Dental Traumatology

Evaluation of laser phototherapy (λ 780 nm) after dental replantation in rats

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Abstract – *Background/Aim:* Tooth replantation is the treatment of choice in cases of avulsion although the outcomes are variable. The teeth can be lost due to external root resorption. The aim of this study was to histologically assess of the effect of laser phototherapy (λ780 nm) on replanted teeth in rats. *Material and Methods:* Sixty Wistar Albinus rats had their maxillary right incisors extracted and were then divided into four groups: G1–absence of storage medium; G2–milk as storage medium; G3–milk as storage medium followed by a laser irradiation of the root surfaces and entrance of the alveolus (λ = 780 nm; P = 70 mW; CW; DE = 21 J/cm^2); G4–milk as storage medium, laser irradiation as in G3 before replantation. After this procedure, laser irradiation was performed on the buccal and palatal mucosa (8.4 J/cm^2 per session) every 48 h for 15 days. The animals were euthanized 15, 30, and 60 days after replantation. *Results:* The histological results showed that after 15 days, G4 exhibited intense chronic inflammation with the presence of clastic cells and moderate external inflammatory root resorption ($P < 0.05$) when compared with G3, in which these outcomes were not observed. At the 30th day, G1, G2, and G4 showed chronic inflammation varying from discrete to moderate, as well as intense external inflammatory root resorption. G3 remained without any inflammation and external inflammatory root resorption up to the 60th day. *Conclusions:* The use of laser phototherapy on the root surface and at the entrance of the alveolus prior to replantation had a positive biomodulative effect on alveolar repair after tooth replantation in rats.

Traumatic injuries affecting the teeth are common. They may result in a wide range of damage to dental structures varying from crown fractures to complete displacement of the tooth from its alveolus, commonly known as tooth avulsion (1). About 16% of all traumatic lesions result in permanent avulsion of the teeth (2). The procedure for the treatment of tooth avulsion is replantation, namely the repositioning of the tooth in its alveolus, allowing restoration of function and esthetics (3).

External root resorption is the most frequent complication of avulsion with subsequent replantation and its development depends on some factors such as length of time the tooth remains outside of the alveolus; the storage medium used for maintaining the avulsed tooth; microbial contamination, and stage of root formation. Preservation of the cementoblasts in an avulsed tooth is one of the key factors for a good prognosis (4). Control of extra-oral time of the avulsed tooth and its maintenance in an appropriate storage medium during this period will also influence the prognosis of the replantation.

Regeneration of the periodontal ligament is the most desirable outcome following tooth replantation. In all the proposed treatment protocols, the aim was to avoid or minimize the inflammatory process. It is known that the inflammatory reaction is directly related to the severity of periodontal damage and pulp infection (5).

The injured supporting tissue triggers an inflammatory response to remove the damaged tissue before the reparative process begins. Therefore, modulation of the inflammatory process is important to attenuate external root resorption (6).

Recently, laser phototherapy has been investigated as a clinical procedure for use after tooth replantation (6, 7). Previous studies have shown that laser phototherapy increases local microcirculation (8), fibroblast proliferation (9, 10), and collagen synthesis (11) in many healing processes.

It was hypothesized that the use of laser phototherapy may positively affect the outcome of tooth replantation (6). The aim of this study was to assess the effect of laser phototherapy (λ780 nm) on replanted teeth in rats using light microscopy.

Materials and methods

The Animal Research Ethics Committee of the Faculty of Dentistry – Federal University of Bahia, Brazil (Protocol number 06.10) approved this work. Sixty male Wistar rats (*Rattus norvegicus, albinus*) weighing 200–250 g were used. The animals were fed with standard pelleted diet (Purina, São Paulo, São Paulo, Brazil) and had water *ad libitum*, except 12 h before the procedure. After replantation, the animals received a crushed pellet diet for 72 h (6).

Surgical Procedure

The surgical procedure was carried out under intramuscular general anesthesia (2% xylazine hydrochloride[1]; 0.03 ml per 100 g body weight; ketamine hydrochloride[2]; 0.06 ml per 100 g body weight). Asepsis of the anterior maxilla was performed with 0.12% chlorhexidine gluconate solution[3] followed by syndesmotomy, luxation, and non-traumatic extraction of the maxillary right incisor of all animals (12).

Study groups

The animals were randomly distributed into four groups of 15 animals each (Table 1).

In all Groups, before replantation the dental papilla and enamel organ of each tooth were removed with a no 15 scalpel blade (Embramac, Itapira, São Paulo, Brazil) and the pulp tissue was extirpated through a retrograde route with a slightly curved no 25 K file.[4] Root canals were irrigated with saline solution, dried with absorbent paper points (Tanari, Manaus, Amazonas, Brazil) and filled with calcium hydroxide associated with polyethylene glycol 400 (Calen®, SS White, Rio de Janeiro, Rio de Janeiro, Brazil) using a 27G needle coupled to a syringe ML (SS White, Rio de Janeiro, Rio de Janeiro, Brazil). Asepsis of the anterior maxilla was performed again, the alveolus was irrigated with 2 ml of saline solution for clot removal and then the teeth were replanted into their respective sockets.

Phototherapy

In Groups 3 and 4, to perform laser irradiation on root surface, at the entrance of the alveolus and on the buccal and palatal mucosa, a GaAlAs laser device (Twinflex Evolution®, MMOptics, São Carlos, São Paulo, Brazil) was used according to the parameters shown in Table 2.

Laser irradiation on the root surface was performed on the mesial and distal surfaces with the tip positioned at a distance of 3.5 cm from the surface to be irradiated, with the aid of a device to keep this distance. In Group 4, for laser irradiation on the buccal and palatal mucosa, 4.2 J/cm^2 per point was used every 48 h for 15 days totaling 67.2 J/cm^2. No type of splinting was used. After replantation, all the animals received a single intramuscular dose of penicillin 20.000 UI.[5] The animals were sacrificed in a carbon dioxide gas chamber (EB 248, Insight Equipamentos–Ribeirão Preto–SP–Brazil), according to experimental time intervals of 15, 30, and 60 days after replantation. The replanted teeth were removed, fixed in 10% formalin for 48 h, decalcified in 5% nitric acid, dehydrated, clarified, and embedded in paraffin. Semi-serial longitudinal sections, 5 μm-thick, were obtained and stained with hematoxylin and eosin for histologic analyses under optical microscopy using the criteria shown in Table 3. Only the middle third of the palatal surface of the root was examined, because this region was not damaged by the surgical procedure. The cervical and apical thirds of the roots, on the other hand, were affected by the gripping effects of the beaks of the forceps and the cutting action of the scalpel blade during tooth extraction and dental papilla removal, respectively.

For statistical analysis and intra- and intergroup comparison, the Fisher test was used, with a 5% level of significance.

Results

Histological analysis

The general histological findings in all groups are shown in Fig. 1.

Group 1
At the 15th day, all specimens exhibited discrete chronic inflammation and scattered lymphocyte cells,

Table 1. Study Groups

Groups	Treatment of root surface and alveolus
Group 1 (G1)	Absence of storage medium–the teeth were kept dry at room temperature for a period of 40 min
Group 2 (G2)	Milk as storage medium–after extraction the teeth were stored in 20 ml of UHT skimmed milk (Cotochés® – Ribeirão das Neves – MG – Brazil) at room temperature for 40 min
Group 3 (G3)	Milk as storage medium followed by laser irradiation–after extraction the teeth were stored in 20 ml of UHT skim milk (Cotochés® – Ribeirão das Neves – MG – Brazil) at room temperature for 40 min. After this period, laser irradiation was applied on root surfaces and at the entrance of alveolus before replantation
Group 4 (G4)	Milk as storage medium, laser irradiation before and after replantation–the teeth received the same treatment as Group 3 and after replantation, laser irradiation was performed on the buccal and palatal mucosa every 48 h for 15 days.

Table 2. Summary of the parameters used in the study

Parameters	Root surface (mesial and distal)	Entrance of the alveolus	Buccal and palatal mucosa
Wavelength (nm)	780	780	780
Power output (mW)	70	70	70
Mode	CW	CW	CW
Spot (cm^2)	0.04	0.04	0.04
Fluence (per point) (J/cm^2)	8.4	4.2	4.2
Fluence (per session) (J/cm^2)	16.8	4.2	8.4
Exposure time, s (per session)	320	60	120

Table 3. Semi quantitative criteria used for the light microscopy analysis

Criteria	Absent	Discrete	Moderate	Intense
External inflammatory root resorption	–	Presence of external root resorption in <25% of area observed	Presence of external root resorption in 25–50% of area observed	Presence of external root resorption in 50–100% of area observed
Ankylosis	–	Presence of junction between bone and root in <25% of area observed	Presence of junction between bone and root in 25–50% of area observed	Presence of junction between bone and root in 50–100% of area observed
Inflammation	–	Presence of <25% of lymphocytes and plasmocytes in relation to the total of cells in the area observed	Presence of <25–50% of lymphocytes and plasmocytes in relation to the total of cells in the area observed	Presence of >50% of lymphocytes and plasmocytes in relation to the total of cells in the area observed
Osteoclasts	–	Presence of <25% of osteoclasts	Presence of <25–50% of osteoclasts	Presence of >50% of osteoclasts

while osteoclasts, external inflammatory root resorption, and ankylosis were absent (Fig. 1a). At the 30th day, there was a moderate level of increase in the inflammatory process and lymphocytes. The number of osteoclasts was high and inflammatory resorption was intense in 60% of the specimens (Fig. 1b). Ankylosis was classified as discrete in 60% of the specimens. At the end of the experimental period, both the inflammatory process and resorption were moderate. Forty percent of the specimens showed intense ankylosis (Fig. 1c), and all of them were characterized by a discrete number of osteoclastic cells.

Group 2
At the 15th day, discrete chronic inflammatory cells and external inflammatory root resorption were observed. Osteoclasts and ankylosis were absent (Fig. 1d). At the 30th day, the inflammatory process and osteoclast cells were discrete. There was intense external inflammatory root resorption (Fig. 1e) and over 50% of the specimens showed ankylosis classified as intense. At the 60th day, the inflammatory process remained discrete and showed scattered cells. Despite external inflammatory root resorption being present (moderate), there were neither osteoclasts nor ankylosis (Fig. 1f).

Group 3
At the 15th day, a discrete chronic inflammatory infiltrate was observed. Osteoclasts, ankylosis, and external inflammatory root resorption were absent (Fig. 1g). At the 30th day, the intensity of the inflammatory process decreased and the absence of lymphocytes in all specimens was seen. Osteoclasts, ankylosis, and external inflammatory root resorption were also absent (Fig. 1h). No major change was seen at the end of the experimental period (Fig. 1i).

Group 4
At the 15th day, a moderate chronic inflammatory process was present. Osteoclasts, external inflammatory root resorption (Fig. 1j), and ankylosis were rarely seen. At the 30th day the level of the inflammatory process and the amount of lymphocyte cells and osteoclasts were scored as moderate. The external inflammatory root resorption was intense in 80% of the specimens (Fig. 1k). Moderate ankylosis was seen. At the 60th day, the inflammatory process was intense

with an increase in the numbers of chronic inflammatory cells. No clastic cells were observed and the external inflammatory root resorption decreased to only 40% of the specimens characterized as intense (Fig. 1l).

Statistical analysis
Comparison within groups showed that in G1, the criteria with reference to external inflammatory root resorption, osteoclasts, and inflammation differed significantly ($P < 0.05$) over time. In G2, only the criteria regarding ankylosis and the presence of osteoclasts differed significantly between the time intervals of 15 and 30 days ($P = 0.04$ and $P = 0.03$ respectively). In G3, the level of inflammatory resorption, osteoclasts, and the presence of ankylosis remained absent or was discrete throughout the experimental period. In this group, there was a significant reduction in inflammation from 15 to 30 days ($P = 0.01$). There was no significant difference with regards to inflammation from 30 to 60 days. G4 showed persistence of all criteria with no significant difference between them.

Comparisons between groups at the 15th, 30th, and 60th days are shown in Tables 4, 5, and 6 respectively.

Discussion
At present, the outcome of tooth replantation following avulsion is variable. Many replanted teeth are lost due to external root resorption, and this outcome has motivated the search for new forms of treatment to eliminate this problem (6, 13–15).

The results of this study showed that in the specimens of G1, the inflammatory process and external inflammatory root resorption varied from moderate (30th day) to intense (60th day). These findings corroborate the results of a previous study (6, 16) which demonstrated that the selection of dry storage promotes cell necrosis of the periodontal ligament and cementum contributing to inflammation and resorption.

Sottovia et al. (17) suggest that the presence of necrotic debris from the periodontal ligament adds to the inflammatory process by releasing various enzymes and inflammatory mediators throughout the course of healing. In comparison with dry storage, when the incisors were stored in milk only (G2), this produced conditions that allowed these cells to be less effected, and resulted in less inflammation mainly at 15 days.
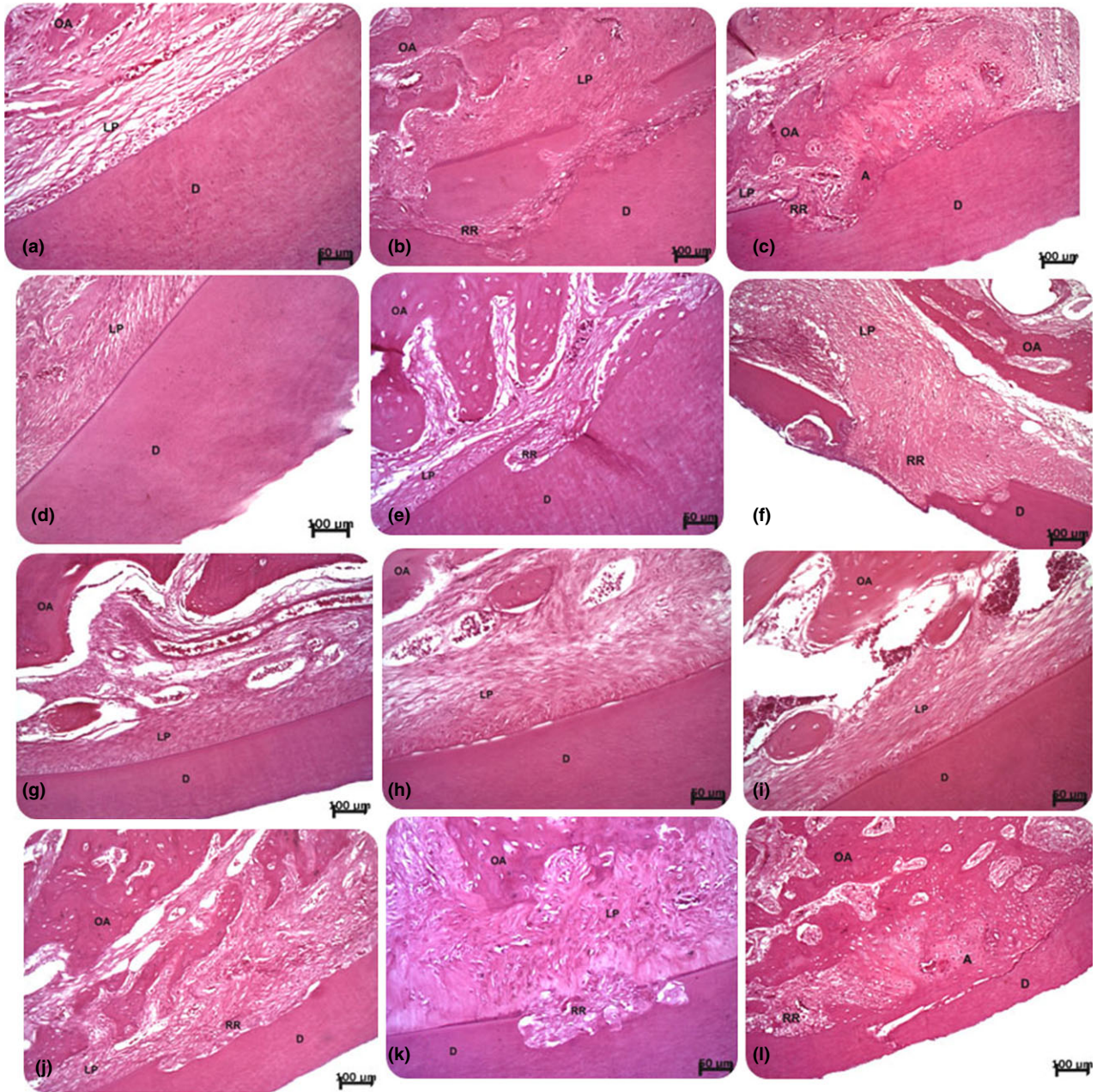
Fig. 1. Photomicrography of the experimental groups. OA, Alveolar bone; LP, Periodontal ligament; D, Dentin; RR, External inflammatory root resorption; A, Ankylosis. (a) G1 15th day–absence of external inflammatory root resorption and ankylosis. (b) G1 30th day–presence of external inflammatory root resorption. (c) G1 60th day–presence of external inflammatory root resorption and ankylosis. (d) G2 15th day–absence of external inflammatory root resorption and ankylosis. (e) G2 30th day–presence of external inflammatory root resorption. (f) G2 60th day–presence of Inflammatory external root resorption. (g) G3 15th day–absence of external inflammatory root resorption and ankylosis. (h) G3 30th day–absence of external inflammatory root resorption and ankylosis. (i) G3 60th day–absence of external inflammatory root resorption and ankyloses. (j) G4 15th day–presence of external inflammatory root resorption. (k) G4 30th day–presence of external inflammatory root resorption. (l) G4 60th day–presence of external inflammatory root resorption and ankylosis.

When immediate replantation is not possible, the tooth should be kept in a storage medium to preserve the viability of periodontal ligament cells and in some cases stimulate their proliferation in an attempt to reduce the presence of resorption (18).

Milk has been recommended as the most effective storage medium (19–22) for avulsed teeth. Its effectiveness in maintaining cell viability is related to its osmolarity, the presence of nutritional substances and growth factors, as well as a lower bacterial content due to the pasteurization process (19, 23). Although storage in milk has shown very favorable results, this medium reduces but does not prevent resorption (24). In an attempt to maintain the integrity of the remaining periodontal ligament, the incisors used in Groups 2, 3, and 4 were stored in UHT skim milk.

Table 4. Comparison between groups at the 15th day

	Group 1	Group 2	Group 3	Group 4
Inflammation	100%[a] discrete	40%[b] discrete	60% discrete	80%[a, b] moderate
Osteoclasts	60% absent	100%[a] absent	100%[b] absent	40%[a, b] absent
External inflammatory root resorption	60% absent	80% discrete	100%[a] absent	40%[a] absent
Ankylosis	80% absent	100% absent	100% absent	80% absent

Same letters on lines denote significant difference ($P < 0.05$, Fisher test).

Table 5. Comparison between groups at the 30th day

	Group 1	Group 2	Group 3	Group 4
Inflammation	80%[a] moderate	60%[b] discrete	100%[a, b, c] absent	100%[c] moderate
Osteoclasts	60%[a] intense	40%[b] discrete	80%[c] absent	100%[a, b, c] discrete
External inflammatory root resorption	60%[a] intense	40% intense	80%[a, b] absent	80%[b] intense
Ankylosis	60% discrete	60% intense	80% absent	40% moderate

Same letters on lines denote significant difference ($P < 0.05$, Fisher test).

Table 6. Comparison between groups at the 60th day

	Group 1	Group 2	Group 3	Group 4
Inflammation	60%[a] moderate	40% discrete	60%[a] discrete	40% intense
Osteoclasts	100%[a, b, c] discrete	80%[a] absent	100%[b] absent	100%[c] absent
External inflammatory root resorption	60%[a] moderate	60%[b] moderate	100%[a, b, c] absent	40%[c] intense
Ankylosis	40% intense	80% absent	100% absent	60% absent

Same letters on lines denote significant difference ($P < 0.05$, Fisher test).

During the experimental period of 15 days, the irradiated groups G3 and G4 showed chronic inflammation classified as discrete and moderate when compared with the non-irradiated groups. This result may be attributed to the laser photobiomodulation increasing vasodilation and local microcirculation (25, 26). Laser irradiation seems to accelerate the exudative stage of inflammation, so that it has a proinflammatory effect in the early periods (27, 28), which was confirmed in this present study.

Inflammation contributes to an environment that promotes the presence of clastic cells and resorption. This was supported by observations made in this present study showing that when inflammation was classified as moderate and intense in Groups 1, 2, and 4 in the evaluated periods, there was also the presence of osteoclasts and inflammatory resorption. In contrast, for Group 3, where inflammation was scored as absent or discrete, and discrete only in the experimental period of 15 days, most specimens of this group showed no

osteoclasts or resorption at any of the experimental periods.

Thus, modulation of the inflammatory process observed in Group 3, which received laser irradiation on the tooth surface and at the entrance of the alveolus before replantation, seems to be important to attenuate the external inflammatory root resorption. Despite using different methodology, Vilela et al. (6) have shown that the anti-inflammatory effect of the laser may have helped to reduce the resorption process when it was applied only at the entrance of alveolus and root surface before replantation. According to these authors, laser irradiation inhibited the clastic action favouring the synthesis of fibroblasts, cementoblasts, and osteoblasts.

The findings of this present study differ from the results of Saito et al. (7), in which the treatment of the root surface and entrance of the alveolus with low power laser did not affect the healing process of tooth replantation in rats. They found no differences between the control group and irradiated group with regards to the occurrence of inflammatory and replacement resorption processes. Although the laser group demonstrated a more advanced stage in the healing process, this was without statistical difference. Different results may be caused by the use of different methodologies, such as evaluation period, extra-alveolar time, and laser parameters.

For the laser to produce its biological effects, some level of cell deficiency must be present, in other words, the cell metabolism might be unbalanced (29). Thus, laser irradiation in Groups 3 and 4 was performed on teeth stored in milk for 40 min, differing from the study of Vilela et al. (6) in which rat incisors kept for 15 min on dry gauze before replantation were irradiated with low-power laser. Saito et al. (7) used the saline solution as a storage medium for periods of 30 and 45 min before laser irradiation.

The effects of laser on cells are wavelength and dose dependent. The study of Kreisler et al. (30) revealed that the infrared laser has a stimulating effect on the proliferation of periodontal ligament fibroblasts. In this present study, the laser used was λ780 nm, based on the target tissue of root and alveolar periodontal ligament cells.

Healing of the periodontal ligament occurs as a series of interactions between gingival fibroblasts, osteoblasts, cementoblasts, and periodontal ligament. Fibroblasts are essential because they can differentiate into osteoblasts and cementoblasts to restore the loss of alveolar bone and cementum (31). In this present study, the effects of the laser on cell stimulation and proliferation were used on the remaining fibroblasts, in addition to its vascular, anti-inflammatory and analgesic properties.

The specimens of Group 3 which received laser irradiation on the root surface and at the entrance of the alveolus, showed positive results in terms of repair after tooth replantation, as osteoclasts and inflammatory resorption were not observed in the analyzed periods. It is believed that the laser parameters used in this group may have contributed to the proliferation and differentiation of fibroblasts. According to Lekic et al. (32), the periodontal ligament has a heterogeneous cell population that can differentiate into osteoblasts or

cementoblasts. The findings of Choi et al. (31) demonstrated that the GaAlAs diode laser could stimulate proliferation and differentiation of human fibroblasts.

Intense external inflammatory root resorption was observed within 30 days after replantation in Group 4. It is believed that the laser irradiation on the palatal mucosa for 15 days stimulated the differentiation and activation of osteoclasts. According to Karu (33), mitochondrial cytochrome c absorbs the laser energy and this absorption increases cell activity via increased ATP synthesis. Because osteoclasts are multinucleated cells with highly active mitochondria, the laser readily affects these cells (34, 35).

Panzarini et al. (36) studying the chronology of the repair process immediately after replantation of incisors in rats, observed a significant increase in TRAP (enzyme of osteoclast activity) from the seventh day after replantation and in all the subsequent periods (15, 28, and 60 days) proving the existence of clastic cells during repair. Based on this notion it is possible that in Group 4, the laser irradiation for 15 days after replantation stimulated the clastic cells present, thus the inflammatory external root resorption was observed in this group in all periods.

The damage to the periodontal ligament observed after tooth avulsion, leads to an inflammatory response to remove the injured tissue before the repair process (37). Thus, modulation of inflammation is important to attenuate subsequent external inflammatory root resorption. According to the results of this study, the use of low intensity laser therapy can be an important tool in controlling external inflammatory root resorption after replantation, thereby increasing the success rates of this procedure.

Conclusions

The use of laser phototherapy on the root surface and at the entrance of the alveolus before replantation (G3) advanced the tissue repair after tooth replantation in rats, with no external inflammatory root resorption, ankylosis, osteoclasts, and inflammation shown, when compared with the other groups. However, laser phototherapy performed on the root surface and at the entrance of the alveolus before replantation, associated with irradiation on the buccal and palatal mucosa after replantation, for 15 days (G4) did not encourage tissue repair and demonstrated the presence of external inflammatory root resorption, ankylosis, osteoclasts, and inflammation at all time periods.

Conflict of interest

The authors confirm that they have no conflict of interest.

Notes

[1]Xylazina® Syntec, Cotia, São Paulo, Brazil.
[2]Cetamin® Syntec, Cotia, São Paulo, Brazil.
[3]FGM, Joinvile, Santa Catarina, Brazil.
[4]25 mm, Dentsply Maillefer, Ballaigues, Switzerland.

[5]Megacilin Super Plus®–Vansil Ind. Com. e Repr. Ltda, Descalvado, São Paulo, Brazil.

References

1. Mori GG, Garcia RB, de Moraes IG, Bramante CM, Bernardineli N. Morphometric and microscopic evaluation of the effect of a solution of alendronate as an intracanal therapeutic agent in rat teeth submitted to late reimplantation. Dent Traumatol 2007;23:218–21.
2. Baginska J, Wilczynska-Borawska M. First-aid algorithms in dental avulsion. J Sch Nurs 2012;28:90–4.
3. Andreasen JO, Lauridsen E, Andreasen FM. Contradictions in the treatment of traumatic dental injuries and ways to proceed in dental trauma research. Dent Traumatol 2010;26:16–22.
4. Donaldson M, Kinirons MJ. Factors affecting the time of onset of resorption in avulsed and replanted incisor teeth in children. Dent Traumatol 2001;17:205–9.
5. Poi WR, Carvalho RM, Panzarini SR, Sonoda CK, Manfrin TM, Rodrigues TS. Influence of enamel matrix derivative (Emdogain) and sodium fluoride on the healing process in delayed tooth replantation: histologic and histometric analysis in rats. Dent Traumatol 2007;23:35–41.
6. Vilela RG, Gjerde K, Frigo L, Leal Junior ECP, Lopes-Martins RAB, Kleine BM, et al. Histomorphometric analysis of inflammatory response and necrosis in re-implanted central incisor of rats treated with low-level laser therapy. Lasers Med Sci 2012;27:551–7.
7. Saito CTMH, Gulinelli JL, Panzarini RS, Garcia VG, Okamoto R, Okamoto T et al. Effect of low-level laser therapy on the healing process after tooth replantation: a histomorphometrical and immunohistochemical analysis. Dent Traumatol 2011;27:30–9.
8. Ribeiro MS, da Silva DF, de Araújo CE, de Oliveira SF, Pelegrini CM, Zorn TM et al. Effects of low-intensity polarized visible laser radiation on skin burns: a light microscopy study. J Clin Laser Med Surg 2004;22:59–66.
9. Santos NR, dos Santos JN, dos Reis JA Jr, Oliveira PC, de Sousa AP, de Carvalho CM et al. Influence of the use of laser phototherapy (l660 or 790 nm) on the survival of cutaneous flaps on diabetic rats. Photomed Laser Surg 2010;28:483–8.
10. Sampaio SCPO, Monteiro JSC, Cangussú MCT, Santos GMP, Santos MAV, Santos JN et al. Effect of laser and LED phototherapies on the healing of cutaneous wound on healthy and iron-deficient Wistar rats and their impact on fibroblastic activity during wound healing. Lasers Med Sci 2013;28:799–806.
11. Parker S. Low-level laser use in dentistry. Br Dent J 2007;202:131–8.
12. Okamoto T, Russo MC. Wound healing following tooth extraction. Histochemical study in rats. Rev Fac Odontol Aracatuba 1973;2:153–69.
13. Kinirons MJ, Gregg TA, Welbury RRR, Cole BOI. Variations in the presenting and treatment features in reimplanted permanent incisors in children and their effect on the prevalence of root resorption. Br Dent J 2000;189:263–6.
14. Soares AJ, Gomes BPFA, Zaia AA, Ferraz CCR, Souza-Filho FJ. Relationship between clinical–radiographic evaluation and outcome of teeth replantation. Dent Traumatol 2008;24:183–8.
15. Hecova H, Tzigkounakis V, Merglova V, Netolicky J. A retrospective study of 889 injured permanent teeth. Dent Traumatol 2010;26:466–75.
16. Mori GG, Nunes DC, Castilho LR, de Moraes IG, Poi WR. Propolis as storage media for avulsed teeth: microscopic and morphometric analysis in rats. Dent Traumatol 2010;26:80–5.
17. Sottovia AD, Sonoda CK, Poi WR, Panzarini SR, Lauris JRP. Delayed tooth replantation after root surface treatment with sodium hypochlorite and sodium fluoride: histomorphometric analysis in rats. J Appl Oral Sci 2006;14:93–9.

18. Andreasen JO, Andreasen FM, Andersson L. Textbook and color atlas of traumatic injuries to the teeth, 4th edn. New Jersey: Wiley-Blackwell, 2007. 912 pp.

19. Marino TG, West LA, Liewehr FR, Mailhot JM, Buxton TB, Runner RR et al. Determination of periodontal ligament cell viability in long shelf-life milk. J Endod 2000;26:699–702.

20. Pearson RM, Liewehr FR, West LA, Patton WR, Mcpherson JC, Runner RR. Human periodontal ligament cell viability in milk and milk substitutes. J Endod 2003;29:184–6.

21. dos Santos CL, Sonoda CK, Poi WR, Panzarini SR, Sundefeld ML, Negri MR. Delayed replantation of rat teeth after use of reconstituted powdered milk as a storage medium. Dent Traumatol 2009;25:51–7.

22. Wang WJ, Zhao YM, Feng XY, Jia WQ, Ge LH. Effect of skimmed pasteurized milk and Hank's balanced salt solution on viability and osteogenic differentiation of human periodontal ligament stem cells. Dent Traumatol 2013;29:365–71.

23. Malhotra N. Current developments in interim transport (storage) media in dentistry: an update. Br Dent J 2011;211:29–33.

24. de Souza BDM, Luckemeyer DD, Felippe WT, Alves AMH, Simões CMO, Felippe MCS. Effect of milk renewal on human periodontal ligament fibroblast viability *in vitro*. Dent Traumatol 2012;28:214–6.

25. Rodrigo SM, Cunha A, Pozza DH, Blaya DS, Moraes JF, Weber JB et al. Analysis of the systemic effect of red and infrared laser therapy on wound repair. Photomed Laser Surg 2009;27:929–35.

26. Ihsan FRM. Low level laser therapy accelerates collateral circulation and enhances microcirculation. Photomed Laser Surg 2005;23:289–94.

27. Mendez TMTV, Pinheiro ALB, Pacheco MTT, Nascimento PM, Ramalho LMP. Dose and Wavelength of Laser Light Have Influence on the Repair of Cutaneous Wounds. J Clin Laser Med Surg 2004;22:19–25.

28. Pereira MCMC, de Pinho CB, Medrado ARP, Andrade ZA, Reis SRA. Influence of 670 nm low-level laser therapy on mast cells and vascular response of cutaneous injuries. J Photochem Photobiol, B 2010;98:188–92.

29. Pinheiro ALB, Brugnera A Jr, Zanin FAA. Aplicação do Laser na Odontologia. São Paulo: Santos; 2010. 428 pp.

30. Kreisler M, Christoffers AB, Willershausen B, d'Hoedt B. Effect of low-level GaAIAs laser irradiation on the proliferation rate of human periodontal ligament fibroblasts: an *in vitro* study. J Clin Periodontol 2003;30:353–8.

31. Choi EJ, Yim JY, Koo KT, Seol YJ, Lee YM, Ku Y et al. Biological effects of a semiconductor diode laser on human periodontal ligament fibroblasts. J Periodontal Implant Sci 2010;40:105–10.

32. Lekic P, Rojas J, Birek C, Tenenbaum H, McCulloch CAG. Phenotypic comparison of periodontal ligament cells *in vivo* and *in vitro*. J Periodontal Res 2001;36:71–9.

33. Karu T. Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J Photochem Photobiol, B 1999;49:1–17.

34. Nicolau RA, Jorgetti V, Rigau J, Pacheco MT, dos Reis LM, Zângaro RA. Effect of low-power GaAlAs laser (660 nm) on bone structure and cell activity: an experimental animal study. Lasers Med Sci 2003;18:89–94.

35. Aihara N, Yamaguchi M, Kasai K. Low-energy irradiation stimulates formation of osteoclast-like cells via RANK expression *in vitro*. Lasers Med Sci 2006;21:24–33.

36. Panzarini SR, Okamoto R, Poi WR, Sonoda CK, Pedrini D, da Silva PE et al. Histological and immunohistochemical analyses of the chronology of healing process after immediate tooth replantation in incisor rat teeth. Dent Traumatol 2013;29:15–22.

37. Consolaro A. The four mechanisms of dental resorption initiation. Dental Press J Orthod 2013;18:7–9.