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## Efficacy of 810 nm Diode Low-Level Laser Treatment on Healing Periodontal Intra-Bony Defects: A Blinded Animal Investigation

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### Abstract

**Background:** Low-power laser treatment demonstrated a bio-stimulatory positive impact during the initial phases of osseous regeneration.

**Purpose:** The study's primary objective is to assess the histological impact of low-power laser therapy (810 nm) on healing intentionally generated intra-bony periodontal defects.

**Methods:** The investigation was evaluated by carrying out 16 identified defects involving eight adult male rabbits (totaling 8 individuals) utilized in this study; categorized into 2 groups using the split-mouth technique. The control group defects were not subjected to any treatment modality, whereas the test group ones were subjected to laser irradiation. Four animals (8 defects) were euthanized after 15 days, and the other four (8 defects) at 30 days post-surgery, followed by histological analyses. Defects were bilaterally produced in each animal, manifesting as bony cavities of 10 mm in occluso-apical length and 4 mm in bucco-lingual length between the first and second molars, utilizing a tapered drill (of fissure type) attached to a high-rpm motor. Following the randomized group allocation, the right-side defects were designated the control group, while the left ones were subjected to laser irradiation.

**Results:** Irradiated defects exhibited diminished inflammatory response, advanced periodontal fiber regeneration and conjunction, and developed bone growth after 15 and 30 days compared to the untreated control group.

**Conclusions:** Low-power laser therapy diminishes the inflammatory response, enhances periodontal fiber regeneration and alignment, and promotes bone maturation and healing.

**Keywords**— low power laser therapy, bio-stimulation, periodontal intra-bony defects, periodontal regeneration, rabbits, histological study

### I. INTRODUCTION

Dysbiotic plaque biofilms are associated with chronic periodontitis, a multifactorial inflammatory disorder characterised by a progressive deterioration of tooth-supporting tissues. [1] Specific pathogenic microorganisms induce progressive degradation of the alveolar bone, periodontal ligament (PDL), or clinical attachment loss (CAL), triggering recession, pocket formation, or both [2, 3]. Periodontal disease is a multifaceted condition marked by root cementum exposure, bone resorption, and degeneration of periodontal tissue resulting from detached connective tissue fibers from the tooth [4]. Periodontal therapy aims to eradicate the inflammatory process, microbiological causes, and risk factors that may exacerbate disease progression and restore damaged periodontal structures.

Periodontal regeneration is considered a complicated, multifactorial process that entails a coordinated scenario of cell activities, including adhesion, migration, proliferation,

and differentiation [5]. Periodontal regenerative techniques such as root bio-modifications, bone substitutes, soft tissue grafts, guided regeneration of tissues (GTR), biomaterials, and physical methods like Low-Power laser therapy (LPLT) or even their combinations are essential for restoring periodontal health and enhancing tooth longevity. These innovative approaches not only promote healing but also improve the overall aesthetics and function of the periodontal structure [6-13].

At present, regenerative periodontal therapies may restore just a part of the diseased tissue, but their capacity for complete periodontal restoration is constrained [14].

Multiple approaches were assessed, yet no singular methodology has been acknowledged as the gold standard for restoring intra-bony defects (IBDs). Periodontal therapy aims to achieve two primary objectives: 1) to avert the onset of different types of periodontal diseases by mitigation of inflammation and infection, and 2) to maintain or enhance the aesthetics, comfort, functionality, and health of the

supporting structures, including the gingival tissues, periodontal ligaments, cementum, and alveolar bone [10].

After Maiman invented the first practical laser system in 1960, two varieties of lasers were used in dentistry and later in periodontology: high-power lasers (HPL) and low-power lasers (LPL). Following Maiman's invention of the first practical laser system in 1960, two categories of lasers were initially employed in dentistry and subsequently in periodontology: high-level lasers (HLL), which are used for surgical procedures, and Low-Power lasers (LPL), which are used for bio-stimulation and tissue repair [15-17]. Due to its ability to enhance collagen synthesis, cellular proliferation, and localized vascular circulation, LPL has lately been used as a bio-stimulant for tissue rehabilitation [11-13]. HLL markedly enhanced biochemical, bioelectric, and bio-energetic functions at the cellular level, leading to elevated metabolism, increased mitotic division of epithelium, proliferation and maturation of fibroblasts, collagen synthesis, augmented granulation tissue, reduced inflammatory mediators, and stimulation of local microcirculation to facilitate healing [16-18].

Particular studies on novel bone formation suggest that the bio-stimulatory effects of lasers arise from a combination of their distinctive characteristics and the creation of specific local circumstances that enhance new osseous tissue development and resolve edema [6, 19].

Before conducting the study, the hypotheses of this work should be clearly defined; 1) Null hypothesis: The treated defects will exhibit no inflammatory changes compared to the non-treated defects after 15 days post-surgery, and after 30 days, there will be no regeneration of periodontal fibers or bone fill relative to the non-treated defects, or 2) Alternative hypothesis: The treated defects will exhibit low inflammation relative to the non-treated defects after 15 days post-surgery, and after 30 days, periodontal fibers and bone fill will regenerate in comparison to the non-treated defects.

This investigation is intended to histologically assess the impact of low-power laser therapy LPLT (810 nm) on the repair of intentionally generated bio-stimulatory periodontal defects.

## II. MATERIALS AND INSTRUMENTS

Given the scarcity of histological studies on IBD healing, especially in rabbits, and the predominance of research focused on bone defects in the femur or calvaria, it is crucial to recognize that the methodology employed in this study, which can be adapted for various experimental contexts, could potentially contribute significantly to a more comprehensive understanding of periodontal healing, thereby enlightening the field of periodontology.

Preparation of animals:

Upon receiving authorization from the Experimental Animal Research Ethics Committee (Cu/I/F/1/19), eight mature New Zealand male white rabbits, aged 7-8 months and averaging over 2.5 kg, were utilized in this investigation to examine sixteen intra-bony defects. Before the procedures, each rabbit was isolated to acclimate to the laboratory environment. They received a distinctive pelleted commercial feed. To ensure the animals' comfort and safety, each rabbit was carefully prepared and received a distinctive

pelleted commercial feed. To attain sedation and general profound anesthesia, subcutaneous injections of Ketamine Hydrochloride (50mg/kg) and Xylazine Hydrochloride (20mg/kg) were administered.

### -Experimental groups and induction of periodontal defects

Sixteen periodontal defects were generated in the region of interest (ROI), with eight in each group and two in the same rabbit, one on each side. The control group (C group): Rabbits received no materials and laser therapy was not administered for defect treatment. Test laser group (TL group): All defects were utterly exposed to laser radiation. Defects in the control group were allocated to the right side of the rabbits, whereas those in the test group were allocated to the left side. Rabbits were assigned to groups at random. Following the thorough shaving of the surgical area, 70% ethanol was employed for disinfection in anticipation of the surgical procedure. And after LA (Artinibsa 40 mg/ 0.01 mg / mL with Epinephrine 1:100,000) was injected at the ROI for hemostasis, a five-centimeter rostrocaudal full-thickness incision was performed through the skin, encompassing the underlying muscles, to reveal the Region of Interest (ROI), the interdental space between the rabbit's first and second mandibular molars.

After retraction of the flap corona-apically, one osseous-wall cavity was established by revealing the distal and mesial surfaces of the respective roots of the first and second molars using a stopper marked-premeasured tapered FG cutting bur attached to a high-rpm motor with copious physiological NaCl irrigation [20, 21]. The cavity was measured at 4 mm in depth (bucco-lingual path) from the buccal end of the alveolus to the lingual surface of the bony cavity and 10 mm in the corona-apical direction from the CEJ to the most apical base of the defect. The exposed roots were thoroughly cleaned using a Gracey curette G5/6 to eradicate the Sharpey's fibers from the cementum surface (Fig.1).



Figure 1. Defect induction

Per blinding regulations, a custom-built laser apparatus was utilized (A custom-made laser device, calibrated with the Melles Griot power meter Model (30 W Broadband)) was used. It was engineered to incorporate both placebo

and active modes. In the placebo mode, only the red LED indication light was activated, devoid of laser radiation. A diode [GaAlAs] 810 nm laser was utilized in continuous mode at a power of 100 mW for 180 seconds, delivering 18 J through a tip with a radius of 0.35 cm and an area of 0.385 cm<sup>2</sup> before flap closure. The energy density applied is approximately 46.8 J/cm<sup>2</sup>.

For suturing, flap repositioned was assured using a basic interrupted technique, using 3-0 silk for the skin and 3-0 vicryl for the muscles. The wound was left uncovered and exposed to the air. Post-surgery, a laser was administered extra-orally and repeated daily for five consecutive days, adhering to the blinding protocols.

#### -Postoperative management

Thereafter, each rabbit was confined to an individual cage. The environment was regulated on a 12-hour light-dark cycle with a relative humidity of 22%. Water and nutrition were accessible. Diclofenac sodium, an analgesic for postoperative care, was provided daily for three days at a dosage of 10 mg/kg. Ceftriaxone (25 mg/kg) was provided once daily for three consecutive days post-surgery. Over a span of five consecutive days, the laser device was applied to each rabbit twice negatively on the right side and twice positively on the left side, with each application lasting 90 seconds. The device was set to laser active mode on the left and non-laser placebo mode on the right (Error! Reference source not found.).



Figure 1. Laser bio-stimulation

#### -Histologic preparation

Thin histological sections, precisely 5 micrometers in thickness, were carefully prepared in a mesiodistal orientation. These sections were then stained with hematoxylin and eosin (H & E), a method commonly used to highlight the cellular structures [22], allowing for detailed examination under an optical microscope (Olympus xc30. Tokyo. Japan).

#### -Evaluation of the histologic sections

The structure of the periodontal ligament (PDL) was categorized according to Behfarnia et al. [23] as **score 0**: disorganized PDL with irregular fiber orientation, **score 1**: organized PDL, which was characterized by dense

connective tissue and **score 2**: fibers are oriented regularly from the alveolar bone towards the surface of the cementum. Also, The inflammatory response was evaluated using an optical microscope and scored based on the presence of inflammatory cells [23] into: **score 0**: < 10% inflammatory cells, **score 1**: 10-30% inflammatory cells, **score 2**: 30-50% inflammatory cells and **score 3**: > 50% inflammatory cells. In addition, Regenerated Bone was graded according to Lucaciu (2015) [24] as **score 0**: absent, **score 1**: present at the borders of the defect and **score 2**: present deep in the defect.

#### -Randomization and Blinding procedures

Initially, each of the eight animals was randomly assigned to either a 15-day or a 30-day sacrifice period. Subsequently, the side was identified as right or left. The site was subsequently chosen randomly as a control or test group. The periodontist researcher conducted all surgical procedures with a double-anonymized methodology. Initially, a different dentist operated the laser equipment in blind mode after the primary operator (the researcher) set it to active or non-laser placebo mode. Secondly, the technician performing the histological preparation and the histologist evaluating it were ignorant of the defect's group classification.

### III. RESULTS

#### -After 15 days

In control group (C), tissue specimens on day 15 exhibited numerous inflammatory chronic cells, predominantly macrophages and lymphocytes (30-50% inflammatory cells); thus, they were classified as Score 2 (Figure 2). Tissue faults were evident in the periodontal ligament (PDL) and the bordering bone. A score of 0 was assigned in the absence of PDL regeneration, connective tissue adhesion, or organized fiber orientation. The acellular type of cementum was entirely excised from the root. Regarding bone, none has been regenerated, which deserved a grade 0 (Fig. 3).

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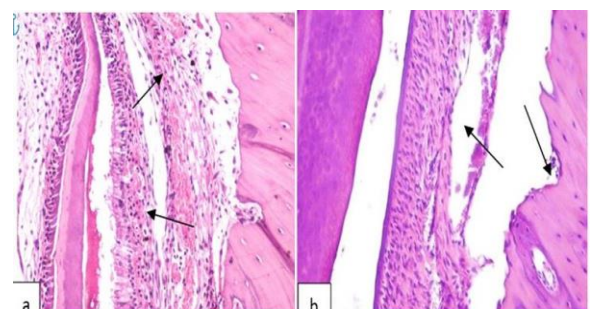
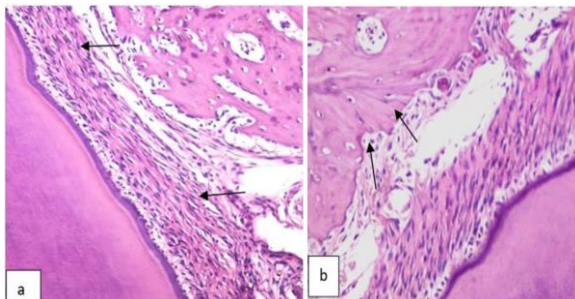


Figure 2. (C) Untreated defect on the 15th day  
Photomicrograph of intra-bony defect showing **arrow (a)**: chronic inflammatory cells mainly lymphocytes and macrophages and **arrow (b)**: tissues defect in both periodontal ligament PDL and adhesive bone (H & E) (200X).

In the test laser group (TL), tissue specimens exhibited a minor inflammatory response characterized by infiltrations of a limited number of mononuclear cells (10-30%



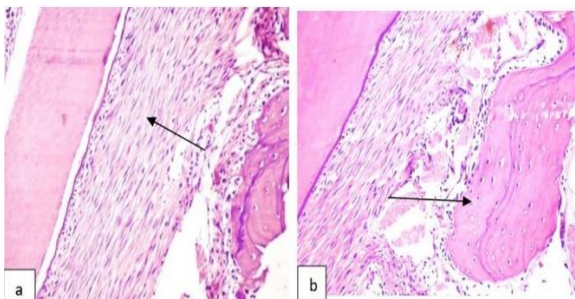
inflammatory cells), receiving a score of 1 (Fig. 4). The PDL zone exhibited structured fiber bundles oriented perpendicularly to the root surface. Nonetheless, fibers adjacent to the bone surface exhibited a haphazard organization, which was indicated by the score 1. Remodeling of old bone's surface was observed in bone resorption regions. No sign of new bone growth was observed. The bone received a rating of 0 (Fig. 4).



**Figure 3.** (TL) Defects treated by Laser on the 15<sup>th</sup> day  
Photomicrograph of intra-bony defect showing **arrow (a)**: mild inflammatory reaction appeared as few mononuclear cells infiltration and connective tissue adhesion with perpendicular fiber orientation supracrestal and **arrow (b)**: resorption of old bone (H & E) (200X).

**-After 30 days**

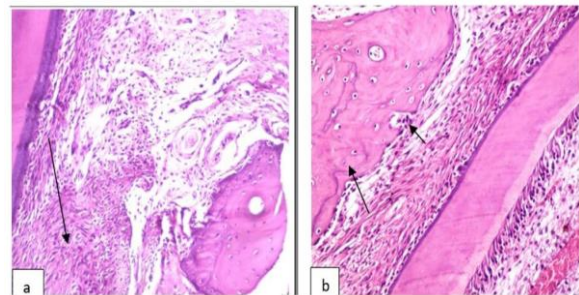
In the control Group (C), the specimens exhibited minimal inflammatory chronic cell infiltrates, predominantly macrophages and lymphocytes (10-30% inflammatory cells), designated as score 1 (Fig. 4). Organized PDL collagen fibers were observed, confined to the apex region, accompanied by connective tissue adhesion exhibiting perpendicular orientation of fibers. Consequently, the score is 1. The acellular cementum was inadequately established on the root surface. Osseous tissue regeneration was observed at the defect's boundary, marked by the significance of osteoblastic cell proliferation and a distinct demarcation between the old and new bone. Consequently, it was evaluated as 1 (Figure 4).



**Figure 4.** (C) Untreated defect on the 30<sup>th</sup> day.  
Photomicrograph of intra-bony defect showing **arrow (a)** few chronic inflammatory cells infiltration and PDL regeneration limited to the apical part of the defect **arrow (b)** regeneration of bony tissues at the surface of the defect (H & E) (200X).

In the test laser group (TL), specimens had a moderate inflammatory response, with fewer than 10% of inflammatory cells in the therapy, receiving a score of zero. The regeneration of the periodontal ligament was confined to the apex, exhibiting a perpendicular orientation of the fibers. Furthermore, the periodontal ligament was more

structured and integrated into the newly developed bone. Consequently, a specific score 1 (Fig. 6). New bone development was observed on the defect's surface, characterized by osteoblast proliferation. This received a grade of 1.



**Figure 5.** (T) Defects treated by Laser on the 30th day  
Photomicrograph of intra-bony defect showing **arrow (a)**: mild inflammatory response and PDL regeneration limited to the apical part of the defect and **arrow (b)**: new bone formation seen at the surface of the defect which characterized by osteoblasts proliferation (H & E) (200X).

**Table 1.** A detailed comparison of the histological scores in the study groups on the 15th and 30th days highlights significant insights crucial for understanding the progression of the condition under investigation

Scoring parameter	(C) group		(T) group	
	15th	30th	15th	30th
Inflammatory cells	2	1	1	0
Periodontal ligament	0	1	1	1
Bone	0	1	0	1

**III. DISCUSSION**

A range of potential limitations must be recognized before conclusions are drawn. The lack of prior analogous studies addressing periodontal intra-bony defects in rabbits constituted restricting factors. While the methodology employed in this study is applicable across various contexts, the results pertain solely to experimental animal research and may lack generalizability. The investigation encompassed sixteen defects, and the study has established consistent and coherent results, reinforcing its external validity.

Although the precise mechanisms underlying the stimulation of bone repair remain poorly understood, they are likely multifaceted. They may involve collagen formation, angiogenesis, osteoblast proliferation and differentiation, and ATP synthesis via mitochondrial respiration [11-13].

Furthermore, LPLT may enhance blood circulation at the application site, augmenting the delivery of circulating cells, nutrients, oxygen, and inorganic salts to the bone defect. The primary factors affecting bone defect repair are the ability of osteogenic precursor cells to invade the defect and differentiate into osteoblasts and their presence in the adjacent soft tissue or bone. Furthermore, fibroblast cells capable of secreting collagen and vital for forming periodontal ligament can be induced to proliferate by LPLT.

Results of this investigation demonstrate that laser irradiation has the potential to significantly improve bone repair independently. The current study showed a reduction in the inflammatory response after LPLT compared to the control group at 15 and 30 days relative to baseline. Also, this study showed improved bone healing with LPLT compared to the control group, with differences in healing observed at 15 and 30 days from baseline. This is concurrent with Ghahroudi et al., who concluded that LPLT may facilitate bone growth in skeletal abnormalities [25].

The current study's results indicated enhancements in periodontal fiber orientation and regeneration following LPLT, as opposed to the control group, after 15 and 30 days relative to baseline measurements. Pereira et al. demonstrated that non-surgical periodontal treatment and photo-biomodulation rehabilitation in experimental periodontitis in rats effectively repaired the periodontal ligament, reduced bone loss, and modulated inflammatory processes [26]. This was consistent with our findings. The results aligned also with Gerbi's conclusions, indicating that laser treatment exerts a synergistic bio-modulating impact on the healing process of a defect, whether accompanied or not by the application of organic lyophilized bone and bovine lyophilized membrane on the rat femur [27, 28]. Similarly, the findings align with Huang's study, demonstrating that LPLT alone enhanced mitochondrial activity, RNA / DNA synthesis in osteoblasts, cell survival, and alkaline phosphatase levels [19]. Furthermore, these findings are consistent with Aboelsaad's work, which demonstrated that LPLT exerted a beneficial local bio-stimulatory impact during the initial phases of bone repair [29, 30]. Moreover, in consistence with our findings, Lopes et al. observed significantly enhanced bone repair in laser-irradiated lesions in rabbit tibiae [31].

#### **-Limitations**

To our knowledge, no prior studies have been conducted on IBD in rabbits or rats. The typical healing capability of bone in rabbits is approximately two weeks, which is regarded as quick; thus, comparing routine healing with Low-Power laser bio-stimulated healing of intra-bony lesions proved hard. The histologic assessment in this study was qualitative and did not include statistical analysis, which may represent a limitation.

#### **IV. CONCLUSION**

Low-power laser therapy may promote the healing of osseous tissues, diminish the inflammatory response, and improve periodontal fiber orientation and tissue regeneration in periodontal bone defect healing. LPLT is considered a non-invasive and safe method for bone augmentation in treating inflammatory bone defects (IBDs). Low-Power Laser Therapy (LPLT) is advantageous for periodontal regeneration in managing Intra-bony defects (IBD). It is recommended that radiographic assessment, in conjunction with histological analysis, be utilized to validate the efficacy of LPLT in improving IBD.

#### **Statements & Declarations**

The authors confirm that no financial support was received to prepare this manuscript.

#### **REFERENCES**

1. P. N. Papapanou et al., "Periodontitis: Consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions," *J. Clin. Periodontol.*, vol. 45, no. December 2017, pp. S162–S170, 2018.
2. J. G. Caton et al., "A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification," *J. Clin. Periodontol.*, vol. 45, no. March, pp. S1–S8, 2018.
3. I. Needleman et al., "Mean annual attachment, bone level, and tooth loss: A systematic review," *J. Clin. Periodontol.*, vol. 45, no. May 2017, pp. S112–S129, 2018.
4. F. J. Hughes, *Periodontium and Periodontal Disease*. Elsevier Inc., 2015.
5. H. N. Woo, Y. J. Cho, S. Tarafder, and C. H. Lee, "The recent advances in scaffolds for integrated periodontal regeneration," *Bioact. Mater.*, vol. 6, no. 10, pp. 3328–3342, 2021.
6. S. Hemaid, A. Saafan, M. Hosny, and G. Wimmer, "Enhancement of Healing of Periodontal Intra-bony Defects Using 810 nm Diode Laser and Different Advanced Treatment Modalities : A Blind Experimental Study," 2019.
7. P. Chandran and A. Sivadas, "Platelet-rich fibrin : Its role in periodontal regeneration," *Saudi J. Dent. Res.*, vol. 5, no. 2, pp. 117–122, 2014.
8. R. J. Miron and Y. F. Zhang, "Osteoinduction: A review of old concepts with new standards," *J. Dent. Res.*, vol. 91, no. 8, pp. 736–744, 2012.
9. F.-M. Chen and Y. Jin, "Periodontal tissue engineering and regeneration: current approaches and expanding opportunities.," *Tissue Eng. Part B. Rev.*, 2010.
10. M. Sialli, D. Chatzopoulou, and D. G. Gillam, "An overview of periodontal regenerative procedures for the general dental practitioner," *Saudi Dent. J.*, vol. 30, no. 1, pp. 26–37, 2018.
11. S. Elavarasu, D. Naveen, and A. Thangavelu, "Lasers in periodontics," *J. Pharm. Bioallied Sci.*, vol. 4, no. 6, p. 260, 2012.
12. K. C. Smith, "Laser (and LED) Therapy Is Phototherapy," *Photomed. Laser Surg.*, vol. 23, no. 1, pp. 78–80, Feb. 2005.
13. Y. A. Vladimirov, A. N. Osipov, and G. I. Klebanov, "Photobiological Principles of Therapeutic Applications of Laser Radiation," *Biochem.*, vol. 69, no. 1, pp. 81–90, 2004.
14. C. A. Ramseier, G. Rasperini, S. Batia, and W. V. Giannobile, "Advanced reconstructive technologies for periodontal tissue repair," *Periodontol.* 2000, vol. 59, no. 1, pp. 185–202, 2012.
15. E. J. Choi et al., "Biological effects of a semiconductor diode laser on human periodontal ligament fibroblasts," *J. Periodontal Implant Sci.*, 2010.
16. L. J. Walsh, "The current status of low level laser therapy in dentistry . Part 1 . Soft tissue applications," no. 4, 1997.

17. L. J. Walsh, "The current status of low level laser therapy in dentistry . Part 2 . Hard tissue applications," no. 5, 1997.
18. P. Anna Mathew, V. More, and J. P. B.S, "Lasers – It's Role in Periodontal Regeneration," *Heal. Informatics - An Int. J.*, vol. 7, no. 2/3/4, pp. 01–07, 2018.
19. Y.-Y. Huang, S. K. Sharma, J. Carroll, and M. R. Hamblin, "Biphasic Dose Response in Low Level Light Therapy – an Update," *Dose-Response*, vol. 9, no. 4, p. dose-response.1, 2011.
20. X. Struillou, H. Boutigny, A. Soueidan, and P. Layrolle, "Experimental Animal Models in Periodontology: A Review," *Open Dent. J.*, vol. 4, no. 1, pp. 37–47, 2010.
21. C.-S. Kim et al., "Periodontal Repair in Surgically Created Intra-bony Defects in Dogs: Influence of the Number of Bone Walls on Healing Response," *J. Periodontol.*, vol. 75, no. 2, pp. 229–235, 2005.
22. S. K. Suvarna, C. Layton, and J. D. Bancroft, *Bancroft's Theory and Practice of Histological Techniques Content Strategist: Michael Houston Content Development Specialists*, vol. 7. 2013.
23. P. Behfarnia, M. Khorasani, R. Birang, and F. Abbas, "Histological and histomorphometric analysis of animal experimental dehiscence defect treated with three bio absorbable GTR collagen membrane," *Dent. Res. J. (Isfahan)*, vol. 9, no. 5, p. 574, 2012.
24. O. Lucaciu, D. Gheban, O. Sorițau, M. Băciuț, R. S. Câmpian, and G. Băciuț, "Comparative assessment of bone regeneration by histometry and a histological scoring system," *Rev. Rom. Med. Lab.*, vol. 23, no. 1, pp. 31–46, 2015.
25. A. A. Rasouli Ghahroudi et al., "Effect of low-level laser therapy irradiation and Bio-Oss graft material on the osteogenesis process in rabbit calvarium defects: A double blind experimental study," *Lasers Med. Sci.*, 2014.
26. S. R. A. Pereira, I. C. V. de Oliveira, R. C. Vieira, M. M. L. Silva, L. S. Branco-de-Almeida, and V. P. Rodrigues, "Effect of photobiomodulation therapy as an adjunct to scaling and root planing in a rat model of ligature-induced periodontitis: a histological and radiographic study," *Lasers Med. Sci.*, vol. 35, no. 4, pp. 991–998, 2020.
27. M. A. Liebert et al., "Assessment of Bone Repair Associated with the Use of Organic Bovine Bone and Membrane Irradiated at 830 nm," vol. 23, no. 4, pp. 382–388, 2005.
28. M. E. M. Gerbi et al., "Assessment of Bone Repair Associated with the Use of Organic Bovine Bone and Membrane Irradiated at 830 nm," *Photomed. Laser Surg.*, 2005.
29. N. S. Aboelsaad et al., "Effect of soft laser and bioactive glass on bone regeneration in the treatment of infra-bony defects (a clinical study)," *Lasers Med. Sci.*, vol. 24, no. 3, pp. 387–395, 2009.
30. N. S. Aboelsaad et al., "Effect of soft laser and bioactive glass on bone regeneration in the treatment of bone defects (an experimental study)," *Lasers Med. Sci.*, vol. 24, no. 4, pp. 527–533, 2009.
31. C. B. Lopes, M. T. T. Pacheco, L. Silveira, J. Duarte, M. C. T. Cangussú, and A. L. B. Pinheiro, "The effect of the association of NIR laser therapy BMPs, and guided bone regeneration on tibial fractures treated with wire osteosynthesis: Raman spectroscopy study," *J. Photochem. Photobiol. B Biol.*, vol. 89, no. 2–3, pp. 125–130, 2007.