



LED photobiomodulation therapy combined with biomaterial as a scaffold promotes better bone quality in the dental alveolus in an experimental extraction model

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Abstract

A bone scaffold added to the dental alveolus immediately after an extraction avoids bone atrophy and deformity at the tooth loss site, enabling rehabilitation with implants. Photobiomodulation accelerates bone healing by stimulating blood flow, activating osteoblasts, diminishing osteoclastic activity, and improving the integration of the biomaterial with the bone tissue. The aim of the present study was to evaluate the effect of photobiomodulation with LED at a wavelength of 850 nm on bone quality in Wistar rats submitted to molar extraction with and without a bone graft using hydroxyapatite biomaterial (Straumann® Cerabone®). Forty-eight rats were distributed among five groups ($n = 12$): basal (no interventions); control (extraction) (basal and control were the same animal, but at different sides); LED (extraction + LED $\lambda = 850$ nm); biomaterial (extraction + biomaterial), and biomaterial + LED (extraction + biomaterial + LED $\lambda = 850$ nm). Euthanasia occurred at 15 and 30 days after the induction of the extraction. The ALP analysis revealed an improvement in bone formation in the control and biomaterial + LED groups at 15 days ($p = 0.0086$ and $p = 0.0379$, Bonferroni). Moreover, the LED group had better bone formation compared to the other groups at 30 days ($p = 0.0007$, Bonferroni). In the analysis of AcP, all groups had less resorption compared to the basal group. Bone volume increased in the biomaterial, biomaterial + LED, and basal groups in comparison to the control group at 15 days ($p < 0.05$, t -test). At 30 days, the basal group had greater volume compared to the control and LED groups ($p < 0.05$, t -test). LED combined with the biomaterial improved bone formation in the histological analysis and diminished bone degeneration (demonstrated by the reduction in AcP), promoting an increase in bone density and volume. LED may be an important therapy to combine with biomaterials to promote bone formation, along with the other known benefits of this therapy, such as the control of pain and the inflammatory process.

Keywords LED · Scaffold · Straumann · Bone density · Micro CT · Extraction · Photobiomodulation · ALP · AcP

Introduction

The World Health Organization recognizes tooth loss as a global public health problem that should be considered in the formulation of health policies [1]. Tooth loss is the main cause of occlusal and oral bone deformities, altering

chewing function, swallowing, phonation, and esthetics [2, 3]. Accentuated changes can occur at the site of the loss, such as the resorption and remodeling of the alveolar process, leading to atrophy, which is a natural, physiological process inherent to the healing process [4–8]. The clinical loss is greater in height than width, and the vestibular wall of the maxilla is resorbed more than that of the mandible [6, 9]. Studies report that this bone loss can reach as much as 50% of the vestibulolingual measurement in the first year after the loss of a tooth [10].

The amount and density of available bone at the edentulous site are primary determinants for predicting the success of rehabilitation with implants [11]. The quality of newly formed bone after an extraction is important to supporting

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chewing forces and the dissipation of these forces in the bone tissue when rehabilitation with implants is performed in the affected region. Studies report that the loss of implants is related to the vascularization, density, and resorption of bone tissue after reconstruction, as the quality of newly formed bone can affect primary stability and the osteointegration process [9]. The primary stability of the implant seems to be mainly influenced by factors related to the bone, such as the density of the spongy portion at the site of osteotomy and the thickness of the cortical plate [11–13].

The bone remodeling process includes the action of several local and systemic factors. Biochemical markers of bone metabolism assist in the evaluation of this process of formation and reabsorption of bone tissue. Among these markers are the alkaline phosphatase (ALP) formation marker and acid phosphatase (AcP) bone resorption marker. ALP is directly related to bone mineralization, as it allows the release of the phosphate group (PO₄) to be used in the formation of hydroxyapatite crystals; ACP make the medium acidic by facilitating the breakdown of hydroxyapatite and releasing the calcium that is trapped in vesicles inside the OC and then these vesicles are released into the blood plasma [14].

Biomaterials have ideal characteristics for use in bone regeneration strategies, as such materials serve as a scaffold for the growth of bone tissue, enabling the proliferation of blood vessels and the delivery of nutrients to cells in the interior of the graft [15]. Studies conducted with the biomaterial Straumann® Cerabone® hydroxyapatite demonstrate a high degree of hydrophilicity, and rigidity [16], less local inflammation compared to other biomaterials and good stability for use in the preservation of the dental alveolus [17]. An increase in the horizontal crest has also been demonstrated with Cerabone® particles followed by the placement of an implant in the anterior region of the maxilla, providing good clinical stability [18].

The use of photobiomodulation with low-power lasers or LED has proven to be effective. Studies have demonstrated an increase in the formation of bone and other tissues, with the enhancement of the entire repair process at the surgical site [19, 20]. Recent studies have shown that photobiomodulation accelerates the repair process and promotes newly formed bone of good quality. Moreover, it is likely that the beneficial effects of LED and laser are similar with regards to the mechanism involved, such as the absorption of light by cytochrome C oxidase in the mitochondrial membrane or a cascade of photophysical metabolic effects in the membrane (probably in calcium channels) [21, 22]. Despite the successful application of photobiomodulation with LED in different fields, its use in bone repair and in combination with biomaterials needs to be studied further [23, 24].

Photobiomodulation for bone repair has demonstrated effectiveness regarding the modulation of both: the local and

systemic responses, improving ionic and mitochondrial cellular exchange, the mineralization of bone, the formation of nitric oxide, lymphatic circulation, the proliferation of osteoblasts, osteoblast gene expression, and the inhibition of osteoclasts (prevention of bone mineral resorption). Photobiomodulation substantially reduces fracture consolidation time and improves both the quality and quantity of newly formed bone [25]. Bone tissue absorbs the irradiation emitted by the light, causing biochemical, bioelectrical, and bioenergy effects. The consequences of this include the stimulation of microcirculation and cellular tropism as well as therapeutic properties, such as analgesic, anti-inflammatory, anti-edema effects, and the stimulation of the tropism of tissues. Several studies in the literature have demonstrated positive effects on the bone repair process [26], such as accelerated healing [27], the stimulation of bone formation, the formation of fibrovascular tissue, and angiogenesis [28].

Despite the beneficial and promising effects of photobiomodulation with LED on the acceleration and improvement in the quality of newly formed bone demonstrated in studies published in the literature [29–31], further studies are needed to establish a safe protocol for its use in bone repair and regeneration in the alveolus following extraction and the placement of bone graft with biomaterial. Therefore, the aim of the present study was to evaluate the impact of photobiomodulation on bone quality of the dental alveolus of Wistar rats following extraction with and without the biomaterial Straumann® Cerabone® hydroxyapatite.

The great similarity and homology between the genomes of rodents and humans make these animal models a major tool to study conditions affecting humans, which can be simulated in rats [32].

Methods

Ethical considerations

This study received approval from the Animal Research Ethics Committee of *Universidade Nove de Julho* (certificate number: 4123300818).

Experimental procedure

Forty-eight male *Wistar* rats (± 300 g) were randomly distributed among five experimental groups ($n = 12$). The basal group received no intervention. In the other groups, extraction of the mandibular's first molar was performed on the first day. The biomaterial and biomaterial + LED groups received the Straumann® Cerabone® biomaterial at the edentulous site immediately after extraction. The LED and biomaterial + LED groups received treatments with LED on alternating days (every 48 h) for a period of 15 days (Fig. 1).

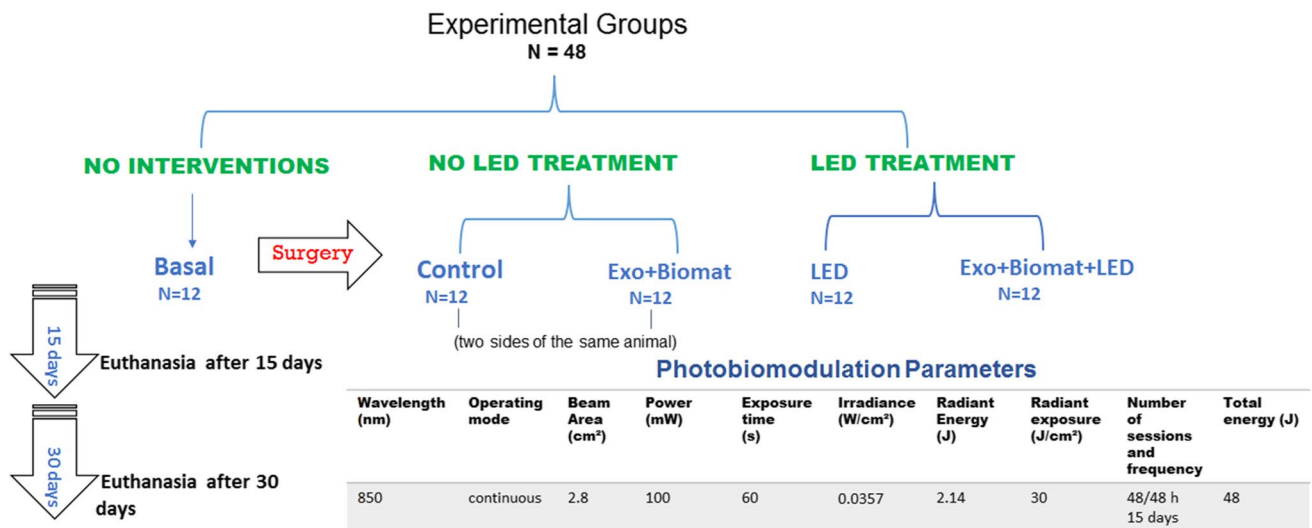


Fig. 1 Study groups, flowchart, and dosimetry parameters

Table 1 Dosimetry parameters

Dosimetry parameters	
Wavelength (nm)	850
Operating mode	Continuous
Power (mW)	100
Beam area (cm ²)	2.8
Exposure time (s)	60
Irradiance (mW/cm ²)	35.7
Radiant exposure (J/cm ²)	2.14
Radiant energy (J)	6
Number of irradiant points	Single point
Application location	Extra oral buccal face
Application techniques	Contact
Number of sessions and frequency	8 sessions—48/48 h 15 days
Total energy (J)	48

Euthanasia was performed on Days 15 and 30. Figure 1 shows the flowchart of the study. It is important to mention that the basal and control group were two sides of the same animal. The complete radiometric parameters for the irradiated groups are listed in Table 1. Blood and the mandible were collected for the biochemical, morphological, and structural analyses.

Experimental extraction

After being anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg) through intraperitoneal injection, the mandible was supported on a flat support and the assistant moved the tongue either to the right or left with gauze. Gingival

syndesmotomy was performed with a modified syndesmotome (worn with a silicon carbide disk and sandpaper) adjusted to the size of the operating region. The extraction was executed by performing luxation of the first molar with a modified Hollenback instrument and periosteal elevator (worn with a silicon carbide disk and sandpaper). After extraction, residual roots were removed with number 5 pediatric forceps.

Biomaterial Straumann

After extraction, the biomaterial groups received Straumann® Cerabone® (0.5–1.0 mm, 1 × 0.5 cc; origin: spongy bovine bone; composition: calcium phosphate [100% pure hydroxyapatite, mineral phase]; porosity: 65–80%, mean pore size: 600–900 μm) applied to the alveolus immediately after extraction with an appropriate curette a pressed slightly into the alveolus with a pediatric spatula. The suture was performed in a simple pattern with 6–0 nylon thread.

Postoperative pain was controlled with tramadol (15 mg/kg, subcutaneously every 8 h for 3 days) and dipyron (50 mg/kg, subcutaneously every 8 h for 3 days).

Statistical analysis

The Shapiro–Wilk test was used to determine the distribution of the data. Two-way analysis of variance (ANOVA) was used for data with approximately normal distribution, whereas Kruskal–Wallis ANOVA was used for data with non-normal distribution. An adequate post-hoc test was used for multiple comparisons of groups for which statistically significant differences were found. The level of significance was set at $\alpha = 0.05$.

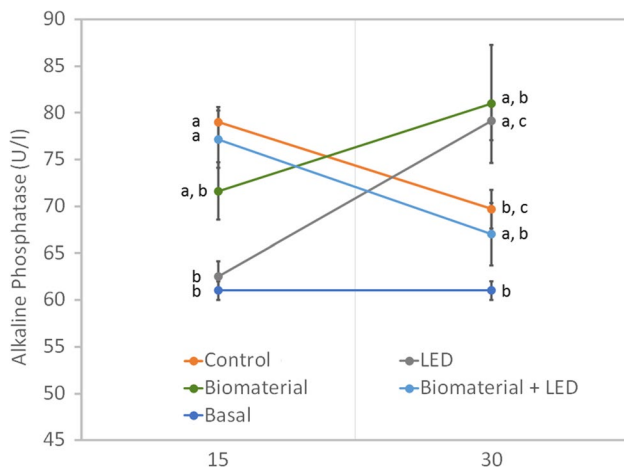


Fig. 2 Mean alkaline phosphatase in experimental groups. Bars represent standard error. Different letters denote statistical difference ($p < 0.05$) and equal letters correspond to similar statistical results ($p > 0.05$)

Results

Analysis of acid and (AcP) alkaline (ALP) phosphatase

Figure 2 shows the mean alkaline phosphatase (ALP) levels in the groups studied. Two-way ANOVA revealed a correlation between the proposed treatment and evaluation time ($p = 0.0002$), demonstrating that the treatments had different impacts on ALP. At 15 days, the control and biomaterial + LED groups differed significantly from the LED group ($p = 0.0086$ and 0.0379 , respectively, Bonferroni) and basal group ($p = 0.0001$ and 0.0179 , respectively, Bonferroni). At 30 days, the basal group differed significantly from the LED group ($p = 0.0007$, Bonferroni). In the analysis of the two evaluation periods within groups (15 and 30 days), statistically significant differences were found in the control and LED groups ($p = 0.0291$ and 0.0030 , respectively, Bonferroni).

Figure 3 shows the mean acid phosphatase (AcP) in the different experimental groups. Two-way ANOVA revealed an interaction between the proposed treatment and evaluation time ($p = 0.0488$), demonstrating that the treatments had different impacts on AcP. Statistically significant differences were found among the study groups ($p = 0.0271$, two-way ANOVA). At 15 days, the LED and biomaterial groups differed significantly from the basal group ($p = 0.0016$ and 0.0281 , respectively, Bonferroni). At 30 days, the control group differed significantly from the biomaterial group ($p = 0.0175$, Bonferroni) and all groups differed from the basal group ($p < 0.05$ for all, Bonferroni). In the comparison of the evaluation periods

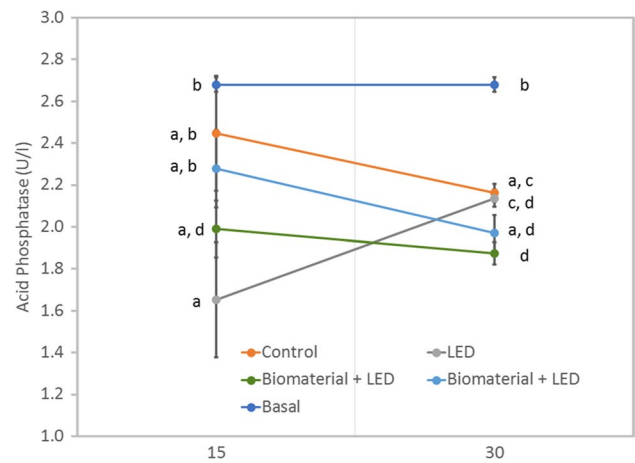


Fig. 3 Mean acid phosphatase in experimental groups. Bars represent standard error. Different letters denote statistical difference ($p < 0.05$) and equal letters correspond to similar statistical results ($p > 0.05$)

(15 and 30 days), a statistically significant difference was found in the LED group ($p = 0.0356$, Bonferroni).

Microtomography (micro CT)

Microtomography was used for the analysis of bone density and volume. Regarding density, two-way ANOVA revealed no statistically significant differences in time or interactions for all groups ($p = 0.535$ and 1.0000 , respectively). However, a significant difference was found among the proposed treatments ($p = 0.0001$), as shown in Fig. 4. At 30 days, the control group differed significantly from the biomaterial and biomaterial + LED groups ($p < 0.05$, t -test), and the LED also differed significantly from the biomaterial and biomaterial + LED groups ($p < 0.05$, t -test). Lack of significant difference among groups was found at 15 days.

Regarding bone volume, two-way ANOVA revealed no statistically significant differences in the interactions for all groups ($p = 0.9753$). However, differences were found among the proposed treatments and between the evaluation times ($p = 0.0001$ and 0.0026 , respectively), as shown in Fig. 5. At 15 days, the control group differed from the basal, biomaterial and biomaterial + LED groups ($p < 0.05$, t -test). At 30 days, the basal group differed significantly from the control and LED groups ($p < 0.05$, t -test); the control group differed significantly from the biomaterial and biomaterial + LED groups ($p < 0.05$, t -test); and the LED group differed significantly from the biomaterial and biomaterial + LED groups ($p < 0.05$, t -test). In the comparison of the evaluation periods (15 and 30 days), a statistically significant difference was found in the biomaterial + LED group ($p = 0.0362$, t -test).

Fig. 4 Mean bone density in experimental groups. Bars represent standard error. Different letters denote statistical difference ($p < 0.05$) and equal letters correspond to similar statistical results ($p > 0.05$)

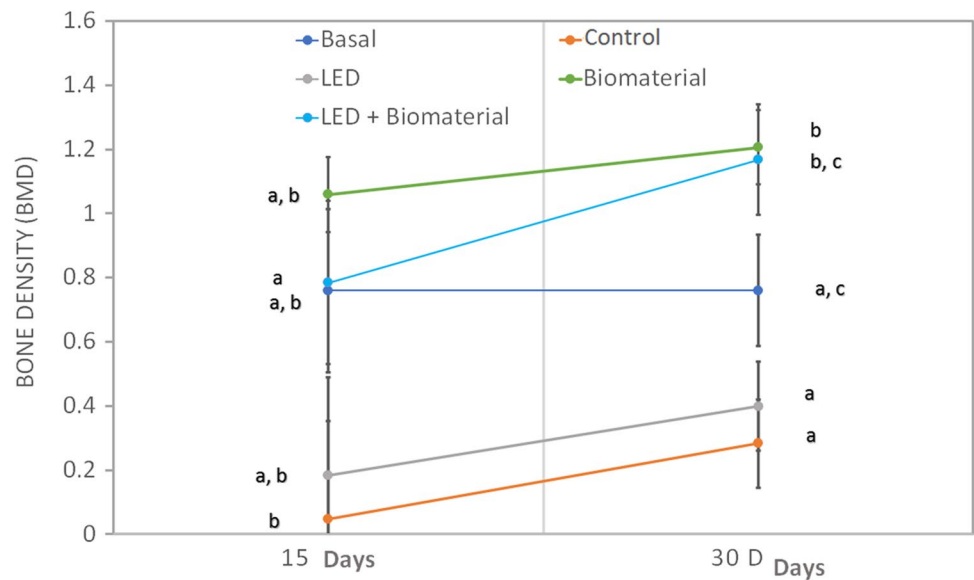
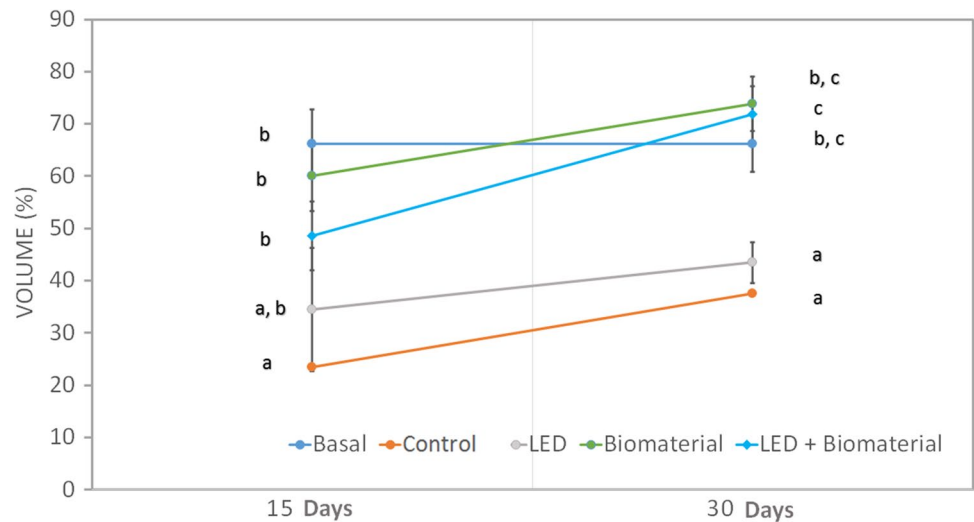


Fig. 5 Mean bone volume in experimental groups. Bars represent standard error. Different letters denote statistical difference ($p < 0.05$) and equal letters correspond to similar statistical results ($p > 0.05$)



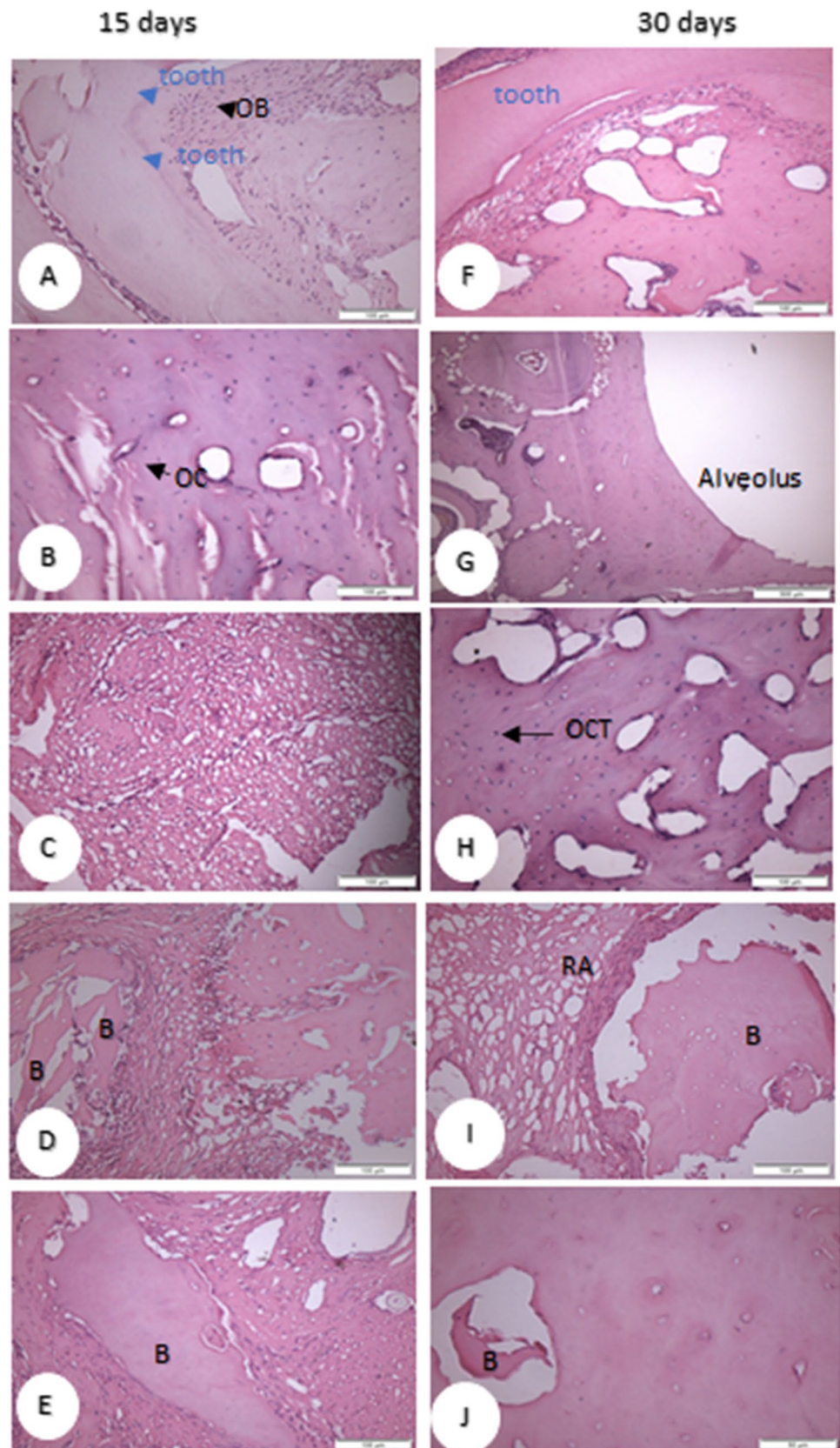
Histopathological analysis

The histopathological analysis revealed considerable structural loss of alveolar bone in the control group after 30 days. In the LED group, intense bone remodeling activity was found, with the presence of several areas of resorption and many osteoblasts at 15 days, whereas mature bones with osteocytes and more organized trabecular bone were found at 30 days. The biomaterial group exhibited quite disorganized regions in the interior of the biomaterial and adjacent bone tissue, with various areas of resorption at both 15 and 30 days. In the biomaterial + LED, the adjacent bone tissue was more organized and mature at both 15 and 30 days, but there were no areas of resorption in the interior of the biomaterial, as shown in Fig. 6.

Discussion

The present study evaluated the effect of LED 850 nm on bone quality in Wistar rats submitted to molar extraction with and without a bone graft using the biomaterial Straumann® Cerabone® hydroxyapatite. The results of the alkaline (ALP) and acid (AcP) phosphatase, microtomographic, and histological analyses agreed, demonstrating that treatment with LED promoted the acceleration of the remodeling and maturation of bone tissue in the alveoli with and without the graft. The use of scaffolds of biomaterial naturally enables greater density and volume in the dental alveolus compared to edentulous sites without the addition of biomaterial. However, the action of LED in alveoli with and without this material led to better tissue organization, more

Fig. 6 Images from histological analysis. A) Aspect of bone with presence of tooth, B) dental alveolus 15 days after extraction, presence of osteoclasts (OC), C) dental alveolus 15 days after extraction and treated with LED, intense remodeling activity with areas of resorption and bone formation, D) area of resorption (AR) around biomaterial (B) and in alveolar bone tissue, E) bone tissue, better organization around biomaterial (B), fewer areas of resorption (AR) in alveolar bone tissue, F) aspect of bone with presence of tooth at 30 days, basal trabecular bone present, G) mature bone tissue with presence of several osteocytes (OCT) and area of alveolus (AL) completely resorbed, H) mature bone with presence of osteocytes and trabecular bone, I) biomaterial (B) with areas of resorption (AR) in interior and in alveolar tissue still present at 30 days, J) mature, well-organized alveolar bone ties with biomaterial (B) in process of resorption



mature bone tissue, and less resorption, as demonstrated by the histological analysis and the evaluation of ALP and AcP. Moreover, the maintenance and indication of growth in both bone volume and density at 30 days demonstrated the prolonged effect of the LED after the 15 days of treatment.

Soares et al. (2015) and Rosa et al. (2014) conducted studies using LED and combined with grafts using biomaterials, reporting an improvement in the healing of bone defects because of the increase in the deposition of hydroxyapatite [29, 30]. Similar effects were found in the present study.

An increase in bone formation demonstrated by the analysis of ALP was found at 15 days in the control and biomaterial + LED groups and the LED was similar to the basal group, in which the tooth was not extracted and there was similar homeostasis of the tissue regarding bone resorption and formation. At 30 days, a significant increase in ALP occurred in the LED group compared to the other groups. This shows that LED enabled the formation of bone formation and repair even after treatment had ceased. In the biomaterial + LED group, bone formation was found at 30 days similar to that in the basal group, demonstrating that LED promoted the acceleration of bone healing and maturation in this group. The action of ALP diminished in the control group at 30 days compared to 15 days, whereas the formation of bone increased significantly in the LED group at 30 days compared to 15 days. Although LED did not significantly improve bone formation in comparison to the other groups, it had a positive effect on bone remodeling and maturation.

These results are compatible with the data described in the literature. Pagim et al. (2014) [33] compared the effects of LED and laser on the proliferation and differentiation of MC3T3 preosteoblasts. The cells were irradiated with laser (red and infrared) and red LED (3 and 5 J/cm²). The lasers had irradiance of 1 W/cm² and an irradiation time of 2 and 5 s. The LEDs had irradiance of 60 mW/cm² and an irradiation time of 50 and 83. The authors found no increase in ALP with either laser or LED.

Li and Leu (2010) [34] investigated the effects of LED with irradiation in the red region ($\lambda = 630$ nm) and irradiance of 5 and 15 mW/cm² (radiant exposure of 2 and 4 J/cm²) on the proliferation and osteogenic differentiation of mesenchymal stem cells (rat MSCs). The authors found an improvement in osteoblasts evaluated based on ALP, which differs from the results of the present investigation.

Another study with data compatible with the present findings was conducted by Pinheiro et al. (2017) [31], who evaluated biochemical changes induced by laser ($\lambda = 780$ nm; 70 mW) and LED ($\lambda = 850 \pm 10$ nm; 150 mW) during the mineralization of a bone defect in an animal model. The authors found that photobiomodulation with laser improved the repair of the bone defects grafted with biomaterial due to the increase in the deposition of phosphate (hydroxyapatite).

However, LED was not as effective at promoting bone maturation as laser in the biomaterial groups. This was likely related to less collagen formation induced by light irradiation, which occurs prior to the resorption and conversion of the biomaterial in the phosphate bone.

In the analysis of AcP, the LED and biomaterial groups differed from the basal group at 15 days, demonstrating less bone resorption in these groups in relation to the condition of homeostasis of the tissue that occurs in the presence of the tooth. At 30 days, the control group differed from the biomaterial group, with less resorption in the latter group, and all groups differed from the basal group, exhibiting lower osteoclastic activity. Based on these findings, we cannot associate the use of LED using the parameters employed in the present study with the reduction in tissue resorption, as similar AcP action was found in the groups with and without LED and all groups had lower AcP in comparison to the basal group, demonstrating low osteoclastic activity in the bone repair process. This suggests that ALP is more active at this time in the remodeling process than resorptive action.

Other studies also report low AcP activity. Zambuzzi et al. (2005) [35] found that tartrate-resistant acid phosphatase (TRAP) was extremely low. The authors correlated the profile of acid phosphatase activity with histological aspects of reactional tissues in response to the subcutaneous installment of graft biomaterial prepared with demineralized bovine bone matrix and concluded that the measurement of phosphatase activity is a useful tool for understanding phenomena involved in the cellular response to bone material implants in connective tissue. The authors state that the specific activities evaluated in their study reflect cellular variations in the different phases of tissue repair. The fact that the resorption of the material occurred in the presence of low TRAP activity suggests that other enzymes, such as matrix metalloproteinases, are more important to this process. The bovine bone matrix studied did not exhibit osteoinduction potential, as reported in the literature, possibly due to the incomplete demineralization of the material during its production, leading to the preservation of inhibitory factors, such as calcium salts and some antigen radicals.

Acid phosphatases are hydrolases involved in different processes, such as bone resorption (TRAP), control of the cell cycle (Tyr-P), and intracellular digestion (FAL)2. Alkaline phosphatase (ALP) is clearly associated with osteogenesis and is an important marker of this process. However, due to the complexity of cell responses to different stimuli, it is often difficult to determine the precise function of a certain enzyme in different physiopathological processes. Moreover, it has recently been demonstrated that the profile of these enzymes varies during the repair process and as a function of the type of material implanted [36].

The action of LED on bone density and volume was evaluated using microtomography in the present study. A

significant improvement in bone density was found in the biomaterial + LED and biomaterial groups in comparison to the control and LED groups at 30 days, whereas no significant differences among groups were found at 15 days, indicating the progressive action of LED in the biomaterial + LED group over time, even after the cessation of the applications.

Similar results were found with regards to bone volume, with an increase in volume in the biomaterial, biomaterial + LED, and basal groups in comparison to the control group at 15 days; at 30 days, the basal group had a greater volume than the control and LED groups, in which the tooth had been extracted and no biomaterial was applied. Tooth loss results in a significant loss of bone volume, as demonstrated in numerous studies, and the presence of biomaterial (with or without LED) maintains bone volume and density in the alveolar process. No difference in volume was found in the comparison of the groups that received the biomaterial with and without LED treatment (biomaterial and biomaterial + LED groups). Moreover, bone volume improved significantly between 15 and 30 days in the biomaterial + LED group, indicating progressive action even after the cessation of the applications.

The clinical analysis of the slides confirmed that tooth loss leads to substantial atrophy of alveolar bone, as seen in the control and LED groups. However, this atrophy was even more evident in the control group at 30 days.

Treatment with LED (LED and biomaterial + LED groups) led to improvements in bone maturation and tissue organization, with smaller areas of alveolar bone resorption. Thus, photobiomodulation with LED was beneficial to the bone tissue. Similar findings are described

by Pinheiro (2011) [37] in a study with 90 Wistar rats divided into 10 groups. The author used grafts with different biomaterials treated or not with LED and submitted it to histological analysis, concluding that the use of LED either alone or combined with biomaterial caused less inflammation.

Studies to verify bone remodeling with biomaterials in rats had shown, over a period of 21 and 30 days, a certain delay in the healing process in the presence of xenografts [38]. At this time, bone remodeling is more active than bone neoformation, and both inorganic bovine bone and bioactive glass particles grafted into the extraction cavity of rat incisors, although biocompatible and capable of osseointegration, delayed the formation of new bone [39]. Thus, the study carried out evidence the improvement in bone maturation and neoformation in the graft model in alveoli of Wistar rats associated with photobiomodulation.

Reports confirming the effects of photobiomodulation on bone repair report benefits regarding osteoblast viability, collagen deposition, and the formation of new bone, especially with infrared irradiation, as can be seen in Table 2. Moreover, the total dose is not the only aspect responsible for the stimulating action; the exposure time and mode of irradiation are also important [40]. Indeed, the parameters and properties of photobiomodulation devices are directly responsible for the effects on cells and tissues. Thus, the selection of these attributes must be appropriate [41, 42]. The literature reports that it is difficult to compare studies investigating the effects of low-level laser therapy on bone tissue due to the variety of dosimetric parameters, experimental models, and duration of treatment. Moreover, the lack of a specific therapeutic window for dosimetry in the

Table 2 Studies with photobiomodulation and bone tissue. ↑ means favorable to photobiomodulation; ↓ means favorable to placebo; ⊕ means lack of significant difference among groups

Authors	Experimental model	Type of light	Wave-length Laser	Wave-length LED	Power Laser	Power LED	Radiant exposure Laser	Radiant exposure LED	Results Laser	Results LED
Soares (2015)	Rats	LED	850 nm	-	150 mW	-	20 J/cm ²	-	-	↑
Rosa (2014)	Rats	Laser	780 nm	-	70 mW	-	16 J/cm ²	-	↑	-
Pagim (2014)	cells	Laser/LED	660 and 780 nm	630 nm	1W	60 mW	3/5 J/cm ²	3/5 J/cm ²	⊕	⊕
Li, Leu (2010)	Rats	LED	630 nm	-	5/15 mW	-	2/4 J/cm ²	-	-	↑
Pinheiro (2017)	Rats	Laser/LED	780 nm	850 nm	70 mW	150 mW	16 J/cm ²	16 J/cm ²	↑	↓
Pinheiro (2012)	Rats	LED	850 nm	-	150 mW	-	16 J/cm ²	-	-	↑

treatment of bone consolidation makes the clinical use of laser therapy quite limited [43].

Although the mechanisms of photobiomodulation have not been clarified and there is no consensus on the best protocol for the use of LED in bone regeneration involving biomaterials, the results of the present study did not demonstrate a substantial improvement in bone quality in comparison to the use of the biomaterial alone. Nonetheless, the findings suggest the positive influence of LED on both tissue organization after injury and bone maturation over time. Thus, further studies are needed to clarify its action and use for improving the bone quality of biomaterial in grafts for the preservation of alveolar bone and local bone architecture.

Final considerations

The results of the groups submitted to LED therapy revealed better tissue organization and the acceleration of the maturation of alveolar bone, demonstrating the positive impact of this light source on bone tissues. Further studies are needed with different dosimetric parameters to enable the establishment of a safe protocol for the improvement in bone formation with or without the use of biomaterials, especially when translating our results from mice to human experiments. Although the radiometric parameters used here were well within the range of those used in human trials, the accelerated metabolism of mice may play an important role, meaning, for a human trial, instead of two weeks of irradiation, a longer period is advised.

LED $\lambda = 850$ nm combined with the biomaterial Straumann hydroxyapatite led to an improvement in bone formation, as demonstrated by the histological analysis, as well as diminished bone degradation due to the reduction in AcP, thereby promoting an increase in bone density and volume. In conclusion, LED may be an important therapy to combine with biomaterials to promote bone formation, along with the other known benefits of this therapy, such as the control of pain and the inflammatory process.

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Declarations

Ethical approval This study received approval from the Animal Research Ethics Committee of *Universidade Nove de Julho* (certificate number: 4123300818).

Conflict of interest The authors declare no competing interests.

References

- Petersen PE (2003) (2003) The World Oral Health Report 2003: continuous improvement of oral health in the 21st century—the approach of the WHO Global Oral Health Programme. *Commun Dent Oral Epidemiol* 31(Suppl 1):3–24
- De Marchi RJ, Hugo FN, Hilgert JB, Padilha DMP (2008) Association between oral health status and nutritional status in south Brazilian independent-living older people. *Nutrition* 24:546–553
- Gerritsen AE, Allen PF, Witter DJ, Bronkhorst EM, Creugers NHJ (2010) Tooth loss and oral health-related quality of life: a systematic review and meta-analysis. *Health Qual Life Outcomes* 8:126–136
- Barone A, Aldini NN, Fini M, Giardino R, Guirado JLC, Covani U (2008) Xenograft versus Extractional one for ridge preservation after tooth removal: a clinical and histomorphometric study. *J Periodontol* 79(8):1370–77
- Araújo M, Lindhe J (2009) Ridge preservation with the use of Bio-Oss collagen: a 6 months study in the dog. *Clin Oral Impl Res* 20:433–440
- Araújo MG, Sukekava F, Wenntiom JL LJ (2005) Ridge alterations following implant placement in fresh extraction sockets: an experimental study in the dog. *J Clin periodontol* 32:645–652
- Pietrovsy J, Massler M (1967) Alveolar ridge resorption following tooth extraction. *J Pros Dent* 17(1):21–27
- Ye F, Lu X, Wang J, Shi Y, Zhang L, Chen J, Li Y, Bu H (2007) A long-term evaluation of osteo inductive HA/ β -Tcp ceramics in vivo: 4, 5 year study in pigs. *J. Mater Sci Mater Med* 18:2173–2178
- Javed F, Romanos GE (2010) The role of primary stability for successful immediate load in goof dental implants. A literature review. *J Dent* 38:612–620
- Araújo MG, Liljenberg B, Lindhe J (2010) Dynamics of Bio-Oss Collagen in corporation in fresh extraction sounds: one experimental study in the dog. *Clin Oral Implants Res* 21(1):55–64
- Linkon LI, Chercheve R (1970) Theories and techniques of implantology, vol 1. Mosby, St Louis
- Saito S, Shimizu N (1997) Stimulatory effects on flow-power laser irradiation on bone regeneration in mid palatal suture during expansion in the rat. *Am J Orthod Dentofac Orthop* 111:525–532
- Lekholm U, Zarb GA (1985) Patient selection and preparation. In: Brånemark P-I, Zarb GA, Albrektsson T (eds) *Tissue Integrated Prostheses: Osseointegration in Clinical Dentistry*. Quintessence Publ Co, Chicago, pp 199–209
- Vieira JGH (2007) Laboratory diagnosis and follow-up in osteometabolic diseases. *J Bras Patol Med Lab* 43(2):75–82
- Davies N, Dobner S, Bezuidenhout D, Schmidt C, Beck M, Zisch AH, Zilla P (2008) The dosage dependence of VEGF stimulation on scaffold neovascularisation. *Biomaterials*
- Trajkovski B, Jaunich M, Müller W-D, Beuer F, Zafropoulos G-G, Housmand A (2018) hydrophilicity, viscoelastic, and physicochemical properties variations in dental bone grafting substitutes. *Materiais* 11:215
- Sargolzaie N, Rafiee M, Sedigh HS, Mahmoudabadi RZ, Keshavarz H (2018) Comparison of the effect of hemihydrate calcium sulfate granules and Cerabone on dental socket preservation: an animal experimente. *J Dent Res Dent Clin Dent Prospect* 12(4):238–244
- Kamadajaja DB, Mira Sumarta NP, Hindawi RA (2019) Stability of tissue augmented with deproteinized bovine bone mineral particles associated with implant placement in anterior maxilla. *Case Reports in Dentistry Article ID 5431752*, 5 pages
- Ns A et al (2009) Effect of soft laser and bioactive glass on bone regeneration in the treatment of infra-bony defects (a clinical study). *Lasers Med Sci* 24:387–395

20. Freitas NR, Guerrini LB, Esper LA, Sbrana MC, Dalben GS, Soares S, Almeida ALPF (2018) Evaluation of photobiomodulation therapy associate with guided bone regeneration in critical size defects. In vivo study. *J Appl Oral Sci* 26
21. Karu T (1989) Photobiology of low power laser effects. *Health Phys* 56(5):691–704
22. Smith R (1993) Bone physiology and the osteoporotic process. *Respir Med* 87(A):3–7
23. Nitzan Y, Gutterman M, Malik Z, Ehrenberg B (1992) Inactivation of Gram-negative bacteria by photosensitised porphyrins. *Photochem Photobiol* 55(1):89–96
24. Nitzan Y, Pechatnikov I (2011) Approaches to kill gram-negative bacteria by photosensitized processes. In: Hamblin MR, Jori G (eds) *Photodynamic inactivation of microbial pathogens: medical and environmental applications*. RSC Publishing, Reino Unido, pp 45–67
25. Islam MN, Zakaria GA, Sirazee FH (2017) Effects of low level laser (Diode- 830 nm) therapy on-human bone regeneration. 3^oInternational Conference on Advanced Clinical Research and Clinical Trials. *J Clin Res Bioeth*
26. Romão MMA, Marques MM, Cortes ARG, Horliana ACRT, Moreira MS, Lascala CA (2015) Micro-computed tomography and histomorphometric analysis of human alveolar bone repair induced by laser phototherapy: a pilot study. *Clinical Paper Oral Surgery* 44:1521–1528
27. Cardoso MV, do Vale Placa R, Sant'Ana ACP et al (2020) Laser and LED photobiomodulation effects in osteogenic or regular medium on rat calvaria osteoblasts obtained by newly forming bone technique. *Lasers Med Sci*. <https://doi.org/10.1007/s10103-020-03056-5>
28. Bossini OS, Rennó ACM, Ribeiro DA, Fangel R, Ribeiro AC, de Assis Lahoz M, Parizotto NA (2012) Low level laser therapy (830 nm) improves bone repair in osteoporotic rats: similar out comes at two different dosages. *Exp Gerontol* 47:136–142
29. Soares LGP, Marques AMC, Guarda MG, Acirole JMS, Pinheiro ALB, Santos JN (2015) Repair of surgical bone defects grafted with hydroxylapatite + β -TCP and irradiated with $\lambda=850$ nm LED light. *Braz Dent J* 26(1):19–25
30. Rosa CB, Castro VIC, Reis Júnior JA et al (2014) The efficacy of the use of IR laser phototherapy (LPT) on bone defect grafted with biphasic ceramic on rats with iron deficiency anemia: Raman spectroscopy analysis. *Lasers Med Sci* 29(3):1251–1259
31. Pinheiro ALB et al (2017) Biomechanical changes of the repair of surgical bone defects grafted with biophasic synthetic micro-granular $H\ddot{A}+\beta$ tricalcium phosphate induced by laser and LED phototerapias and assessed by Raman spectroscopy. *Lasers Med Sci* 32:663–672
32. Diemen VV, Trindade EM, Trindade MRM (2006) Experimental model to induce obesity in rats. *Acta Cir Bras* 21(6):425–429
33. Pagin MT, Oliveira FA, Oliveira RC (2014) Laser and light-emitting diode effects on pre-osteoblast growth and differentiation. *Lasers Med Sci* 29:55–59
34. Li WT, Leu YC, Wu JL (2010) Red-light light-emitting diode irradiation increases the proliferation and osteogenic differentiation of rat bone marrow mesenchymal stem cells. *Photomed Laser Surg* 28:157–165
35. Zambuzzi WF, Neves MC, Oliveira RC, Silva TL, Cestari TM, Busalaf MA, Granjeiro JM, Taga R, Taga EM (2005) Tissue reaction and phosphatases profile after implant of xenogenic demineralized bone matrix in rat muscle. *Cienc Odontol Bras* 8(2):90–98
36. Lutolf MP, Weber FE, Schmoekel HG, Schense JC, Kohler T, Muller R et al (2010) Repair of bone defects using synthetic mimetics of collagenous extracellular matrices. *Nat Biotechnol* 21(5):513–8
37. Pinheiro ALB, Soares LG, Barbosa FB, Ramalho LM, dos Santos JN (2012) Does LED phototherapy influence the repair of bone defects grafted with MTA, bone morphogenetic proteins, and guided bone regeneration? A description of the repair process on rodents. *Lasers Med Sci* 27:1013–1024
38. Luca RE, Giuliani A, Mănescu A, Heredea R, Hoinoiu B, Constantin GD, Duma VF, Todea CD (2020) Osteogenic potential of bovine bone graft in combination with laser photobiomodulation: an ex vivo demonstrative study in Wistar rats by cross-linked studies based on synchrotron microtomography and histology. *Int J Mol Sci* 21(3):778. <https://doi.org/10.3390/ijms21030778>
39. Calixto RFE, Teófilo JM, Brentegani LG, Lamasano-Carvalho TL (2007) Grafting of tooth extraction socket with inorganic bovine bone or bioactive glass particles: comparative histometric study in rats. *Implant Dentistry* 16(3):260–269
40. Pinheiro ALB, Gerbi ME (2006) Photoengineering of bone repair process. *Photomed and Laser Surgery* 4:169–178
41. Arany PR (2016) Craniofacial wound healing with photobiomodulation therapy new insights and current challenges. *J Dent Res* 95(9):977–984
42. de Freitas LF, Hamblin MR (2016) Proposed mechanisms of photobiomodulation or low-level light therapy. *IEEE Sel Top Quantum Electron* 22(3):7000417
43. Bossini PS, Rennó AC, Ribeiro DA, Fangel R, Peitl O, Zanotto ED, Parizotto NA (2011) (2010) Biosilicate® and low-level laser therapy improve bone repair in osteoporotic rats. *J Tissue Eng Regen Med* 5(3):229–237. <https://doi.org/10.1002/term.309>

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