

Low-level laser therapy for the regeneration of mandibular bone defects in rabbits

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Abstract

Objective: This study aimed to assess the effect of low-level laser therapy (LLLT) on the regeneration of mandibular bone defects in rabbits.

Methods: This animal study was conducted on 14 male albino rabbits. Two circular defects 5 mm in diameter were created bilaterally at the angles of the mandible. One side was randomly selected to undergo laser therapy at a wavelength of 905 nm and a power of 20 mW for 2 minutes daily, starting immediately after surgery. The other side served as the sham-irradiated control. Half of the rabbits were sacrificed on day 8, and the other half on day 15. The mandibles of sacrificed animals were resected and subjected to histopathological analysis to measure the percentage of mineralized bone, osteoid matrix and fibrous tissue as well as the number of blood vessels (as the indicator of angiogenesis), fibroblasts, and inflammatory cells. Data were analyzed by paired t-test and independent samples t-test at the significance level of $P < 0.05$.

Results: No statistically significant differences were observed between the laser-treated and control sides in any of the measured histopathological parameters at either the 8-day or 15-day time points ($P > 0.05$). On the laser side, a significant increase was observed in the mean percentage of mineralized bone ($P = 0.022$) and osteoid matrix ($P = 0.002$) from day 8 to day 15. The control side revealed no significant changes in the evaluated parameters over time ($P > 0.05$).

Conclusions: Low-level laser irradiation may accelerate the regeneration of mandibular bone defects in rabbits.

Keywords: Biostimulation, Bone defect, Bone regeneration, Laser therapy, Low-level laser, Osteogenesis

Introduction

The regeneration of bone defects caused by trauma, pathological conditions, or surgical procedures is a challenge in contemporary dentistry. The slow healing of bone defects often leads to clinical burdens for patients, including a higher risk of infection, chronic pain, and discomfort, as well as economic burdens due to increased medical costs (1-3). Thus, accelerating the healing and regeneration of bone defects is an interesting topic of research.

Several strategies have been proposed for accelerating the healing of bone defects, such as the application of allografts, autografts, growth factors, and polymer membranes. However, these techniques have high technical sensitivity and complexity, may cause complications such as pain and infection, and provide unpredictable results. Therefore, researchers seek to find alternatives with less complexity and fewer complications for enhancing regeneration in bone defects (4, 5).

The biological effects of lasers were first demonstrated in 1967 (6), leading to the introduction of laser therapy into clinical practice by 1971 (7). The beneficial effects of low-level laser therapy (LLLT) in bone regeneration include increased blood flow, activation of osteoblasts, decreased activity of osteoclasts, and anti-inflammatory effects. Several studies demonstrated that LLLT affects the proliferation and differentiation of osteoblasts and chondrocytes and accelerates the healing of bone defects (8, 9). Other studies have reported the acceleration of fracture healing, increased formation of calluses, and greater bone mineralization as a result of LLLT (10, 11). The upregulation of osteogenic markers has also been reported following LLLT (12).

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The therapeutic effects of low-power lasers can vary depending on the applied laser parameters (13-15). Evidence suggests that LLLT with an energy density between 1 and 10 J/cm² can promote the healing of soft and hard tissues (16, 17). Furthermore, extending the irradiation time up to 5 minutes has been shown to enhance bone regeneration and accelerate early-stage healing of extraction sockets (18).

The optimal efficacy of LLLT in accelerating wound healing, reducing inflammation, and alleviating pain has been well documented (10, 19-21). However, limited research has investigated the effectiveness of LLLT in regenerating jaw bone defects. Therefore, this study aimed to assess the effect of LLLT on the regeneration of mandibular bone defects in rabbits.

Materials and methods

Study design and sample size calculation

The experimental procedures and study protocol were approved by the ethics committee of Rafsanjan University of Medical Sciences (Approval No: IR.RUMS.REC.1396.20). The study was performed according to the principles of the Basel Declaration.

The sample size was determined based on data from a previous study (22) investigating the effects of low-level laser therapy (LLLT) on bone regeneration in an animal model. Assuming a significance level of $\alpha = 0.05$, and a statistical power of 80% ($1 - \beta = 0.80$), a minimum of 7 rabbits per group was required to detect a statistically significant difference in bone healing between groups. Sample size calculation was performed using G*Power software (version 3.1, Heinrich Heine University, Düsseldorf, Germany).

Experimental Design

A total of 14 rabbits were randomly divided into two

- 15-day group

Each group was further divided into two subgroups:

- Laser group (LLLT): One side of each rabbit received low-level laser therapy.
- Control group: Another side of each rabbit received sham irradiation with the laser device turned off.

Surgical procedure

The rabbits were kept in separate cages with ad libitum access to food and water under standard conditions with 12 h light/12 h dark cycles. Additionally, they were examined by a veterinarian to ensure their general health. The rabbits were housed in the university's animal facility for one week to allow for acclimation.

General anesthesia was induced via intramuscular injection of 5% ketamine hydrochloride (100 mg/kg; Rotexmedica, Trittau, Germany) and 100 mg/kg xylazine (Rotexmedica, Trittau, Germany). Additionally, local anesthesia was achieved by the injection of 2% lidocaine plus 1/100,000 epinephrine (Daroupakhsh, Tehran, Iran). The surgical site was shaved and aseptically draped to maintain a sterile field.

A semilunar flap approximately 2 cm in length was elevated through the skin and deep fascia at the mandibular angle on both sides. Following flap elevation and exposure of the surgical site, a 5 mm diameter bone defect was created at the bisector of the mandibular angle, 1 cm above the inferior border of the mandible, using a round carbide bur and a low-speed handpiece (Teezkavan, Tehran, Iran) under continuous saline irrigation. A standardized defect was created to involve both the medial and lateral cortical plates (Figure 1a). Subsequently, the muscles, fascia, and skin were repositioned. Deep tissues were sutured using

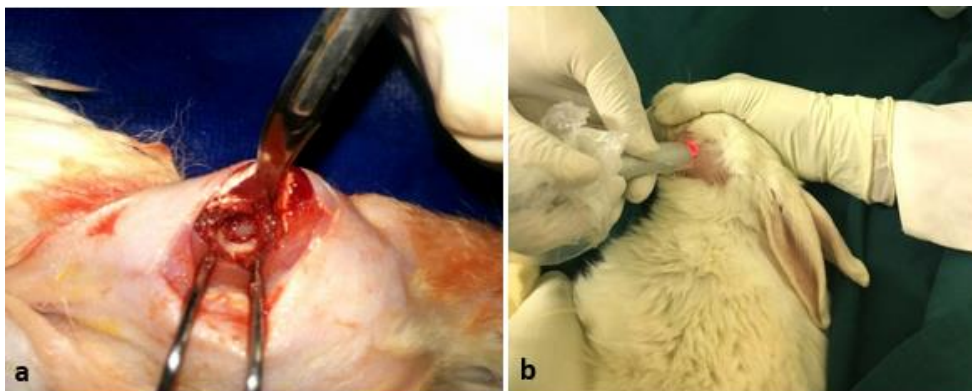


Figure 1. Surgical intervention and laser irradiation in bone defects: a) Creation of bone defects at the angle of the mandible. b) Laser irradiation of the surgical site on the test side

groups based on the sacrifice interval (n=7):

- 8-day group

resorbable sutures, while the skin was closed with non-resorbable nylon sutures.

The rabbits were placed back in their cages and kept under standard conditions. Cefazolin (100 mg/kg; Bristol Meyer Squibb, Sermoneta, Italy) was injected intramuscularly before surgery, immediately after surgery, and 24 hours later (23).

Grouping and intervention

The animals were randomly assigned to two groups (n=7 per group) based on the time of sacrifice.

The 8-day group: This group received daily laser irradiation for 7 consecutive days and was sacrificed on day 8.

The 15-day group: The animals in this group received daily laser irradiation for 14 consecutive days and were sacrificed on day 15.

In each animal, one side was randomly selected as the test (laser) side by flipping a coin. A diode laser (LAMBDA SPA, Sermoneta, Italy) irradiated the surgical site on the test side immediately after surgery. A technician held the rabbits still during irradiation. Low-level laser therapy (LLLT) was applied in continuous wave (CW) mode with a wavelength of 905 nm, output power of 20 mW, for 2 minutes per day, giving an energy density of 4.8 J/cm² (8 mm probe diameter). The laser handpiece was held perpendicular to the surface and in direct contact with the tissue (Figure 1.b). The contralateral side served as the sham-irradiated control, in which the laser device was applied in the same manner, but remained turned off (10). Both the control and experimental groups were maintained under identical environmental conditions and received the same handling and care.

After each laser irradiation session, the rabbits were returned to their cages.

Euthanasia and sample preparation

At the designated time points, all rabbits were deeply anesthetized with ketamine. Euthanasia was performed using the vital perfusion technique to ensure rapid tissue fixation. A vertical incision was made in the neck to expose the common carotid arteries (CCAs), which were isolated and clamped. Saline containing 10% formalin was then perfused through the arteries. Signs of successful perfusion included reduced heart and respiratory rates, pallor of the mucosa, and muscle stiffness.

Following perfusion, the mandibles, including the surrounding medial soft tissues at the mandibular angles, were surgically dissected (10, 24). The samples

were immediately immersed in 4% buffered paraformaldehyde (Mojallali, Tehran, Iran) for 48 hours. They were then decalcified in 4% ethylenediaminetetraacetic acid (EDTA) solution (Merck, Darmstadt, Germany), which was refreshed twice weekly. After two months, bone softening was confirmed with a surgical scalpel (25).

Subsequently, the specimens were processed using a tissue processor (Sakura Fine Technical, Tokyo, Japan), including dehydration in graded ethanol, clearing, and paraffin embedding. Paraffin blocks were sectioned into 5-µm slices using a microtome (SLEE Med, MAINZ, Germany). The sections were stained with hematoxylin and eosin for histomorphometric evaluation. Two samples were processed per rabbit: one from the laser-treated side and one from the sham-irradiated control side.

Histomorphometric assessment

Histomorphometric evaluation was conducted by a pathologist blinded to the experimental groups. Tissue sections were examined under a light microscope (Carl Zeiss GmbH, Jena, Germany) at 400× magnification. For each section, five randomly selected high-power fields (HPFs) within the central region of the defect were evaluated. Photomicrographs were captured, and the measurements were averaged to obtain mean values per parameter for each sample.

The following parameters were assessed in the photomicrographs:

Mineralized bone (%): The proportion of newly formed mineralized bone within the defect area was estimated using light microscopy by comparing stained (eosinophilic) mineralized regions to the total defect area. The percentage was visually calculated.

Osteoid matrix (%): The non-mineralized bone matrix (osteoid) was identified as lightly stained areas adjacent to mineralized bone. It was quantified similarly to mineralized bone and expressed as a percentage of the total defect area.

Fibrous tissue (%): Fibrous connective tissue occupying the defect site was evaluated by identifying collagenous, unstained, or lightly stained regions lacking bone or osteoid features, and quantified as a percentage of the total area.

Number of blood vessels (as an indicator of angiogenesis): Blood vessels were counted manually in each HPF based on their circular or oval shape, presence of an endothelial lining, and lumen. The mean number per field was calculated across the five fields.

Fibroblasts (cells/field): Fibroblasts were identified by their elongated nuclei and spindle-shaped morphology. The number of fibroblasts was counted per field and averaged.

Inflammatory cells (cells/field): Mononuclear inflammatory cells (e.g., lymphocytes, macrophages) were counted based on their round nuclei and darker staining, and their mean number per field was calculated.

Statistical analysis

Statistical analysis was performed using SPSS version 21 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro–Wilk test ($P>0.05$). A paired t-test was used to compare histomorphometric parameters between the laser-treated and sham-irradiated control sides within each group (8-day and 15-day). An independent samples t-test was used to compare values between the 8-day and 15-day groups for both the laser-treated and control sides. A p -value <0.05 was considered statistically significant.

Results

Table 1 presents the mean and standard deviation (SD) of the histopathological parameters in the laser-treated and control groups at both the 8-day and 15-day time points.

No statistically significant differences were observed between the laser-treated and control sides in any of the measured histopathological parameters at either the 8-day or 15-day time points ($P>0.05$; Table 1).

The mean percentage of mineralized bone in the laser-treated group significantly increased from $10.71 \pm 5.34\%$ on day 8 to $17.14 \pm 4.88\%$ on day 15 ($P=0.022$). The control group showed an increase from $9.29 \pm 3.45\%$ to $13.57 \pm 4.75\%$, which was not statistically significant ($P=0.111$).

Similarly, the percentage of osteoid matrix in the laser group increased significantly from $16.43 \pm 10.69\%$ on day 8 to $33.57 \pm 1.35\%$ on day 15 ($P=0.002$). The control group also showed an increase, from $23.57 \pm 7.48\%$ to $33.57 \pm 13.75\%$, but this change was not statistically significant ($P=0.128$). No significant changes were observed in the remaining variables between the 8 and 15 days in the laser or control groups ($P>0.05$; Table 1).

Discussion

This study evaluated the effect of low-level laser therapy (LLLT) on the regeneration of mandibular bone defects in rabbits. The findings revealed no significant differences between the laser-treated and control sides at either time point in any histopathological parameters. In the laser-treated group, both mineralized bone and osteoid matrix percentages significantly increased from day 8 to day 15, indicating progressive bone formation.

Table 1. Mean and standard deviation (SD) of histopathological variables in laser and control sides on days 8 and 15

Variables	Side	8 -day group Mean $\pm$ SD	15-day group Mean $\pm$ SD	P-value
Mineralized bone (%)	Laser	10.71 ± 5.34	17.14 ± 4.88	0.022*
	Control	9.29 ± 3.45	13.57 ± 4.75	0.111
	P-value	0.563	0.191	
Osteoid matrix (%)	Laser	16.43 ± 10.69	33.57 ± 1.35	0.002*
	Control	23.57 ± 7.48	33.57 ± 13.75	0.128
	P-value	0.173	0.998	
Fibroblasts (Cell umber)	Laser	67.14 ± 13.49	64.29 ± 16.18	0.738
	Control	60.00 ± 9.57	63.57 ± 13.75	0.652
	P-value	0.276	0.931	
Fibrous tissue (%)	Laser	65.71 ± 11.34	66.43 ± 18.86	0.927
	Control	61.43 ± 14.92	56.29 ± 19.02	0.591
	P-value	0.556	0.149	
Angiogenesis (Number of blood vessels)	Laser	4.43 ± 0.78	4.71 ± 1.38	0.689
	Control	5.14 ± 2.03	4.43 ± 1.51	0.466
	P-value	0.403	0.718	
Inflammatory cells (Cell number)	Laser	32.86 ± 13.49	37.14 ± 14.96	0.607
	Control	40.00 ± 9.57	36.43 ± 13.75	0.607
	P-value	0.276	0.927	

* P-values less than 0.05 were considered statistically significant.

The control group showed similar trends, but the changes were lower and not statistically significant. The within-group improvement in the laser group implies that LLLT may accelerate the early phases of bone regeneration by promoting mineralization and matrix deposition over time.

The observed increase in mineralized bone and osteoid matrix in the laser-treated group is consistent with previous studies demonstrating the osteogenic potential of LLLT. Laser irradiation has been shown to stimulate osteoblast activity and enhance extracellular matrix production, contributing to bone regeneration (9, 26). Bai et al. (27), observed enhanced bone formation following LLLT in a rat calvarial defect model. Similarly, Khadra et al. (28) indicated increased bone density and improved histomorphometric parameters in laser-treated rabbit bone defects, corroborating the stimulatory effect of LLLT on bone regeneration.

In the present study, no significant differences were observed in either laser-treated or control sides in the percentage of fibrous tissue, number of blood vessels, fibroblasts, or inflammatory cells over time. These parameters are commonly used to assess tissue remodeling, angiogenesis, and inflammatory response during healing. Therefore, under the conditions of this study, low-level laser therapy did not significantly influence the fibrotic response, early angiogenic activity, or inflammatory phase, associated with bone regeneration.

The laser-treated and control sides revealed no significant difference in any of the histopathological parameters on either 8 or 15 days. Although the laser group experienced a significantly higher increase in mineralized and osteoid tissue over time, the difference between groups failed to achieve statistical significance. In contrast to the outcomes of this study, Ribeiro et al (29) observed a significant increase in osteoblast and osteoclast activity and the area percentage of cancellous bone in the lased alveolus of rats post-tooth extraction compared to the control group.

The lack of a significant difference between the laser and control groups may be related to the selected laser parameters and the treatment protocol applied. It is believed that optimizing factors such as wavelength, power output, energy density, and duration of application is essential to achieving consistent and clinically meaningful results (30).

Fibrous tissue forms part of the granulation tissue that fills the defect. It provides a temporary scaffold for cell migration, including fibroblasts, inflammatory cells, and eventually osteoprogenitor cells and it also provides a

matrix for angiogenesis. In the early stages (first few days), the presence of moderate fibrous tissue is normal and necessary, but in later healing stages (e.g., day 15 in this study), the presence of high amounts of fibrous tissue may indicate an incomplete transition to bone or osteoid, which is representative for a weaker or slower regenerative response.

The anti-inflammatory effect of LLLT has been documented in previous studies through modulation of cytokine profiles and reduction of oxidative stress (31). Maintaining a balanced inflammatory environment is critical for optimal bone healing, as excessive inflammation can delay tissue repair (31, 32). However, the anti-inflammatory effect of LLLT was not observed in this study possibly due to the low number of animals or selected laser parameters.

Several studies demonstrated that LLLT can enhance local blood flow, thereby improving the delivery of nutrients, mineral salts, and oxygen to bone defect sites (33). Kobu (34) reported an 80% increase in blood supply and a 15% rise in bone oxygenation following LLLT. However, this increased blood flow may result from systemic factors rather than angiogenesis and the associated increase in blood vessel number. Ozcelik et al. (35) reported that LLLT increased angiogenesis and wound healing. Maiya et al. (36) found that LLLT accelerated the onset of angiogenesis during wound healing in diabetic patients compared to a control group. Although angiogenesis did not show significant changes in the laser side, vascular responses may have occurred earlier or later than the time points examined in this study. The selected laser parameters may also be ineffective in stimulating neovascularization. The differences in the experimental model (bone defects in rabbits versus diabetic wounds in humans in the study of Maiya et al (36) may also account for the differences observed in the results of these studies.

The outcomes of this study contrast with several reports that support the beneficial effects of LLLT on bone healing. Song et al. (37) investigated the effects of 905 nm Ga-Al-As laser irradiation (500 mW, 51.7 J/cm²) applied immediately after extraction and continued daily for 7 days on hard tissue healing in extraction sockets of rats. They found that laser treatment significantly promoted hard tissue healing in the maxillary first molar sockets. Ueda and Shimizu (38) investigated the effects of Ga-Al-As laser irradiation on rat calvaria and reported a significant increase in alkaline phosphatase activity 12–15 days after treatment, indicating enhanced bone regeneration. Similarly, Rando et al. (39), in a review study, concluded that LLLT

positively influences alveolar bone healing by reducing pain and inflammation, promoting angiogenesis, and accelerating new bone formation. These effects contribute to the preservation or even increase of alveolar ridge height and/or thickness.

This study was conducted in rabbits; therefore, the results should not be directly generalized to humans. Additional limitations include the small sample size in each group and the short-term evaluation period. Future studies with larger sample sizes and longer follow-up periods are recommended to better assess the effects of various laser wavelengths and exposure parameters on wound healing and bone regeneration. Moreover, histochemical analysis of serum markers such as calcium, phosphorus, and alkaline phosphatase levels is suggested to further evaluate the efficacy of LLLT in bone healing.

Conclusions

- There were no significant differences between the laser-treated and control sides at either time point in any histopathological parameters.
- Low-level laser therapy caused a significant increase in both mineralized bone and osteoid matrix percentages from day 8 to day 15.
- The control group revealed no significant changes in any of the histopathological variables over time.
- LLLT may accelerate the early phases of bone regeneration by promoting mineralization and matrix deposition over time

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Not applicable.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Ethical consideration

The experimental procedures and study protocol were approved by the ethics committee of Rafsanjan University of Medical Sciences (Approval No: IR.RUMS.REC.1396.20). The study was performed according to the principles of the Basel Declaration.

Author contributions

A.P. Contributed to the management and supervision of research; M.I. contributed to the design and conceptualization of the study, data collection, analysis, and manuscript preparation; S.K. assisted with the care of laboratory animals, laser irradiation, surgeries, and data collection; M.A. conducted the animal surgeries; M.K. Performed the histopathological analysis and data analysis. All authors read and approved the final manuscript.

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