

Effect of low-level laser therapy and mineral trioxide aggregate on alveolar bone repair

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Abstract

Aim: This study aimed to evaluate the effect of low-level laser therapy (LLLT) associated to mineral trioxide aggregate (MTA) on the alveolar bone repair process. **Methods:** Forty Wistar rats had the upper right incisor extracted and were assigned to 4 groups according to the treatment of the alveolar wound: G1 - no treatment (control group); G2 - sockets were filled with MTA; G3 - sockets were treated with LLLT (1780 nm, 40 mW, 16 J/cm²); and G4 - sockets were treated with LLLT and filled with MTA. The animals were sacrificed 14 days after the surgical procedures. The maxillas were removed, fixed in formalin, decalcified in 0.5% nitric acid and processed histologically. **Results:** In G1, there was bone formation on the bottom of the socket, but it was not noticeable on the superficial areas, as well as the presence of an intense granulation tissue. In G2, the bone trabeculae were irregular and thin, and were associated with intense vascular hyperemia and chronic inflammation. In G3, there was substantial formation of thick interwoven osteocyte-rich trabecular bone, with an evident osteoblastic rimming. In G4, it was observed an intense deposition of thin irregular bone trabeculae, but vascular hyperemia was quite distinguishable. **Conclusion:** LLLT was the most successful treatment to improve alveolar bone repair

Key Words: Biomaterials, bone regeneration, low level laser therapy.

Introduction

Many techniques and materials have been tested in the last decades for the maintenance of the alveolar ridge after dental extraction. However, none of them have proven to be completely effective¹.

It has been reported that the use of materials to increase new bone formation in sockets immediately following tooth extraction, such as microgranular hydroxyapatite (HA), tricalcium phosphate, *Ricinus communis* and glass ionomer cement, seems to delay the alveolar repair process¹⁻³. Nevertheless, recent studies have described promising results with the use of biomaterials in promoting bone tissue replacement and regeneration⁴⁻⁶.

Mineral trioxide aggregate (MTA) has been developed for

endodontics and consistently allows for cementum overgrowth and may facilitate periodontal ligament regeneration⁷. Furthermore, MTA resists bacterial leakage and may provide protection for the pulp, allowing repair and maintenance of pulp vitality when used in combination with a sealed restoration⁸. Studies have demonstrated that MTA seems to stimulate calcium deposition in the connective tissue and hence it may work as an important osteogenesis inductor agent^{9,10}.

Low-level laser therapy (LLLT) in the far red to near-infrared (NIR) range is has been shown to modulate several biological processes, phenomenon known as photobiomodulation. Furthermore, LLLT has been reported as an important tool to positively stimulate bone formation in vivo and in vitro¹¹.

The positive results with the use of osteoconductive materials in association with LLLT to promote significant improvements in bone repair seem very encouraging^{12,13}. Even though there are some reports looking at the biological effects of MTA and LLLT association^{14,15}, they

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are focused on endodontics procedures. Therefore, the aim of this study was to evaluate the effect of LLLT ($\lambda 780$ nm) associated to MTA on the alveolar bone repair process after tooth extraction in rats.

Material and Methods

Twenty male Wistar rats weighing 250 ± 50 g supplied by the Central Vivarium of the University Tiradentes, Brazil were housed in cages under controlled lighting (lights on from 6:00 am to 6:00 pm) and temperature (23.2°C) conditions. Laboratory chow (Nuvilab[®]) and tap water were given *ad libitum*. The animals were anesthetized with an intraperitoneal injection of 2.5% tribromoethanol (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 1 mL/100g body weight. Under sterile conditions, the upper right incisor was extracted with an adapted forceps after separation of the surrounding gingival tissue and luxation with an enamel hatchet with cutting edge. The animals were assigned to 4 groups (n=5): G1 - no treatment; G2 - sockets were filled with MTA (Angelus[®]; Londrina, PR, Brazil); G3 - sockets were treated with LLLT; G4 - sockets were treated with LLLT and filled with MTA. After the surgical procedures, 5.0 nylon sutures were placed (Techsynt Medical Express Ltda., São Paulo, SP, Brazil). LLLT procedures were performed at 1780 nm (Twin Laser; MMOptics, São Carlos, SP, Brazil), 40 mW and CW 16 J/cm² *per* session, distributed in four points of 4 J/cm² each. The animals were irradiated every 48 h, totalizing 4 sessions. The first session was accomplished immediately after the surgical procedures. The animals were sacrificed 14 days after tooth extraction with an intramuscular injection of zolazepam (Zoletil[®]; Virbac Laboratories, Carros, France; 0.8 mL/kg), Sodium thiopental (Cristália, Itapera, SP, Brazil; 0.43 mL/kg) and KCl solution (Ariston 19.1%, 2.559 mEq/mL). The right maxilla was separated from the left maxilla and the samples were fixed in 10% formalin, decalcified in a solution of 5% nitric acid, dehydrated in increasing concentrations of ethanol solutions, cleared and embedded in paraffin (Figure 1). All procedures were carried out in compliance with the standards for animal experimentation and were previously approved by the local Research Ethics Committee.

Five-micrometer-thick longitudinal histological sections were cut at 60 μm intervals and were subsequently stained in hematoxylin and eosin (HE). The local response to material implantation, identification of cell type and wound healing were evaluated under light microscopy. The morphological features of the bone trabeculae deposited within the alveolar wounds were also described. The areas of new bone formation within the sockets were determined by the analysis of digitized images of the histological sections ($\times 100$ magnification) using an image-analysis software specific for morphometric evaluation (Imagelab[®]; Softium Sistemas de Informática, São Paulo, SP, Brazil). Data were analyzed statistically by

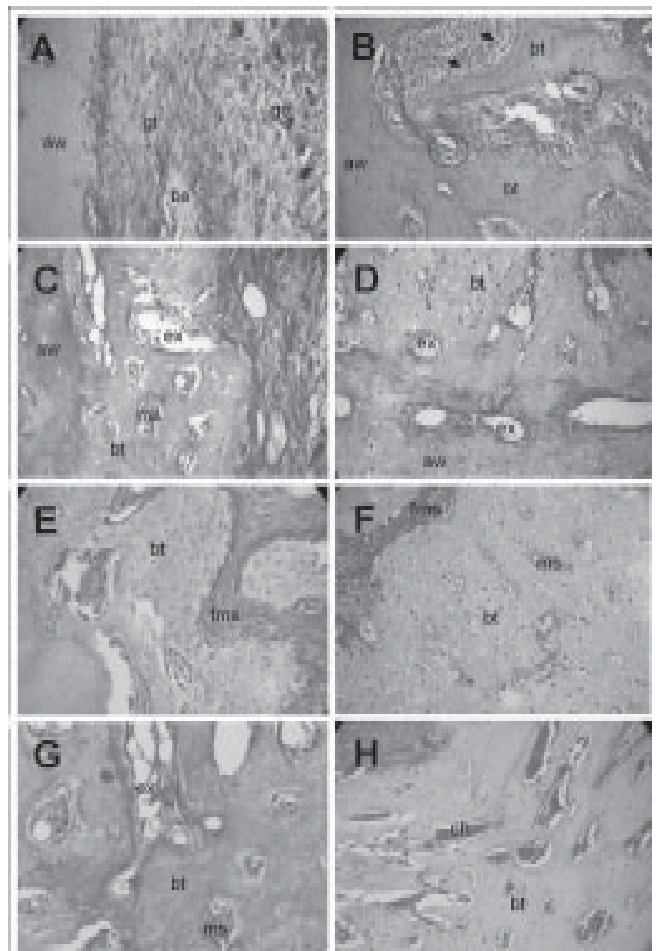


Fig. 1. (A) Granulation tissue (gt), sparse bone spicules and osteoclast-like giant cells (gc) adjacent to the alveolar wall (aw) in the superficial region of G1. (B) Trabecular bone (bt) exhibiting osteoblastic rimming (arrow) and osteoclastic activity (circle) in the deep region of the same group. (C and D) Marginal thin bone trabeculae (bt), residual exogenous material (ex) in the superficial and deeper regions of G2. (E) Medullar fibrosis (fms) in G3. (F) Substantial thick trabecular bone deposition exhibiting narrow medullar spaces (ms). (G) Residual exogenous material (ex) surrounded by intense deposition of thick bone trabeculae and (H) evident capillary hyperemia in G4. (HE, $\times 200$).

ANOVA and Tukey's post-hoc test for intergroup comparisons. Significance level was set at 5%.

Results

Bone trabeculae deposition was observed in all groups, but in different extensions. In general, there was higher density of bone deposition in the cervical region of the sockets unlike the superficial region. In addition, inflammatory infiltrate was scarce and the inflammatory cells were more easily perceived in the superficial region of the socket (Figure 1).

In G1, the bone trabeculae were limited to the cervical region of the sockets, and presented an irregular, thin and

highly cellular appearance. Osteoblastic rimming intercalated with osteoclastic activity was also observed. In the superficial region, however, there was interstitial edema and exuberant granulation tissue. Furthermore, focus of hemorrhagic areas and remaining blood clot were also seen. Inflammatory infiltrate was composed of sparse macrophages and some lymphocytes and plasma cells.

In the cervical region of G2's specimens, there was distinguishable formation of thick bone trabeculae involving the exogenous material (MTA), which presented a prominent osteoblastic rimming. In the superficial region, bone formation was less evident, and it was clearly limited to the margins of the sockets. The connective tissue was loosely arranged, edematous and presented intense hyperemia and moderate mononuclear infiltrate. In addition, foci of globular mineralization consistent with psammomatoid bodies (dystrophic calcification) were also observed.

In G3, remarkable deposition of thick mature anastomosed bone trabeculae was observed in the cervical region, presenting a highly evident osteoblastic rimming. In some sparse areas, foci of interstitial medullar fibrosis were verified. In the superficial region, there was also intense new bone formation, but the medullar spaces were larger and permeated by remaining hyperemic blood capillaries. Rare plasma cells and lymphocytes were seen around the congested capillaries.

The cervical region of G4's specimens was characterized by evident thick trabecular bone formation, with a notable osteoblastic rimming. Inwardly, sparse deposits of exogenous material (MTA) were seen, as well as foci of interstitial medullar fibrosis. In the superficial region, the bone trabeculae were thinner, but evident. Inflammatory mononuclear infiltrate was scarce, but vascular hyperemia was clearly observed. Areas of coalescent psammomatoid bodies, occasionally forming larger calcified amorphous or trabecular-like masses were also observed, but they were not frequent.

As shown in the Figure 2, the morphometric analysis of the alveolar bone repair revealed that the area of bone

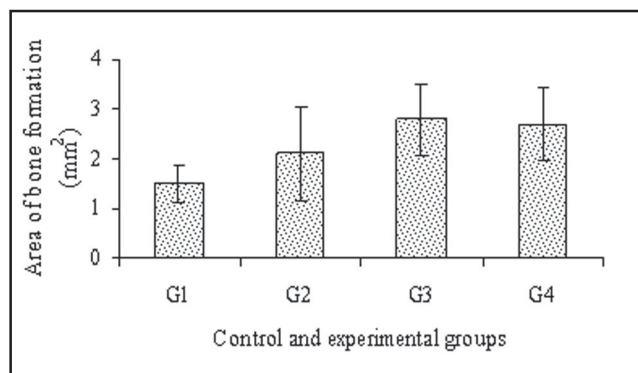


Fig. 2. Mean area of bone formation within the alveolar wound in the control and experimental groups after 14 days of tooth extraction.

formation in G1 ($1.5 \pm 0.41 \text{ mm}^2$) was significantly smaller than that in G2 ($2.1 \pm 0.93 \text{ mm}^2$), G3 ($2.8 \pm 0.71 \text{ mm}^2$) and G4 ($2.7 \pm 0.74 \text{ mm}^2$) ($p=0.00$). G3 and G4 showed a significantly larger bone repair area than G2 ($p=0.03$ and 0.05 , respectively), but they had statistically similar results ($p=0.16$).

Discussion

In the present study, substantial bone formation was found in the sockets of all groups 14 days after dental extraction. These findings are supported by previous reports asserting that, in rats, the alveolar wound is subsequently filled with newly formed bone tissue and the alveolar ridge is remodeled within 15 to 21 days^{2,3}. Furthermore, bone formation was more evident in the bottom of the sockets than in the superficial regions. These findings are in agreement with those of previous studies that showed that the alveolar bone repair develops first in the bottom of the socket and then extends to the surface^{3,16,17}.

Previous investigations have indicated that the persistence of the inflammatory response in the later phases of alveolar bone repair might be a result of a phlogistic activity of residual blood clots¹⁶. These data seem to support our findings of substantial mononuclear infiltrate in association to hemorrhagic areas and little residual clot in the control group. Surprisingly, the group treated with MTA (G2) also presented moderate infiltration of mononuclear cells and interstitial edema in the superficial regions of the sockets. Notwithstanding, it has been suggested that the manipulation of exogenous biomaterial within the alveolar wound may act as a phlogiston and cause persistence of the inflammation, though with low intensity^{2,3}, which would support our findings.

On the other hand, in both groups treated with LLLT (G3 and G4), little inflammatory reaction was observed. This might be related to the fact that the LLLT has a sort of biomodulatory properties, including antiinflammatory action¹⁸⁻²⁰.

Among all groups analyzed in this study, G1 exhibited the less amount of new bone formation, particularly in the surface, which could be related to the maintenance of the inflammatory infiltrate and interstitial edema in this area. Similar findings have been reported in other recent studies^{16,17}.

The pattern of bone regeneration in G2 (treated with MTA) was better than that in G1 (control), particularly in the deep zones, where bone trabeculae was formed around the exogenous material. Moreover, there was significantly greater bone formation in this group than in the control group. These findings suggest that the presence of MTA may have facilitated the process of bone deposition. Hard tissue deposition next to MTA may occur as a result of its biochemical properties, such as alkalinity and high content of calcium phosphate, calcium oxide and silica^{21,22}, which are important inorganic constituents widely required for

bone mineralization²³. Although bone formation in the superficial regions of the sockets was less substantial, probably due to some phlogistic activity promoted by handling of the biomaterial during alveolar wound filling, it was clearly greater than in the control group. Moreover, the areas of globular mineralization (“psammomatoid bodies”) might represent foci of dystrophic calcification, induced by tissue lesion secondary to the residual inflammation, which progressed to larger masses in response to the abundant local offer of minerals provided by the implanted biomaterial. Similar findings were reported with the use of other implantable material containing high calcium content¹⁶.

The group treated with LLLT presented the best morphological pattern of bone deposition, with bone trabeculae extending from the bottom to the surface of the sockets. Furthermore, the content of bone trabeculae deposited in both groups was significantly greater than in G1 and G2. Considering that the bone matrix (osteoid) is extensively rich in type I collagen²⁴, the beneficial effects promoted by LLLT in this study might be based on its capability of inducing collagen synthesis^{25,26}. Similar positive findings have been reported attesting the biomodulatory role of LLLT over bone formation^{15,27,28}. Moreover, the presence of interstitial medullar fibrotic areas in this group could be related to a stimulatory activity of LLLT on fibroblast differentiation and proliferation, as well as on the collagen synthesis^{29,30}.

Although the association of MTA and LLLT induced a more intense trabecular bone deposition than in G1 and G2, it was not as effective as LLLT alone (G3). In G2, the superficial region of the sockets was filled with thinner bone trabeculae, and there was still evidence of capillary hyperemia. In addition, the global content of newly formed bone trabeculae in the core of the sockets was statistically similar to that of G3. Since the results observed in the group treated with LLLT alone were more effective than the LLLT/MTA association, it might be suggested that the presence of MTA could have somehow altered the dynamics of photostimulation of bone repair. However, previous investigations performed in femur of rats have demonstrated positive findings using the association of MTA and LLLT³¹. It may be speculated that MTA handling within the alveolar wound caused a disarrangement of part of the blood clot and altered the bone regeneration process³².

In addition, the foci of globular calcification (“psammomatoid bodies”) were larger and coalescent in G4 (LLLT/MTA) compared to G2 (MTA), suggesting that LLLT may have influenced the increase of these calcified masses. Furthermore, medullar fibrosis was also observed in G4, but they probably developed in response to laser irradiation, since it was also seen in G3 (LLLT).

The findings of the present study suggest that, although MTA has the potential to be used in clinical situations

similar to those simulated in this study, associated or not to laser photobiomodulation, LLLT alone seemed to be more effective in stimulating alveolar bone repair. Further studies involving MTA and other LLLT protocols are necessary to indicate their clinical use.

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