

Tooth preparation and replantation

The extracted teeth were kept dry for 60 min. They were attached by their crowns on a sheet of #7 wax. After this period, the dental papillae were excised with a #11 surgical blade to expose the root canals. The pulp was removed via the apical foramen with a slightly curved #15 Flexofile (Dentsply-Maillefer Instruments S.A., Ballaigues-Suíça) and was prepared with #20 and #25 Flexofiles. The canals were copiously irrigated PDL with a 1% sodium hypochlorite solution (Farmácia Fórmula & Ação, São Paulo-SP, Brazil) and EDTA-T solution (Farmácia Fórmula & Ação) for 3 min using a Luer Look syringe, and they were aspirated with a 30×4 gauge needle.

The extracted teeth were immersed in a 1% sodium hypochlorite solution for 10 min, rubbed with gauze to remove the remaining PDL and, finally, irrigated with saline solution.

Division of groups

According to the root surface treatment before replantation, the teeth were assigned to four groups ($n = 15$):

1. Group 1 (negative control) – no root surface treatment.
2. Group 2 (positive control) – root surfaces were treated following the protocol of the American Association of Endodontics (AAE) (23) for delayed replantation: teeth were immersed in 20 ml of 2% sodium fluoride solution – pH 5.5 (Farmácia Fórmula & Ação), for 20 min.
3. Group 3 – root surfaces were irradiated with a GaAlAs high-power diode laser (Tera Surgery; DMC equipamentos Ltda, São Carlos, Brazil) with a wavelength 810 nm. Laser light was delivered through a 600-μm quartz optic fiber using a 650-nm red light diode guide beam in contact with root surface with a 45° incidence angulation and with scanning movements. Power outputs were measured using a power meter device. Laser parameters at the display were set at 1.2 and 1.0 W at the end of the fiber (output) in a continuous wave mode. The exposition time was 30 s: 5 s for the buccal face, 5 s for the lingual face and 10 s for each proximal face (mesial/distal).
4. Group 4 – root surfaces were irradiated with a GaAlAs high-power diode laser in the same parameters as G3 but in pulsed mode.

After root surface treatments, the root canals were dried with sterile paper points and filled with a calcium hydroxide paste composed of calcium hydroxide, polyethyleneglycol, zinc oxide, and colophonium (Calen-®; S.S. White Artigos Dentários Ltda, Rio de Janeiro, RJ, Brazil). This paste was introduced into the root canal with a proper syringe (ML®; White Artigos Dentários Ltda).

After replantation, the animals received a single intramuscular injection of 20.000 U.I. of benzatine G penicillin. The animals received only water 12 h before and after surgical procedures. After this period, they received grained, solid food for 15 days.

Radiographic analysis

Five animals from each group were euthanized at 15, 30, and 60 days after replantation with an overdose of intraperitoneal injection of sodium thiopental followed by a cervical dislocation to assure death. The right and the left sides of the maxilla were separated in the median line with a #15 surgical blade, and the specimens containing the replanted and the homologous tooth, respectively, were fixed in 10% neutral-buffered formalin for 7 days.

To obtain radiographic images, a Gendex 765 DC X-ray unit (Dentsply International Inc., Des Plaines, IL, USA) was used; it operated at 65 kVp and 7 mA and at a focus-film distance of 30 cm with a perpendicular incidence to the film-object plan. The exposure time was 0.02 s, which was determined by a previous pilot study. The image was obtained with a CCD sensor (2×3 cm active area) of the direct digital system (Visualix, Gendex/Dentsply, Milan, Italy). The two hemimaxillas from each animal were positioned on the optical plate. For image standardization, a device was developed that always fixed the sensor in the same position, controlling the distance and the X-ray incidence angle. After image digitalization, a brightness/contrast adjustment was performed on the Visualix System (gamma = 0.92, brightness = 10 and contrast = 100).

The images of the replanted teeth were analyzed and compared to the images of the homologous teeth by a single examiner to facilitate the identification of the resorption areas. The 'count and tag' tool from the IMAGETOOL software version 2.0 (University of Texas Health Science Center, San Antonio, TX, USA) was used for recognizing and counting the number of resorption areas. After, the mean number of resorption areas was calculated for each group during the three experimental periods (15, 30, and 60 days), and the data were submitted for statistical analysis (Kruskal–Wallis and Dunn *post hoc* tests, $P < 0.05$).

Histomorphometrical analysis

After radiographic analysis, the specimens were decalcified in 5% EDTA (pH 7), processed and embedded in paraffin. After that, 6-μm transversal cuts of the cervical, middle and apical thirds were obtained and stained by hematoxylin and eosin. Nine histological transversal sections from each specimen were analyzed – three of each radicular third. Images were captured with a digital camera (Canon Powershot A640-Japão) attached to a microscope (Carl Zeiss, AxiolaB, Jena, Germany) and connected to a computer (50× magnification). Then, they were stored in JPEG format.

IMAGE-PRO PLUS 7.0 software (Media Cybernetics, SilverSpring, EUA) was used to perform the measurement of root resorption areas (inflammatory and replacement) and the total area of each section. To determine the mean percentage of the occurrence of each root resorption, data were exported to EXCEL software (Microsoft Corp., Redmond, WA, USA), and the resorption areas of each section were calculated in relation to the total area.

Histomorphometric analysis was assigned by a single blinded and experienced examiner. The results were recorded on specific tables, and statistical analyses were carried out using Kruskal–Wallis and Dunn *post hoc* tests with a significance level set at 5% ($P < 0.05$).

Histological event analysis

For the descriptive analysis of histological events, sections of 20× and 40× the original magnification were examined, and the following parameters were considered: the characteristics of the connective tissue formed in the periodontal space, the presence of cement, the reinsertion and the direction of periodontal fibers, the presence of inflammatory and replacement resorption as well as ankylosis. Resorption involving dentin and resorption accompanied by inflammatory cells in the adjacent periodontium was defined as inflammatory resorption. Those in which bone obliterated the area were defined as replacement resorption. Ankylosis was defined as direct bone union to the intact cementum plus union to the previously resorbed cementum or dentin.

Results

Histomorphometric and radiographic analyses

Data of histomorphometric and radiographic analyses as well as the comparison among the experimental groups regarding the occurrence of root resorption at 15, 30, and 60 days are described in Tables 1, 2 and 3, respectively.

Microscopic analysis of histological events

15 Days

An analysis of the histological sections revealed the formation of disorganized and sparse connective tissue with the absence or presence of a thin PDL parallel to the root surface in most specimens of all the experimental groups (Fig. 1a–c). The formation of dense connective tissue oriented in a perpendicular direction to the root surface occurred in one specimen of group 3 (Fig. 1c).

Examination of the root surface revealed the absence of cementum, which happened only in places where root resorption occurred in all experimental groups.

Small lacunae of inflammatory resorption were observed in all groups, and group 1 showed more areas of inflammatory resorption (Fig. 1a). During this period, none of the groups presented replacement resorption or ankylosis.

30 Days

Analysis of the histological sections also revealed the formation of disorganized and sparse connective tissue with an absence or presence of a thin PDL parallel to the root surface in most specimens of all the experimental groups. Examination of the radicular surfaces revealed an absence of cementum, which happened only in places where root resorption occurred, in all groups.

In 30 days, there was an increase in the size of resorption lacunae in groups 1 and 2 with a great number of inflammatory cells (Fig. 2a). Lacunae of replacement resorption were observed during this period in groups 1, 2, and 4 (Fig. 2b–d). Ankylosis occurred in only one specimen of group 1 that had the PDL space obliterated by bone attached to the root surface (Fig. 2b).

60 Days

The presence of a PDL like connective tissue was reduced in 60 days for all groups. Many specimens from groups 1, 2, and 4 showed a predominance of neoformation of bone tissue filling the periodontal space (Fig. 3a–d).

Examination of the root surface revealed an absence of cementum in all places where root resorption occurred in all experimental groups.

During the 60-day period, only group 3 did not present replacement resorption and ankylosis (Fig. 3b). Groups 1 and 2 showed an increase in the lacunae of inflammatory and replacement resorption. A higher occurrence of ankylosis was observed in groups 1, 2, and 3 when compared to the 30-day period (Fig. 3a,b,d).

Table 1. The mean values of the percentages of the occurrence of root resorption, the number of teeth with dental ankylosis and the mean values of the number of root resorption areas in the histomorphometric and radiographic analyses conducted over a period of 15 days

	Histomorphometric analysis				Radiographic analysis	
	Root resorption (%)					
Groups	Inflammatory resorption	Replacement resorption	Total of resorption	Dental ankylosis (teeth)	Number of resorption areas	Dental ankylosis (teeth)
G1	0.33[1]	0	0.33[1]	0	5.7[a]	0
G2	0.25[1]	0	0.25[1]	0	4.9[a]	0
G3	0.10[2]	0	0.10[2]	0	3.5[b]	0
G4	0.14[2]	0	0.14[2]	0	3.1[b]	0

[1]Significant statistical difference from[2]; [a]Significant statistical difference from[b] (Kruskal–Wallis test, $P < 0.05$).

Table 2. The mean values of the percentages of the occurrence of root resorption, the number of teeth with dental ankylosis and the mean values of the number of root resorption areas, in the histomorphometric and radiographic analyses conducted over the period of 30 days

	Histomorphometric analysis				Radiographic analysis	
	Root resorption (%)					
Groups	Inflammatory resorption	Replacement resorption	Total of resorption	Dental ankylosis (teeth)	Number of resorption areas	Dental ankylosis (teeth)
G1	0.99[1]	0.77[3]	1.76[5]	1	6.3[a]	1
G2	0.09[2]	0.77[3]	0.86[5]	0	4.7[ab]	0
G3	0.17[2]	0.00[4]	0.17[6]	0	3.3[b]	0
G4	0.12[2]	0.07[4]	0.19[6]	0	3.0[b]	0

[1]Significant statistical difference from[2]; [3]significant statistical difference from[4]; [5]significant statistical difference from[6]; [a]significant statistical difference from[b] (Kruskal–Wallis test, $P < 0.05$).

Table 3. The mean values of the percentages of the occurrence of root resorption, the number of teeth with dental ankylosis and the mean values of the number of root resorption areas, in the histomorphometric and radiographic analyses conducted over the period of 60 days

	Histomorphometric analysis				Radiographic analysis	
	Root resorption (%)					
Groups	Inflammatory resorption	Replacement resorption	Total of resorption	Dental ankylosis (teeth)	Number of resorption areas	Dental ankylosis (teeth)
G1	1.48[1]	1.22[3]	2.70[5]	3	8.4[a]	2
G2	0.87[1]	0.68[3]	1.55[5]	1	5.9[b]	1
G3	0.10[2]	0.00[4]	0.10[6]	0	3.0[c]	0
G4	0.10[2]	0.23[3]	0.33[6]	1	3.3[c]	1

[1]Significant statistical difference from[2]; [3]significant statistical difference from[4]; [5]significant statistical difference from[6]; [a]significant statistical difference from[b]; [b]significant statistical difference from[c] (Kruskal–Wallis test, $P < 0.05$).

Discussion

The typical pattern of events following delayed replantation of avulsed incisors was seen in the negative control group (G1), with the highest average of root resorption in both radiographic and histomorphometric analyses in all experimental periods. The replacement resorption associated with delayed replantation of avulsed teeth is a progressive condition resulting in the gradual replacement of normal root structure by bone (24). In fact, such events were evident in the control groups (G1 and G2), mainly over the period of 60 days.

The American Association of Endodontics recommends PDL removal and root surface treatment with acidulated sodium fluoride solution for the treatment of avulsed teeth (23). Some researchers have pointed out that sodium fluoride solution reduces the resorption process because of the conversion of hydroxyapatite from dentin and cementum to fluorapatite, which makes it more resistant to resorption (2, 8, 9). In contrast, in

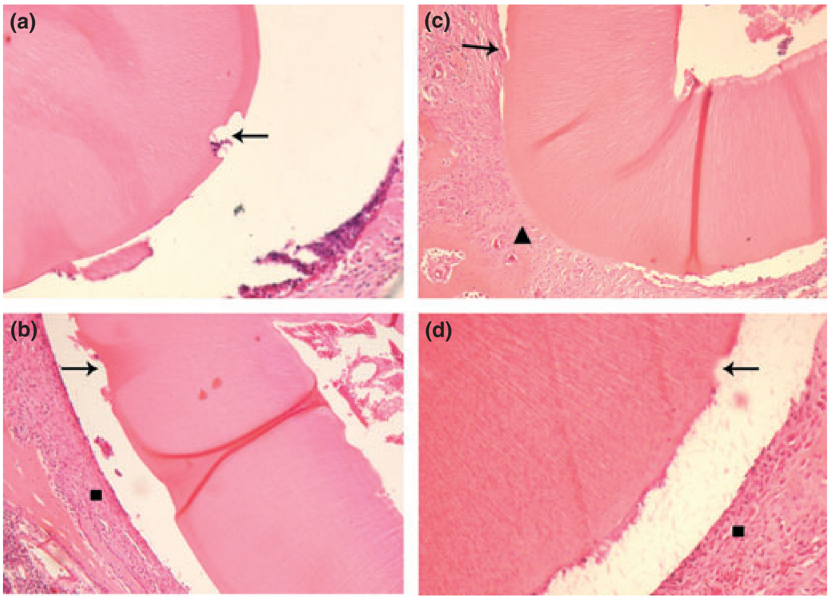

Fig. 1. Representative histological sections with lacunae of inflammatory resorption at 15 days (→): (a) group 1 – middle third; (b) group 2 – apical third; (c) group 3 – cervical third; and (d) group 4 – cervical third. (▲) Connective tissue parallel to the root (■) connective tissue perpendicular to the root. Original magnification 200× (HE staining).

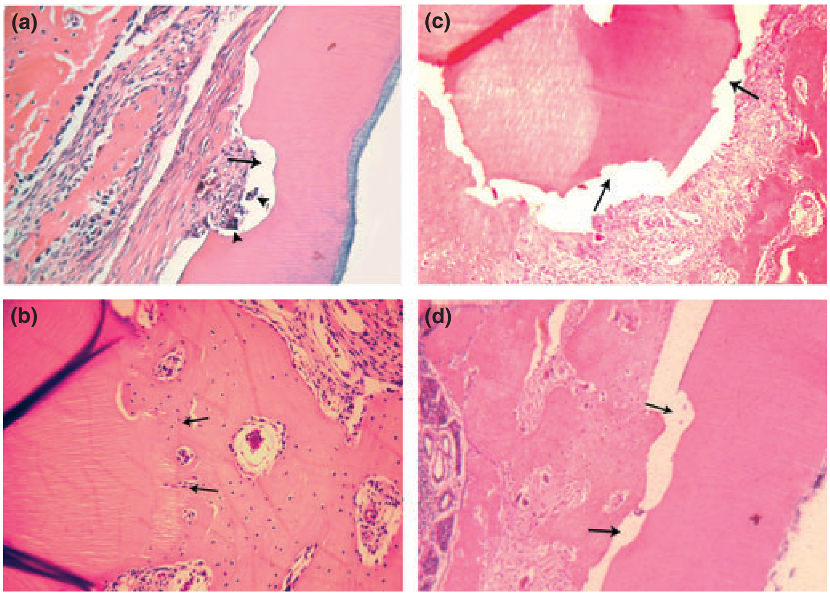

Fig. 2. Representative histological sections of root resorption areas at 30 days (→): (a) inflammatory resorption in group 1 in the apical third with the presence of multinuclear cells (▲); (b) replacement resorption and ankylosis occurrence in group 1 in the middle third; (c) inflammatory resorption in group 2; and (d) replacement resorption in group 4 with neoformed bone tissue. Original magnification 200× (HE staining).

this study, a significant reduction in root resorption and ankylosis in the group treated with sodium fluoride solution was not observed when compared to the negative control group. These findings are in agreement with other studies (8, 10).

In this study, the most promising results in all experimental periods were found in groups where the root surfaces were irradiated with a high-power diode laser (G3 and G4). The high-power diode laser (800–980 nm) as well as Nd:YAG laser irradiation are preferably absorbed by the pigmented tissues and weakly absorbed by water and hydroxyapatite. When these lasers are applied on hard dental tissues, the absorbed energy is transformed into heat, which increases the surface temperature resulting in structural changes on the root surface. The analysis of dental surfaces irradiated with a diode laser using scanning electron microscopy has shown smooth areas intermingled with rough

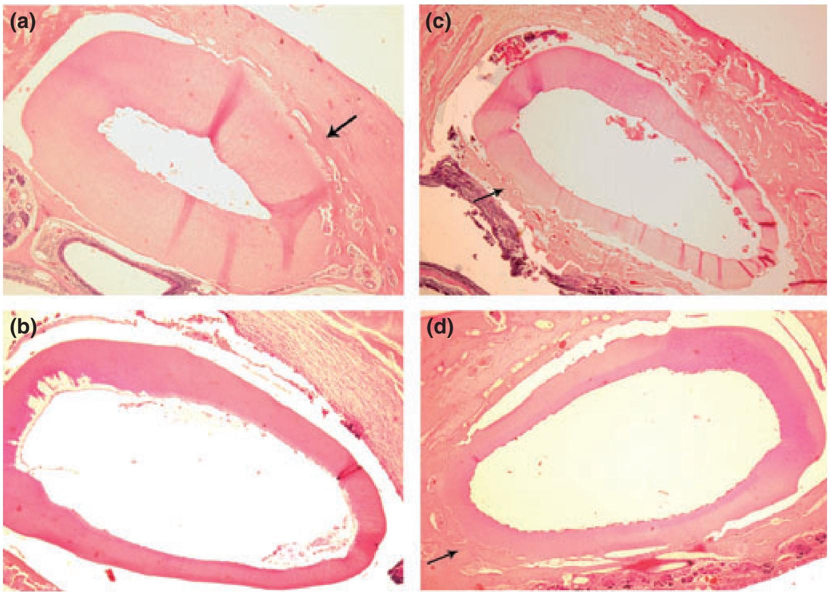

Fig. 3. Representative histological sections of root resorption areas at 60 days: (a) replacement resorption and ankylosis occurrence in group 1 – cervical third; (b) absence of replacement resorption and ankylosis in group 3; (c) replacement resorption and presence of ankylosis in group 2 in the middle third; and (d) replacement resorption and presence of ankylosis in group 4 in the apical third with the periodontal space filled by neoformed bone tissue. Original magnification 50× (HE staining).

areas resembling parallel grooves. The smooth areas exhibited homogeneous fusion and melting, whereas these processes occurred in a more irregular fashion on the rough areas (12, 14, 22). According to recent studies, besides favoring the cell's adhesion on dental surfaces and the subsequent repair process (21, 22), those morphologic alterations promoted by the diode laser may also make these surfaces more resistant to the action of clastic cells of the resorption process. These findings, in association with the bactericidal effect (16, 17), may justify the low values of root resorption in the irradiated groups (G3 and G4), especially in comparison with the negative control group (G1).

Regarding the irradiation mode, the continuous mode has been considered more aggressive than the pulsed mode because of a greater heating of the irradiated area. However, in this study, there was no interference of the irradiation mode in the results. The irradiated groups only diverged statistically in the occurrence of replacement resorption because replacement resorption did not appear in any experimental period in group 3 (continuous mode); however, it was observed in group 4 after 30 days, and ankylosis was observed at 60 days in one specimen. Our findings are in agreement with the ones by Haypek et al. (14) who observed that the use of a high-power diode laser for root surface conditioning produced nonthermal injuries and caused similar, superficial morphological changes regardless of the irradiation mode used (pulsed or continuous). In both modes of laser setup, the root surfaces showed a modified smear layer, with smooth areas intermingled with rough areas resembling parallel grooves. In addition, the smooth areas exhibited fusion and melting in a homogeneous aspect, with cell attachment and proliferation occurring similarly for both modes. According to the authors, these alterations were not able to change the biocompatibility of the root surfaces. On the other hand, Hakki et al. (20) verified that Er, Cr:YSGG laser with short-pulse length seems to provide better conditions for root surface attachment of PDL fibroblasts than long pulsed laser. These opposite results can be attributed to the use of different types of lasers with different wavelengths and parameters, which determine how each laser interacts with the dental tissues. Consequently, it is expected that the morphological features on root surfaces irradiated with Erbium lasers are very different from those with diode lasers. In a prior study, we demonstrated different morphologic changes in root surfaces irradiated with Er:YAG and diode lasers. The samples irradiated with Er:YAG exhibited micro-roughness on the dentinal surface, with aspect of overlapping scales, and open dentinal tubules; those irradiated with diode laser exhibited a smooth surface and closed dentinal tubules. Therefore, both lasers favored cell's adhesion when compared with no irradiated root surfaces (22). These similar results, associated with the compact size of units and the low cost of the diode laser (25), lead us to choose this laser for the present study.

The therapeutic strategy to inhibit replacement resorption would appear to be preventing PDL repair by osseous tissue and/or stimulation of periodontal connective tissue regeneration. Successful replantation is dependent on the regeneration of the PDL, thus preventing ankylosis or any replacement root resorption (26). In this study, although a reduction in replacement resorption and ankylosis was found after the irradiation of the root surfaces with a high-power diode laser (G3 and G4), newly formed fiber bundles with functional orientation of a healthy periodontium was found only in one specimen (G3). According to some studies, periodontal regeneration in delayed replanted teeth involves complex molecular and cellular mechanisms orchestrating multifactorial interactive function events (27), and in these cases, it is necessary to stimulate the cells remaining on the periodontal alveolus because the cells of the PDL of the root are already completely unviable (28, 29). Therefore, in the present study, although diode laser irradiation increased the root surface resistance to the resorption process, it had no role in stimulating those cells to regenerate the periodontium.

Although we have not found an ideal protocol, the present results are promising and lead the way for new studies involving high-power lasers. This study also opens the door for the development of other protocols designed to promote the regeneration of the periodontal structure, which would prevent and control root resorption, benefiting the prognosis of delayed replanted teeth. Progress in tissue engineering has opened the field for periodontal regeneration. With this concept in mind, the irradiation of root surfaces with high-power diode lasers associated with biologic approaches, such the use of mediator growth factors and the stimulation of progenitor cells of the PDL (28, 30), may provide a new perspective in the treatment of delayed replanted teeth.

Our results, using a rat model, suggest that within the parameters of this study, a high-power diode laser produces physical and morphological changes to the root surface that favor reduced root resorption after delayed replantation.

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