Evaluation of laser phototherapy ($\lambda$ 780 nm) after dental replantation in rats

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Key words: laser therapy, low-Level; root resorption; tooth injuries

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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 ($\lambda$ 780 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 ($\lambda$ = 780 nm; $P$ = 70 mW; CW; DE = 21 J/cm²); 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/cm² 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 it 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 ($\lambda$780 nm) on replanted teeth in rats using light microscopy.
Effect of phototherapy on dental replantation

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 hydrochloride1; 0.03 ml per 100 g body weight; ketamine hydrochloride2; 0.06 ml per 100 g body weight). Asepsis of the anterior maxilla was performed with 0.12% chlorhexidine gluconate solution3 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.

Table 1. Study Groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment of root surface and alveolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Absence of storage medium—the teeth were kept dry at room temperature for a period of 40 min</td>
</tr>
<tr>
<td>Group 2</td>
<td>Milk as storage medium—after extraction the teeth were stored in 20 ml of UHT skimmed milk (Cotoches® – Ribeirão das Neves – MG – Brazil) at room temperature for 40 min</td>
</tr>
<tr>
<td>Group 3</td>
<td>Milk as storage medium followed by laser irradiation—after extraction the teeth were stored in 20 ml of UHT skim milk (Cotoches® – 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</td>
</tr>
<tr>
<td>Group 4</td>
<td>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.</td>
</tr>
</tbody>
</table>

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 (Twinfox 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/cm² per point was used every 48 h for 15 days totaling 67.2 J/cm². 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 2. Summary of the parameters used in the study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Root surface (mesial and distal)</th>
<th>Entrance of the alveolus</th>
<th>Buccal and palatal mucosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>780</td>
<td>780</td>
<td>780</td>
</tr>
<tr>
<td>Power output (mW)</td>
<td>70</td>
<td>70</td>
<td>70</td>
</tr>
<tr>
<td>Mode</td>
<td>CW</td>
<td>CW</td>
<td>CW</td>
</tr>
<tr>
<td>Spot (cm²)</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Fluence (per point)</td>
<td>8.4</td>
<td>4.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Fluence (per session) (J/cm²)</td>
<td>16.8</td>
<td>4.2</td>
<td>8.4</td>
</tr>
<tr>
<td>Exposure time, s (per session)</td>
<td>320</td>
<td>60</td>
<td>120</td>
</tr>
</tbody>
</table>
Table 3. Semi quantitative criteria used for the light microscopy analysis

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Absent</th>
<th>Discrete</th>
<th>Moderate</th>
<th>Intense</th>
</tr>
</thead>
<tbody>
<tr>
<td>External inflammatory root resorption</td>
<td>Presence of external root resorption in &lt;25% of area observed</td>
<td>Presence of external root resorption in 25–50% of area observed</td>
<td>Presence of external root resorption in 50–100% of area observed</td>
<td></td>
</tr>
<tr>
<td>Ankylosis</td>
<td>Presence of junction between bone and root in &lt;25% of area observed</td>
<td>Presence of junction between bone and root in 25–50% of area observed</td>
<td>Presence of junction between bone and root in 50–100% of area observed</td>
<td></td>
</tr>
<tr>
<td>Inflammation</td>
<td>Presence of &lt;25% of lymphocytes and plasmocytes in relation to the total of cells in the area observed</td>
<td>Presence of &lt;25–50% of lymphocytes and plasmocytes in relation to the total of cells in the area observed</td>
<td>Presence of &gt;50% of lymphocytes and plasmocytes in relation to the total of cells in the area observed</td>
<td></td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>Presence of &lt;25% of osteoclasts</td>
<td>Presence of &lt;25–50% of osteoclasts</td>
<td>Presence of &gt;50% of osteoclasts</td>
<td></td>
</tr>
</tbody>
</table>

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. 1b). 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.

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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.

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 ankylosis. (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.
Comparison between groups at the 60th day

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>60%</td>
<td>80%</td>
<td>100%</td>
<td>80%</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>discrete</td>
<td>discrete</td>
<td>discrete</td>
<td>moderate</td>
</tr>
<tr>
<td>External inflammatory root resorption</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Ankylosis</td>
<td>80%</td>
<td>100%</td>
<td>100%</td>
<td>80%</td>
</tr>
</tbody>
</table>

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

Comparison between groups at the 30th day

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>80%</td>
<td>60%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>intense</td>
<td>discrete</td>
<td>discrete</td>
<td>moderate</td>
</tr>
<tr>
<td>External inflammatory root resorption</td>
<td>intense</td>
<td>intense</td>
<td>absent</td>
<td>intense</td>
</tr>
<tr>
<td>Ankylosis</td>
<td>60%</td>
<td>60%</td>
<td>80%</td>
<td>40%</td>
</tr>
</tbody>
</table>

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

Comparison between groups at the 15th day

<table>
<thead>
<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>60%</td>
<td>40%</td>
<td>60%</td>
<td>80%</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>discrete</td>
<td>discrete</td>
<td>discrete</td>
<td>moderate</td>
</tr>
<tr>
<td>External inflammatory root resorption</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>Ankylosis</td>
<td>80%</td>
<td>100%</td>
<td>100%</td>
<td>80%</td>
</tr>
</tbody>
</table>

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 intense. This result may be attributed to the laser photobiomodulation increasing vasodilation and local microcirculation. Laser irradiation seems to accelerate the exudative stage of inflammation, so that it has a proinflammatory effect in the early periods. This was supported by observations made in this present study.

Inflammation contributes to an environment that promotes the presence of clastic cells and resorption. The specimens of Group 3 which received laser irradiation on the root surface and at the entrance of the alveolus before replantation, seems to be important to attenuate the external inflammatory root resorption. 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. 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. in which rat incisors kept for 15 min on dry gauze before replantation were irradiated with low-power laser. Saito et al. 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. revealed that the infrared laser has a stimulating effect on the proliferation of periodontal ligament fibroblasts. In this present study, the laser used was 780 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, and periodontal ligament. Fibroblasts are essential because they can differentiate into osteoblasts and cementoblasts to restore the loss of alveolar bone and cementum. 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., 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.

Conflicts of interest

The authors confirm that they have no conflict of interest.

Notes

1. Xylazine® Syntec, Cotia, São Paulo, Brazil.
2. Cetamin® Syntec, Cotia, São Paulo, Brazil.
3. FG M, Joinville, Santa Catarina, Brazil.
4. 25 mm, DeTrey Mailléfer, Ballaigues, Switzerland.

References