

ORIGINAL ARTICLE

Effects of Near-Infrared Laser Biostimulation on Bone Cells Number and Activity during Orthodontic Therapy: An Animal Study

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Abstract

Purpose: Laser photobiostimulation has recently gained recognition as a non-invasive and effective technique for accelerating orthodontic tooth movement and enhancing bone healing. This article evaluates the effects of laser biostimulation at an energy density of 15.9 Joules/cm² on the amount of orthodontic movement and its impact at the histological level.

Materials and Methods: Thirty adult male albino rabbits were randomly chosen to form two groups (n=15 per group): a Control (C) and a Laser Treatment (LT) group. The LT group received laser treatment for three weeks at 976 ± 10 nm and an energy density of 15.9 Joules/cm². Laser irradiation was applied to four specific spots on the lower incisors for 80 seconds, administered on days 0, 3, 6, 9, 11, 13, 16, 18, and 20. Five rabbits from each group were euthanized at 7, 14, and 21 days for subsequent analysis.

Results: The amount of orthodontic movement, the extent of osteogenesis, osteoblasts, and osteoclast counts were significantly larger in the laser-exposed group than in the unexposed group. Notably, bone alkaline phosphatase and tartrate-resistant acid phosphatase 5b activity significantly increased, particularly at two weeks relative to the control group.

Conclusion: Laser biostimulation offered evidence of improved parameters of teeth movement, providing insight to enhance the orthodontic therapy outcome.

Keywords: 976 nm Laser Biostimulation; Osteoblast; Osteoclast; Bone Alkaline Phosphatase; Tartrate-Resistant Acid Phosphatase 5b.

1. Introduction

Over the last decades, numerous studies have investigated the efficacy of different interventions in speeding up tooth movement; one of these interventions is laser biostimulation or low-level laser therapy. Laser exposure can produce a photobiostimulatory effect, causing a directed change in cell behaviors and enhancing tissue healing, it can enhance cell proliferation and stem cell differentiation and contributes to decreasing pain and inflammation in parallel with the acceleration of tissue repair [1]. It is well established that the optimal wavelength ranges for biostimulation, commonly referred to as the "optical window," lie between 550 and 950 nm, where laser beam penetration in the tissue reaches its maximum [2, 3]. Another critical factor influencing the therapeutic efficacy of laser treatment is the energy density (fluence). Previous research indicates that lasers with energy densities between 2 and 12 J/cm² effectively promote biostimulation [2-4].

The novelty of this study resides in its examination of the effects of low-level laser biostimulation, utilizing a combination of a (976 nm) wavelength, an energy dose of (8 J), and a fluence of (15.9 J/cm²), applied to four distinct sites—specifically the mesial, distal, and midline, both labially and lingually—over a total duration of 80 seconds. This wavelength and fluence (energy dose) exceed the range typically considered optimal for biostimulation, thereby contributing a unique perspective to the field. Although the cytochrome c oxidase is considered the main chromophore to absorb red and near-infrared lights, the application of higher near-infrared light (980–1064 nm) also activates heat and light-sensitive ion channels, such as members of the Transient Receptor Potential (TRP) family with the photon absorption range of 980–1064 nm [1, 5]. The study utilizes rabbits as the animal model considered more physiologically comparable to humans than rats, the species mainly used in laser biostimulation literature [5]. This model allows for a more accurate investigation of the histological and immunohistochemical aspects of alveolar bone remodeling during orthodontic force application. Specifically, the study examines the effects of laser biostimulation during orthodontic tooth movement by evaluating new bone formation, as well as osteoblast

and osteoclast number and activity, through the measurement of bone formation markers (bone alkaline phosphatase, BALP) and bone resorption markers (tartrate-resistant acid phosphatase 5b, TRAP 5b).

2. Materials and Methods

2.1. Study Settings

This research was performed from July 2023 to May 2024. The experimental phase of this research received approval from the Ethics Committee of the College of Dentistry at Mosul University (approval code: UoM.Dent. 23/35, No. 35 on 4/6/2023). All required measures were taken to minimize animal pain or discomfort during the whole study period. The animal housing and follow-up were done in the College of Veterinary Medicine (University of Mosul, Mosul, Iraq), while experimental parameters and histology were conducted in the College of Dentistry (University of Mosul, Mosul, Iraq). No financial interests existed.

2.2. Animals

The study sample consisted of 30 adult male white rabbits, which were assigned into two groups (15 rabbits per group):

Group A: unexposed (or control) Group (C): This group received an orthodontic appliance that applied a 50-gram distalizing force to the lower incisors.

Group B: Laser treatment group (LT): This group received the same orthodontic appliance used in the control group, in addition to laser exposure.

The orthodontic force was administered for three weeks, and at the end of each week, ten rabbits (5 from each group) were euthanized for further study.

2.3. Orthodontic Appliance

The orthodontic appliance (Dentaurum, Germany) consisted of two stainless steel bands with tubes (2.5 mm wide, 0.022 x 0.030 inches) attached to the lower incisors. The setup included two bands, an open coil spring, an archwire, and a ligature wire pre-assembled outside the rabbit's mouth and inserted as a single unit. A 7.5 mm nickel-titanium open coil spring (light force,

0.010 x 0.030 inches) generated 50 grams of force when compressed into a 3.5 mm space (initially present between the two adjacent tubes). The 3.5 mm spacing was crucial for maintaining a consistent 50-gram force across all samples. This appliance is similar to the appliance used by Al-Fakhry and Al-Sayagh [6] using the same magnitude of orthodontic force.

2.4. Laser Therapy Exposure Procedure

Gallium Aluminum Arsenide (GaAlAs) semiconductor laser (Solase Pro Dental Diode Laser, China) in bio-stimulation continuous mode is used as the source of laser therapy. The laser had a wavelength of $(976 \pm 10 \text{ nm})$, a power of (100 mW), and delivered energy of (8 joules). The total fluence was (15.9 J/cm^2) . The laser beam was delivered using an optical fiberglass rod (8 mm in diameter) held just in contact with the target; the bio-stimulation handpiece provided a red light guide for more accurate invisible laser beam positioning on the target area (Figure 1).

Experimental laser doses were administered at four sites on the lower incisors (midline labially and lingually, right and left distally), with each site receiving 20 seconds of laser irradiation (totaling 80 seconds). The four target areas were irradiated on days 0, 3, 6, 9, 11, 13, 16, 18, and 20. This exposure schedule mirrors the patterns employed by several researchers [7-11] and is similar to the 48-hour interval regimen used by others [12-14], but has been modified to span the 3 weeks of orthodontic force application. This adjustment was made to prevent any potential interference with the euthanasia procedures conducted at 7, 14, and 21 days.

2.5. Measurement of the Rate of Orthodontic Movement

The orthodontic movement was measured using a direct measurement method with a digital caliper between the mesioincisal angle of the lower incisors [6]. Measurements were taken at 10 intervals: days 1, 3, 5, 7, 9, 12, 14, 16, 18, and 21.

2.6. Histological Analysis

Histological sections of the lower incisor teeth, including the periodontium and osseous tissue, were examined under a light microscope. The histological sections were divided horizontally into two equal regions (each 4500 μm in length): the upper region (U), comprising the alveolar bone and PDL around the upper portion of the roots in the histological section both mesially and distally in cervical direction, and the lower region (L), encompassing the lower portion of the roots in the histological section also both mesially and distally in apical direction.

2.7. Immunohistochemistry Analysis

The specimens were cut into 4 μm sections, and the Immunohistochemistry (IHC) technique was performed to detect the expression levels of BALP and TRAP 5b in the upper and lower regions. BALP primary antibody (Elabscience, USA), TRAP 5b primary antibody (Biovendor, Czech Republic), and Poly excel HRP/DAB detection system (Elabscience, USA) were used. The procedure of IHC was performed according to Magaki *et al.* and Suvarna *et al.* [15, 16], BALP and TRAP 5b activity scores used the method of Hernández-Rodríguez *et al.* and Jammal *et al.* [17, 18] as follows:



Figure 1. Representative Images for Low-level Laser Application in the LT Group

0 = No reaction or very weak positive reaction.

1 = Weak positive reaction.

2 = Moderate positive reaction.

3 = Strong positive reaction.

4 = Very strong positive reaction.

2.8. Statistical Analysis

Statistical analysis was conducted using SPSS Statistics 25. The normality distribution of the data was evaluated using the Shapiro-Wilk test, confirming normal distribution across all data. Comparisons between the LT and control groups regarding tooth movement were performed using an independent samples t-test at $p \leq 0.05$. Comparisons of new bone formation area, as well as osteoblast and osteoclast counts, were analyzed using a one-way analysis of variance followed by Duncan's multiple comparisons test at $p \leq 0.05$. The non-parametric data of the BALP and TRAP 5b immunohistochemistry biomarker scores were analyzed as median and IQR (Inter-Quartile-Range) by the Kruskal-Wallis Test using the Pairwise Multiple Comparison Dunn's Test at $p \leq 0.05$.

3. Results

3.1. Rate of Tooth Movement

The Laser Treatment (LT) group exhibited a significantly higher rate of tooth movement from day 7 to day 18 compared to the Control (C) group. However, no significant difference was observed in orthodontic movement on days 1, 3, 5, and 21 (Figure 2) using an independent sample t-test at $p \leq 0.05$.

3.2. Histological Analysis

New bone formation: The laser treatment (LT) group showed a significantly larger area of new bone formation (osteoid) compared to the control (C) group at all-time points. No significant difference was observed between the upper and lower regions in the LT group in the 1- and 2-week period, but the upper region had significantly higher results than the lower region in week 3 (Figures 3 and 4).

Osteoblast count: Results indicated significant differences in osteoblast counts between the laser treatment (LT) group and control group at 1, 2, and 3 weeks, with the LT group showing the highest counts. No significant difference was found between the upper and lower regions in the LT group in all periods (Figures 5 and 7).

Osteoclast count: The laser treatment (LT) group demonstrated a significant dominance in osteoclast

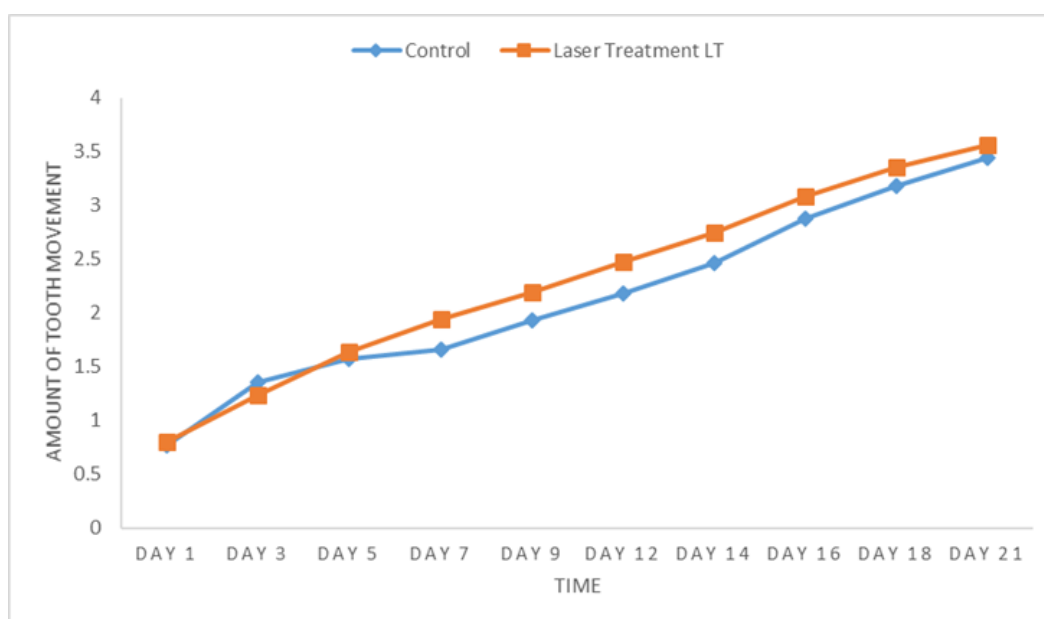


Figure 2. Comparison of the Amount of Orthodontic Movement Between the Laser Treatment and Control Groups

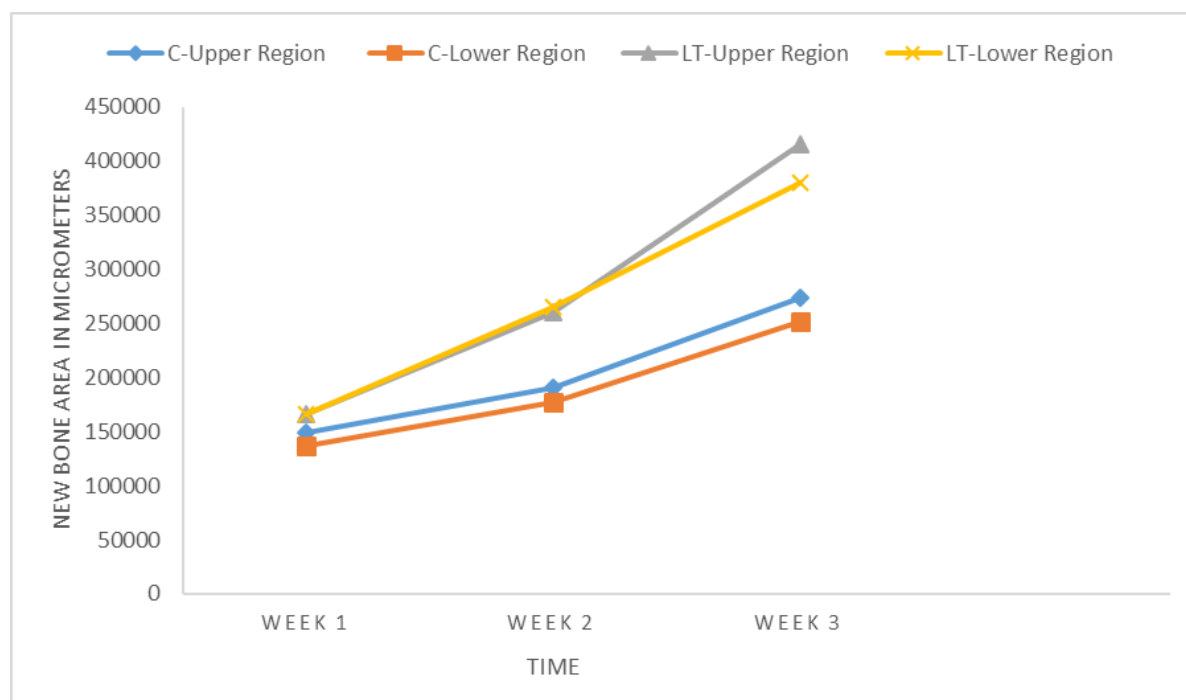


Figure 3. Comparison of Surface Area of the New Bone Between the Laser Treatment (LT) and Control (C) Groups at 1, 2, and 3 Weeks

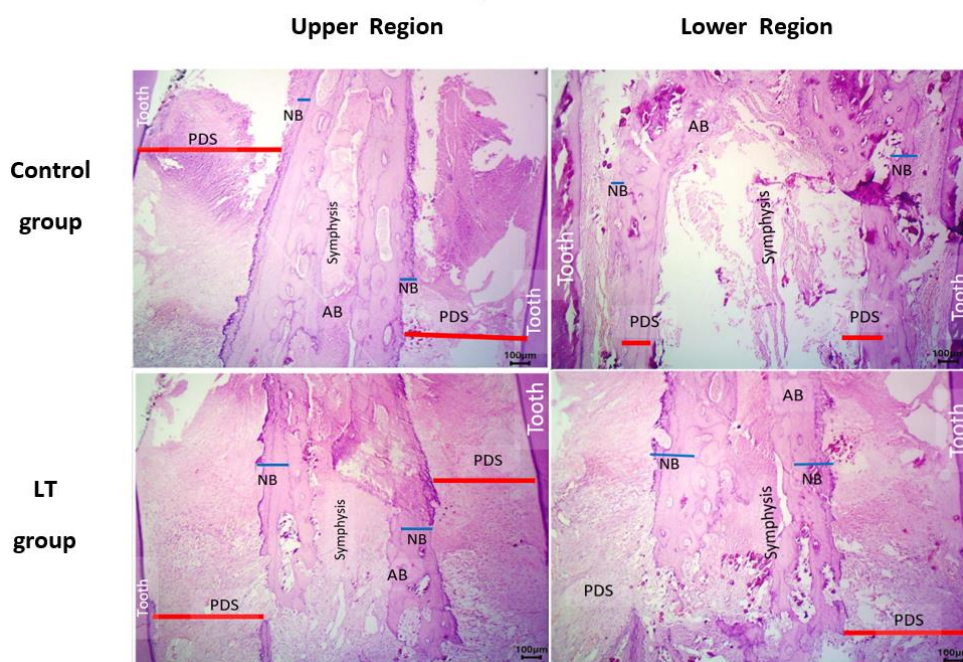


Figure 4. Histological Section from the Control Group Versus Laser Treatment (LT) Group at Week 3 in the Upper Region and Lower Region Showed New Bone (NB, blue line), Alveolar Bone (AB), Periodontal Space (PDS), H&E stain, 40X, Scale bar=100µm

numbers compared to the control group throughout all periods, with a significant difference between upper and lower regions in the LT group at the 2 weeks (non-significant difference in the 1 and 3-week periods) (Figures 6 and 7).

3.3. Immunohistochemistry (IHC) Analysis

Bone Alkaline Phosphatase: The BALP activity score was significantly greater in the Laser Treatment (LT) group in both upper and lower regions only at 2-

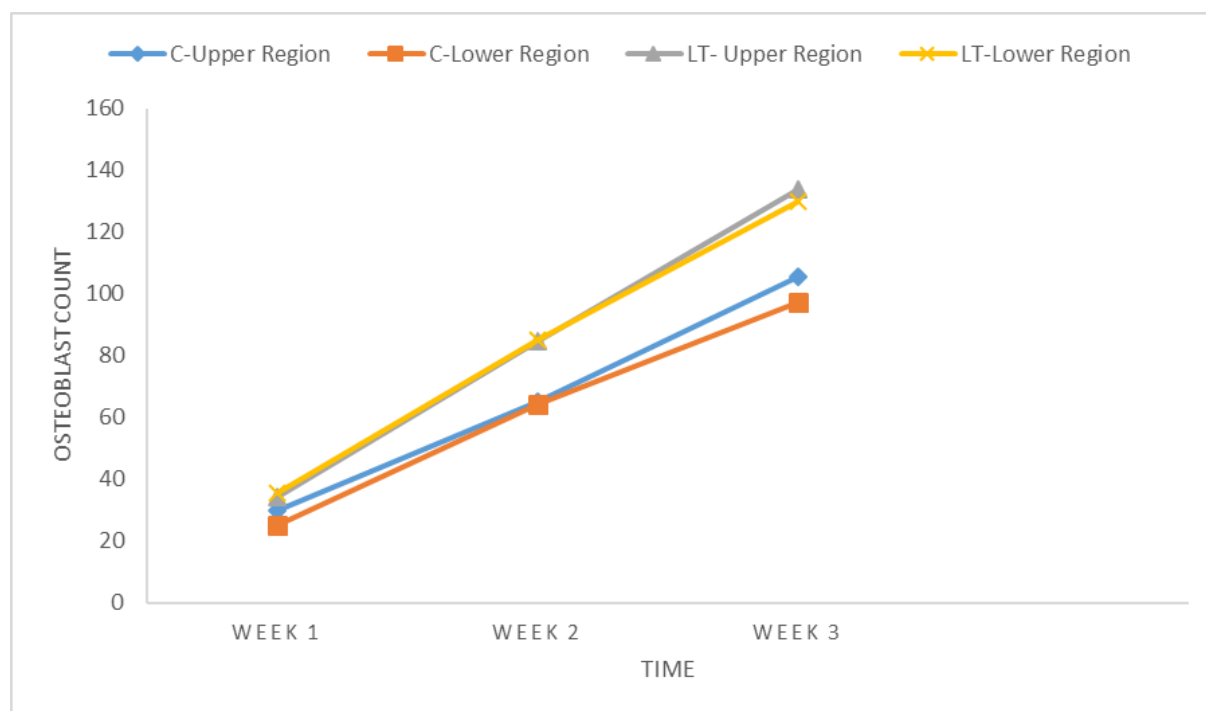


Figure 5. Comparison of the Osteoblast Count Between the Laser Treatment (LT) and Control Groups at 1, 2, and 3 Weeks

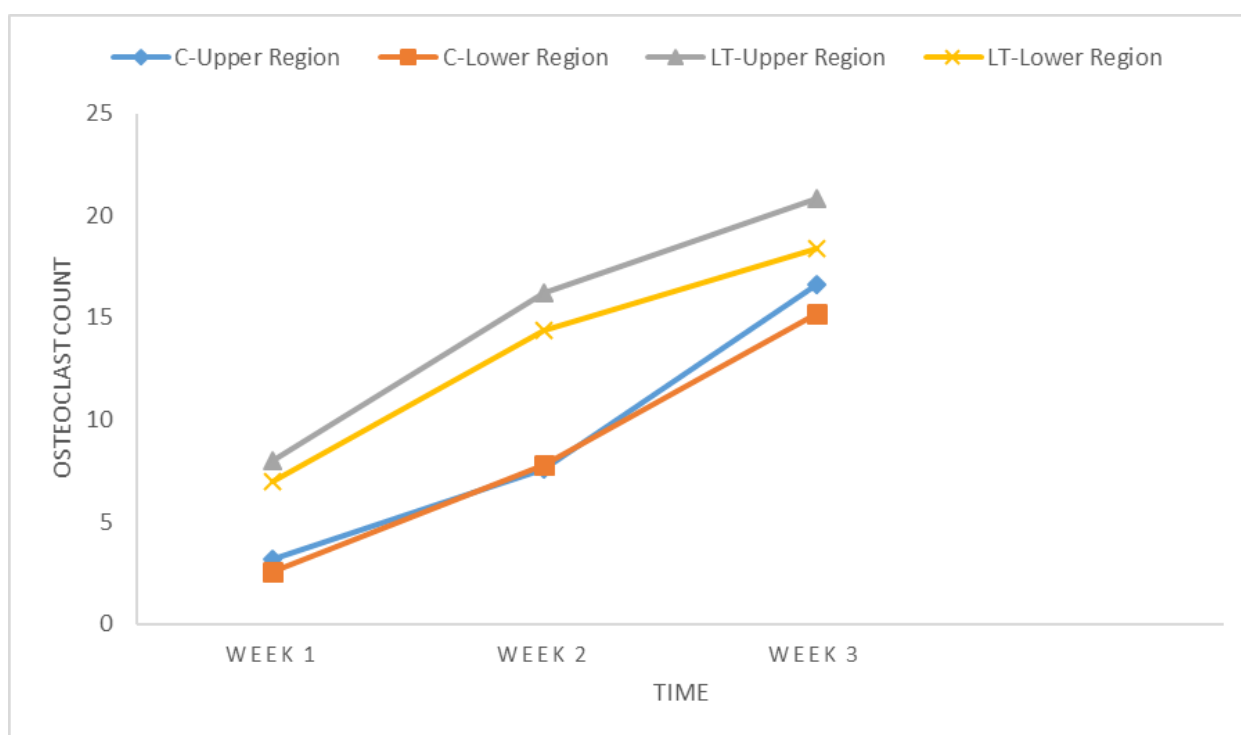


Figure 6. Comparison of the Osteoclast Count Between the Laser Treatment (LT) and Control (C) Groups at 1, 2, and 3 Weeks

week periods; non-significant differences were observed between control and LT groups in upper and lower regions at 1 and 3-week periods (Table 1, Figure 8).

4. Discussion

The mentioned wavelength, power, and energy used in this study cause no temperature changes in exposed tissue, Hsu *et al.* use a similar wavelength (970nm) but

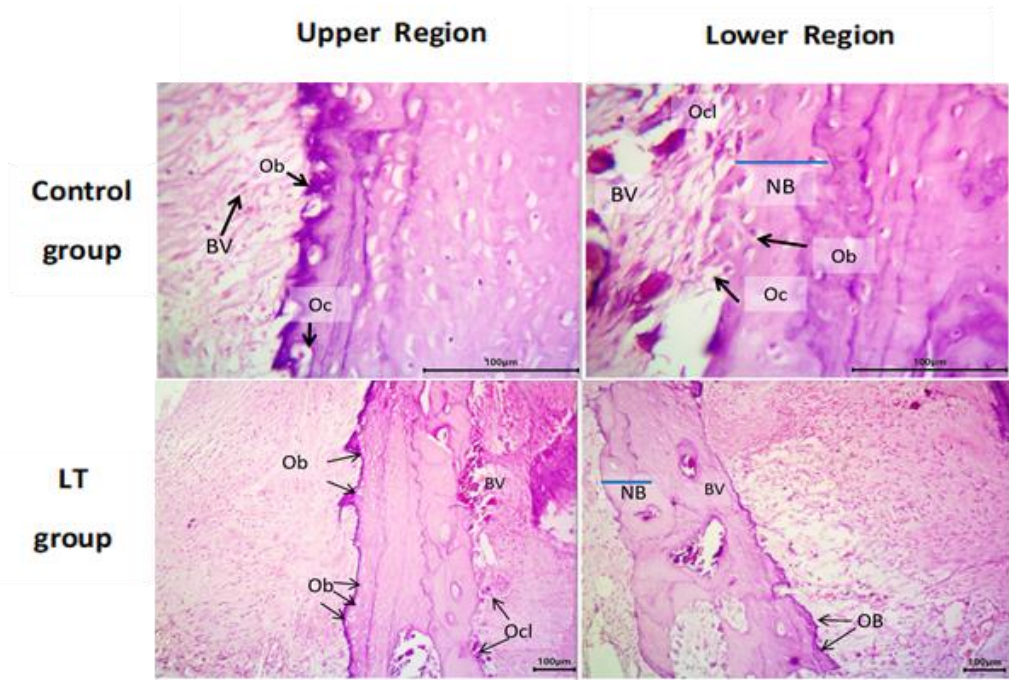


Figure 7. Histological Section from the Control Group versus the Laser Treatment (LT) Group at Week 3 in the Upper Region and Lower Region Showed New Bone (NB, blue line), Osteoblasts (Ob), Osteoclasts (Oc), and Blood Vessels (BV). H&E stain (below: 100X, above: 400X), Scale bar=100µm

Table 1. The IHC Scores of Bone Alkaline Phosphatase in Control and Laser Treatment (LT) Groups After 1, 2, and 3 Weeks

Region	Group	1 Week	<i>P</i> -Value	2 Weeks	<i>P</i> -Value	3 Weeks	<i>P</i> -Value
Upper Region	Control group	0 (0.5) A	0.151	1 (1) A	0.035	2 (1) A	0.961
	LT group	1 (0.5) A		2 (1) B		2 (0.5) A	
Lower Region	Control group	0 (1) A	0.690	1 (0.5) A	0.008	2 (1) A	1.000
	LT group	1 (1) A		2 (0) B		2 (1) A	

* Data expressed as Median & IQR ((Inter-Quartile-Range), (N= 5).
** Different capital letters between groups in columns within same region and period mean there is significant difference at $p\leq0.05$

with much higher power and energy perform thermal change tests, and observed transient and mild rises in temperature locally, with no detectable change on the surface of the irradiated area [19]. power output located below 500 mW lies within photobiostimulatory effects where the laser radiation is not accompanied by a local temperature increase in tissues by more than 1 °C [20-22]. Laser biostimulation at spectral width between 600nm and 1000nm and energy density between 1 and 20 J/cm²

have no ablative or thermal mechanism, but rather photochemical effects [23].

The significant difference in tooth movement between the laser treatment (LT) and the control group started to appear on day 7 and lasted until day 18. This suggests that biostimulation laser treatment likely began providing a clinical benefit by day 7. After day 18, the lack of significant difference may be attributed to the reduced force in the orthodontic appliance,

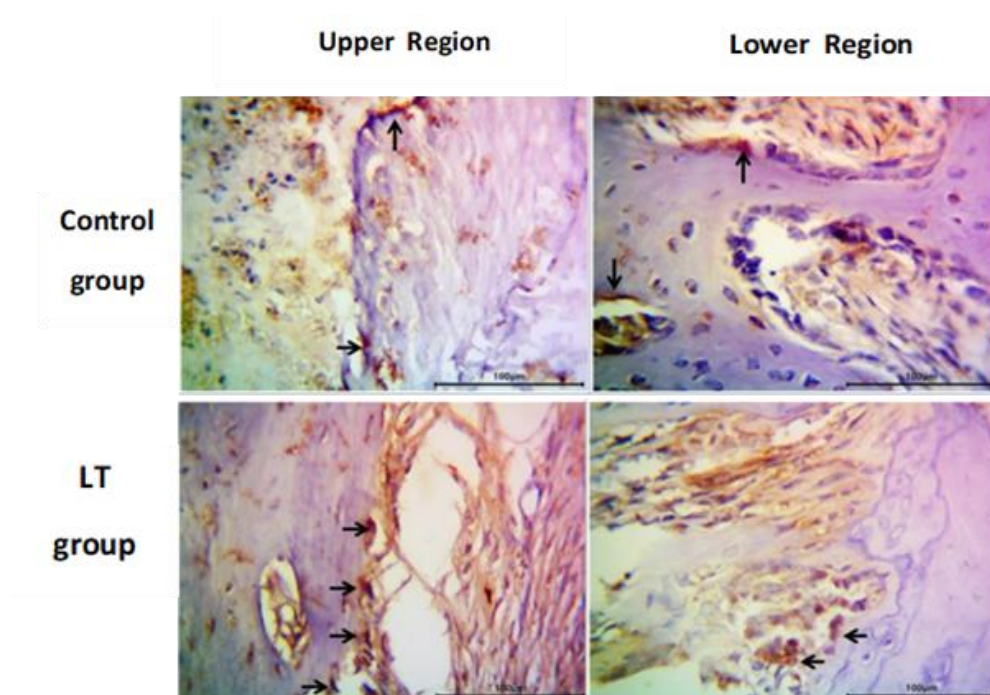


Figure 8. Immunohistochemistry Expression of Bone Alkaline Phosphatase of the Control Group versus the Laser Treatment (LT) Group at the 2-week in the Upper and Lower Regions (Brown Color of Osteoblasts is a Positive Reaction). 400X, Scale bar = 100 µm

Table. 2. The IHC Scores of Tartrate-Resistant - Acid Phosphatase 5b in Control and Laser Treatment (LT) Groups After 1, 2, and 3 Weeks

Region	Group	1 Week	P-Value	2 Weeks	P-Value	3 Weeks	P-Value
Upper Region	Control group	1 (1)	0.981	1 (0.5)	0.981	1 (1)	.021
	LT group	1 (0.5)		1 (0)		2 (1)	
		A		A		B	
Lower Region	Control group	1 (1)	0.981	1 (0.5)	.033	2 (1)	1.000
	LT group	1 (0.5)		2 (0.5)		2 (1)	
		A		B		A	

*Data expressed as Median & IQR ((Inter-Quartile-Range), (N= 5).

** Different capital letters between groups in columns within the same region and period mean there is a significant difference at $p \leq 0.05$.

which might have been insufficient to show a difference between the two groups.

These findings might agree with Hsu *et al.* who observe that using 970 nm laser treatment with various energy densities increases the amount of orthodontic movement [19] and agree with Yassaei *et al.* who found similar results using the laser at 810 nm, 100 mW power, and 6.29 Joule/cm² fluence [3]. This acceleration in tooth movement may be related to higher Interleukin-1 β (IL-1 β), Receptor Activator of Nuclear Factor Kappa (RANK), RANK ligand

(RANKL), and lower Osteoprotegerin (OPG) levels [2, 3, 24, 25], and increased levels of TRAP, Matrix Metalloproteinase-9 (MMP-9), cathepsin K, and integrin subunits [26].

In terms of new bone formation, the LT group reflected significantly superior results than the control group in all periods; this agrees with Alazzawi *et al.* who found that 940 nm and a fluence of 45.85 Joule/cm² laser can stimulate orthodontic movement, enhance gene expressions, and increase alveolar bone turnover [27]. Hsu *et al.* found increased osteocalcin

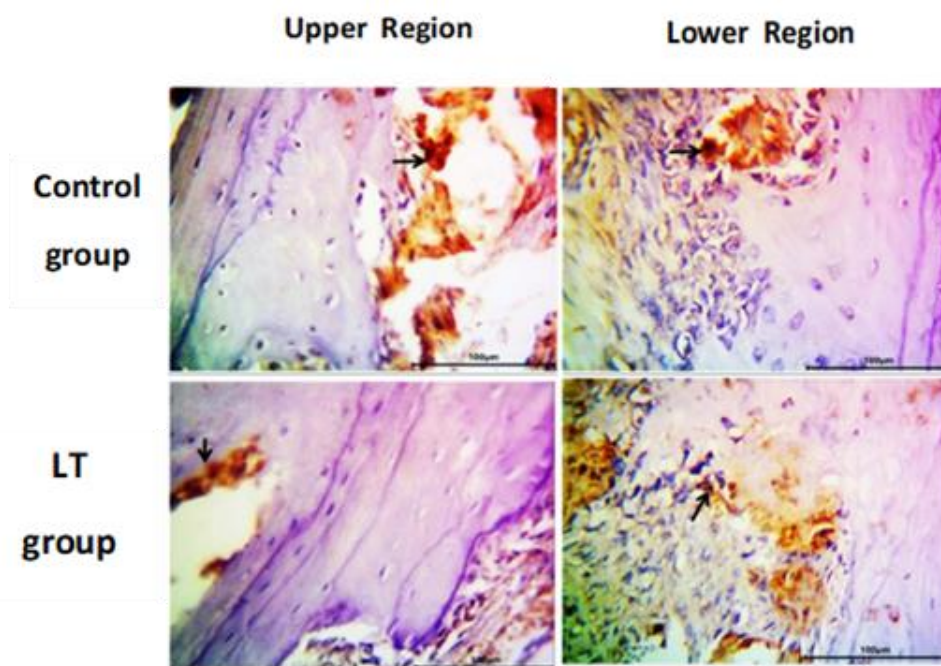


Figure 9. Immunohistochemistry Expression of Tartrate Resistant - Acid Phosphatase 5b of the Control Group versus the Laser Treatment (LT) Group at the 2-week in the Upper and Lower Regions (Brown Color of Osteoclasts is a Positive Reaction). 400X, Scale bar = 100 μ m

levels, indicating increased levels of bone formation [19]. Similar results were also found by Chang *et al.* and Zhong *et al.* [28, 29]; wavelengths higher than 800 nm resulted in better bone healing by improving osteoproliferation and osteoinduction [30]. This enhancement in bone formation may be due to improving the production of vascular endothelial growth factor, Runx2, bone morphogenic protein-2, osteopontin, and osteocalcin and increasing the expression of genes responsible for matrix and collagen formation and maturation [31, 32], elevated COL1a gene expression resulted in faster bone healing, accompanied by the formation of mature collagen bundles [33, 34].

Regarding osteoblast counts, the laser-exposed group showed a larger osteoblast number than the unexposed group, with the highest count observed at the 3-week mark with no significant difference between upper and lower regions. This finding is consistent with Dominguez *et al.* who reported that osteoblast cells are responsive to laser exposure, which increases bone remodeling by upregulating the proliferation and function of both osteoblasts and osteoclasts during orthodontic tooth movement [35]. Altan *et al.* observed a noticeable increase in osteoclast count, osteoblasts, and new bone formation

following exposure to 820-nm wavelength low-level laser therapy at a power of 100 mW [4]. The 980 nm wavelength can biostimulate pre-osteoblasts and regulate their metabolic schedule [36], increasing osteoblastic proliferation, decreasing apoptosis, stimulating osteogenesis, and increasing bone density [3, 28]. Bone alkaline phosphatase, which considers osteoblast primary mineralization marker showed larger activity in the laser-exposed than the non-exposed group in the upper and lower regions in 2 weeks, with a non-significant difference in the 1- and 3-week periods. This result may align with studies that found that photobiostimulation therapy increases the proliferation and maturation of osteoblasts and increases alkaline phosphatase and osteocalcin expression [28, 29, 37]; this finding may also be supported by Wang *et al.* who investigated the effects of laser therapy at 1064 nm on osteogenesis, their study demonstrated that laser exposure could enhance bone formation by increasing levels of Runx2, alkaline phosphatase, osteocalcin, and mineralized nodule formation. They found that Bone Morphogenic Protein 2 (BMP2) levels were particularly sensitive to laser treatment and that it promoted osteogenic differentiation and mineralization through the activation of the BMP/Smad signaling pathway [38].

When laser exposure stimulates osteoblasts, it is accompanied by increased alkaline phosphatase and osteocalcin expression. Increasing the proliferation of osteoblasts and increasing circulating RANKL so enhances osteoclast genesis and increases the speed of dental movement [24, 33, 39].

The osteoclast count was larger in the laser-exposed group compared to the unexposed group across all three time periods studied. Regarding TRAP 5b activity, a significant difference was observed only in the upper region at 3 weeks and in the lower region at 2 weeks; no significant differences were found in the remaining regions and periods. This result may be because of the increased activity of RANK and RANKL increasing the osteoclastic action; the results indicated that laser enhances osteoblast and osteoclast action, but it is more effective towards osteoclast action [27]. More osteoclasts in the initial days after exposure are the ground for bone remodeling acceleration [19, 25]. Huang *et al.* study demonstrates that laser therapy of 808 nm and at a fluence of 4 Joule/cm² effectively increases osteoclast differentiation due to RANKL activation and increases not only TRAP activity but also the expression of other osteoclast functional genes, including matrix metalloproteinase 9, nuclear factor of activated T-cells cytoplasmic 1, and cathepsin K [40].

The observed acceleration of tooth movement in the laser-exposed group between days 7 and 18 Compared to the control group, can primarily be attributed to the increased number and activity of osteoblasts and osteoclasts. The 980 nm laser appears to effectively biostimulate pre-osteoblasts, enhancing their proliferation, viability, and differentiation [30, 36]. This biostimulatory effect accelerates bone remodeling by promoting the proliferation and function of both osteoblasts and osteoclasts, thereby offering potential clinical applications for accelerating orthodontic tooth movement and enhancing bone formation [35]. The mechanism of this stimulation involves the modulation of RANK/RANKL signaling in osteoclast precursor cells, detectable early in the laser irradiation process, which underlies the acceleration of bone remodeling [19, 24, 25]. Tabatabaei *et al.* demonstrated that laser treatment at 980 nm, with 100 mW power and energy doses of 3 and 6 J/cm², in combination with orthodontic forces, resulted in the upregulation of bone resorption genes

(RANKL and SOST) on the pressure side and their downregulation on the tension side [41]. Additionally, Huang *et al.* observed that laser treatment not only promoted RANKL-dependent osteoclast differentiation but also enhanced the expression of functional genes associated with osteoclast activity [40].

The cellular response to laser radiation may be attributed to modulating metabolic activity via stimulating the mitochondrial energy cycle [27]; This phenomenon is mediated by mitochondrial chromophores that absorb photons from laser radiation. While cytochrome c oxidase is recognized as the primary chromophore for absorbing red and near-infrared wavelengths, the application of higher near-infrared light (980–1064 nm) also stimulates heat and light-sensitive ion channels, such as members of the transient receptor potential (TRP) channel family, which have a photon absorption spectrum within the range of 980–1064 nm. This interaction triggers the activation of various signaling molecules, including nitric oxide, ATP, calcium ions, reactive oxygen species, and other intracellular mediators [1, 5].

5. Conclusion

Within the scope of this study, laser biostimulation therapy with a near-infrared 976 nm wavelength, energy of 8 J, and energy density of 15.9 J/cm² exhibited statistically significant superiority in most of the evaluated variables, including the rate of tooth movement, new bone formation, and osteoblast and osteoclast counts compared to the control group. The bone alkaline phosphatase and tartrate-resistant - Acid Phosphatase 5b activity were less pronounced, with the majority of significant differences observed during the two weeks.

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