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The impact of concentrated growth factor and low-level laser therapy on osseointegration of dental implants: An experimental study on sheep

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Abstract

This study aims to assess the effect of the Concentrated Growth Factor (CGF) with low-level laser therapy biostimulation (LLL) in the healing of bone around dental implants in an animal model. Four male sheep in good, healthy condition were utilized for the study. Sheep were divided into two groups, each model serving as two observation subgroups. The tibia of each sheep was subjected to random surgical procedures, with a one and two-month gap between each operation. A total of 40 dental implants were implanted, with each sheep receiving ten implants (five implants in each tibia). The groups were divided as follows: group 1 (Control), in which ten implants were placed in each sheep (five dental implants in each tibia), and group 2 (Study group in which ten implants were placed in each sheep (five dental implants in each tibia) with Sacco's Membrane (concentrated growth factor) and LLLT Biostimulation. Densitometric analysis was performed using digital radiography, immunohistochemical, and histopathological assessment. The findings of the present investigation revealed a statistically significant disparity in the extent of bone development surrounding dental implants at four and eight weeks, with the study group exhibiting a more favorable outcome than the control group. In conclusion, using CGF in conjunction with low-level laser treatment biostimulation has contributed to new bone formation and the establishment of osseointegration.

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Introduction

The process of bone healing encompasses a comprehensive method of repairing bone tissue, characterized by a multi-dimensional framework and a timetable that exhibits overlapping stages. To facilitate the fabrication of new bone tissue, it is necessary for cells originating from the adjacent tissues to undergo migration, proliferation, and differentiation on a bone replacement substrate (1). The procedure's efficacy is contingent upon several variables, encompassing the characteristics of bony tissue and the composition of the implant material. The integration of implants into the bone is commonly attributed to two main processes: osteoconduction, which involves the generation of new bone, and remodeling, which entails the reconstruction of pre-existing bone (2). During the process of reconstruction, bone tissue undergoes a series of changes. Initially, it transforms from the fibrous, plexiform repairing bone by limited technical characteristics to the trabecular arte- ture organized as a 3-dimensional meshwork. Subsequently, it develops into a highly mineralized lamellar bone, which facilitates the formation of secondary connection points between the recipient tissues and an implant. This transformation occurs as the new bone replaces the old bone resorptive by osteoclast (2). Develop tissue encompassing an implant surface and residual bone within the drilled bed exhibit the presence of marrow cavities and blood vessels. Multiple studies exhibit that diverse growing and differential agents, such as bone morphogenetic protein and lactoferrin, can potentially enhance the process of bone

repair (3). Autologous PRF, which is the platelet concentrate of the second generation, can be described as a fibrin matrix that facilitates angiogenesis and induces contact inhibition during the healing process of a periodontal lesion (4,5). Concentrated Growth Factor (CGF), a platelet concentrate formulated by Sacco in 2006, is recommended in dental offices due to its potential positive outcomes. The autologous platelet concentrate is an enhanced formulation incorporating blood cells and growth factors, exhibiting higher sophistication (6,7). Another method to improve bone healing is to employ LLLT (8). The diode lasers commonly employed in dentistry are gallium aluminum arsenide (GaAlAs) diode lasers operating at a wavelength of 810 nm, gallium arsenide (GaAs) diode lasers operating at a wavelength of 940 nm, and indium gallium arsenide (InGaAs) diode lasers operating at a wavelength of 980 nm. These particular diode lasers are frequently utilized in dental applications (9). The diode laser can be employed in surgical procedures, provided continuous contact exists between the optical fiber and the tissue (10).

This study aimed to assess the impact of CGF with low-level laser therapy biostimulation (L Biostimulation) on bone healing around dental implants in an animal model.

Materials and methods

Ethical approval

The study received the ethical approval from the Scientific Research Committee, College of Veterinary Medicine, University of Mosul, (UM.VET.2022.089).

Study design and sample size

The study comprised a sample of four male landrace sheep aged between 1.5 and 2.5 years, weighing 35 to 45 kg (mean = 40 kg). All sheep were sourced from a single farm. A veterinarian conducted regular health and dietary assessments. The animals underwent a two-week acclimatization period before each surgical procedure, during which their overall health was assessed to confirm the absence of any general or contagious illnesses. To limit errors mediated by operators, it was ensured that a consistent surgeon executed all procedures conducted throughout the study.

Experimental design

Sheep were separated into two groups, each model serving as two observation subgroups. The tibia of each sheep was subjected to random surgical procedures, with a one and two-month gap between each operation. A total of 40 dental implants were implanted, with each sheep receiving ten implants (five implants in each tibia). The groups were separated in the following manner: The experimental design consisted of two groups: Group 1, serving as the control group, involved the placement of ten implants in each sheep, with five dental implants inserted in each tibia. Group 2, the study group, also placed ten implants in each sheep, with five dental implants in each tibia. However, in addition to the implants, group 2 received Sacco's Membrane, a concentrated growth factor, and LLLT Biostimulation.

Preparation of Sacco's membrane (concentrated growth factor)

Blood collection was performed at the jugular vein. Sheep were positioned upright and restrained at the head and nearest body region before blood collection. To ensure suitable visual and mechanical accessibility and minimize the risk of contamination, the wool surrounding the blood collection location was eliminated. The process involves cleansing the exposed skin with a 10% povidone-iodine solution. Laterally rotating the head and extending the neck, a hypothetical vertical line is established from the midpoint of the sheep's eye, employing a ruler as a tool for approximating the trajectory of the jugular vein. The blood collection process involved BD Vacutainer® Tubes, a 21G needle, and a holder. Two blood samples, each measuring 10 ml, were obtained and promptly subjected to centrifugation. The obtained blood samples were centrifuged using a pre-programmed centrifuge (Medifuge, Silfradent, Sofia, Italy) with a one-step centrifugation procedure. This protocol consisted of the following steps: 30 seconds of acceleration, 2 minutes at 2700 revolutions per minute (rpm), 4 minutes at 2400 rpm, 4 minutes at 2700 rpm, 3 minutes at 3000 rpm, 36 seconds of deceleration, and finally, the centrifuge was stopped. After the centrifugation cycle, half of the membrane, specifically the platelet-rich side proximal to the red end, is subjected to processing and mincing and, afterward, placed in the osteotomy bed before implant insertion.

Surgical procedure

Before surgery, every animal is allowed to graze and provided unrestricted access to drinking water. The surgical site was prepared in an aseptic manner surgical procedures were performed under general anesthesia and adhered to strict sterile conditions. The administration of general anesthesia, encompassing both induction and maintenance, involves the intravenous infusion of a combination of ketamine-hydrochloride, a general anesthetic with a concentration of 10 mg/ /kg, and xylazine, a sedative-analgesic solution with a concentration of 0.02 mg/l/kg. After 10 minutes, the animal was administered sedation for approximately one hour, which was deemed sufficient to facilitate the completion of the treatment. The veterinarian thoroughly assessed the sheep's vital signs, ensuring their monitoring during the surgical procedure. To achieve disinfection, the designated area underwent a cleaning process involving the application of a 10% solution of povidone-iodine, followed by using a towel to remove any residual contaminants. Before making the incision, a local

anesthetic with epinephrine at a concentration of 1:80,000 is administered to achieve hemostasis. A 15 cm incision was made on the skin to expose the borders of the tibia or medial or lateral aspect of the tibia. The skin and face layers were then opened and will be closed separately upon completion while ensuring hemostasis. After exposing the bone, five standard osteotomy sites were created with thorough irrigation using a cooled 0.9% saline solution. These sites were spaced at least 0.5 cm apart and intended to place five dental implants. The implants used were from the (B&B dental implant company, Italy) slim line system manufactured in Italy, and they had an acid etching surface. The implants had a diameter of 3mm and a length of 8mm (Figure 1). The placement procedure followed the recommendations provided by the manufacturer. Twenty implant beds were designated as control groups, in which no additives were applied.

In comparison, another twenty osteotomy beds were assigned as study groups, in which crushed CGF (Sacco's membrane) pieces were inserted. Subsequently, the placement of healing screws occurred (Figure 1). The implant site was subsequently subjected to low-power laser irradiation using a diode laser (Epic Diode Laser / Biolase / USA) with a wavelength of 940 nm, an output power of 0.5 watts, and 10 seconds for a surface area of 1 cm^2. The laser was positioned 1 cm from the target, operated continuously, and delivered a cumulative energy dose of 5 joules/cm^2. To ensure laser protection, individuals utilized goggles. Following the surgical procedure, the soft tissue was meticulously closed using a layered approach. The fascia was sutured with an absorbable suture material known as vicryl, while the skin was sutured using a non-absorbable 3-0 black silk suture. The wound was treated with an aerosol spray containing oxytetracycline to ensure adequate antibiotic coverage, followed by meticulous bandaging.

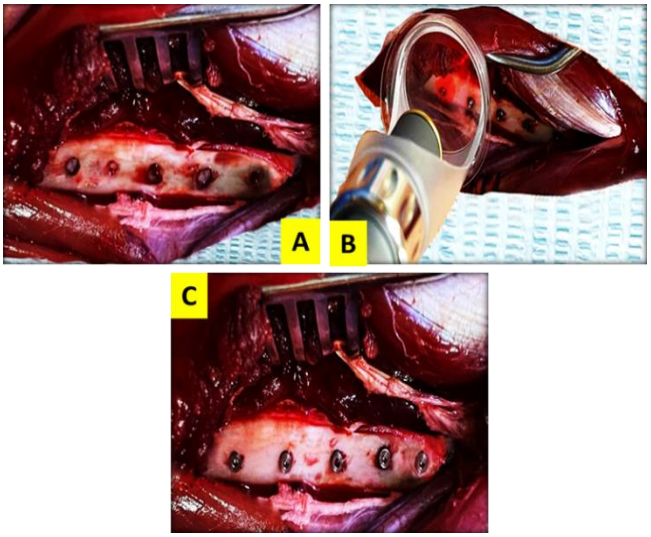

Figure 1: Osteotomy beds for the studied groups.

The postoperative care of the animal involves managing an intermuscular injection of the antibiotic oxytetracycline at 20 mg/ml per 10 kg of body weight. During the initial week, the animals were confined within the animal housing facility, with limited mobility. They were provided with a standardized meal and access to water, and a qualified veterinarian regularly monitored their well-being. The dressing applied to the incision was replaced at three-day intervals, and the wound was regularly examined for any indications of infection until the sutures were removed on the tenth day following the operation.

Radiographic assessment

Following the conclusion of the trial and for radiographic assessment of surgical sites, a sedating analgesic solution containing xylazine at a concentration of 0.02 mg/kg was given to the sheep. No animal was sacrificed. Subsequently, a digital dental radiograph machine equipped with imaging analysis software (version 7.0.3) was employed to assess the x-ray images of each tibia. A parallel technique was employed as an imaging procedure to mitigate any elongation, shortening, or geometrical distortion in the resulting image. The power settings were configured to 50 milliamperes per second and 60 kilovolts. The digital sensor (size = 1 Carestream, RVG 5200) was positioned using a digital sensor positioner, ensuring the specimen was centered on the sensor. The optimal source-object distance was determined by placing the X-ray source five centimeters from the sensor. To obtain precise measurements, the sensor was positioned in a manner that was both horizontally and vertically perpendicular to the elongated axis of the object. Our objective was to assess the densitometric values indicative of the bone density surrounding each dental implant inside the central region.

The densitometric data are visually represented in a graph, where the values span from 0 to 255. In this representation, black is associated with the value 0, while white corresponds to 255. This study focused on the implant fixture and the adjacent bone, which were examined using a central x-ray centered on the implant fixture. After gathering the images, the subsequent analysis involved selecting a linear drawing option from the program. This option was utilized to draw a red line connecting the neighboring serrations in the dental implant, namely at its middle regions encompassing both the mesial and distal sides. This assessment aimed to evaluate the bone density on these two sides. The total densitometric mean of bob one-surrounded-ental implant serrations was determined by the summing means of two-sided middle regions and calculating the rage mean. A positive correlation exists between the mean densitometric value and the density of bone surrounding the dental fixture. In other words, as the mean densitometric value increases, the density of the bone also increases; conversely, as the mean densitometric value decreases, the density of the bone decreases (Figure 2).

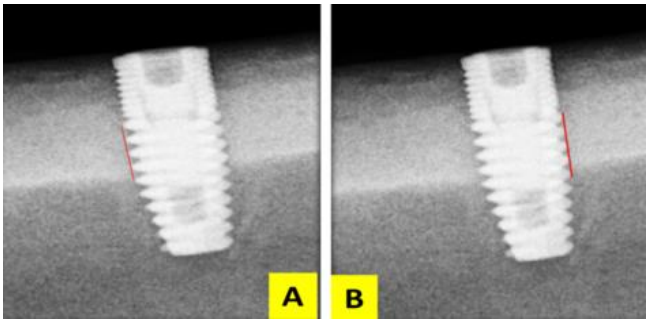

Figure 2: X-ray of the inserted implants.

Immunohistochemical assessments

To perform immunohistochemical assessments, remove wax, provide moisture, and perform heat repair or digesting therapy on the tissue segment's antigen. Remove endogenous peroxidase activity by incubating in a solution containing 3% hydrogen peroxide for 10 minutes. Incubate the Blocking Buffer at 37°C for 30 minutes. Add the primary antibody and incubate at 20 to 37°C for 1 to 2 hours. Apply Polyperoxidase-anti-Mouse/Rabbit IgG and rinse. Prepare the DAB Working Solution by mixing 50 µL of DAB Concentrate with 1 mL of DAB Substrate. Observing a tan or brownish-yellow signal, assume command over the DAB coloration phase. Rinse with deionized water to stop chromogenic reactions.

Histopathological assessments

The process of tissue processing involves collecting tissue samples, placing them in a neutral buffered formalin solution, dehydrating them using ethyl alcohol, clearing the tissue with pure xylene, and infiltrating it with heated paraffin wax. The samples are then placed in a paraffin mold filled with hot wax, submerged in the wax, and sliced into 4-6 µm thick sections using a rotary microtome. The sections are then placed in a water bath and hoisted onto a clean glass slide. The slide is left to cure at room temperature for 24 hours, followed by an additional hour of drying on a hot plate at 55-60ºC. The slide is then ready to be stained. The process ensures that the tissue samples exhibit a robust, sturdy, and authentic color. Harris hematoxylin and alcoholic eosin.

Statistical analysis

Statistical analysis was done using (SPSS)Version 23 software for Windows. The parametric data were expressed as means ± standard error (SE). They analyzed the data using a T-test to compare the periods. Significance level at $P<0.05$, and the non-parametric data of the bone cell counts, and biomarker scores were examined using the Kruskal-Wallis Test with Pairwise Multiple Comparison Tukey Test to compare groups and the Mann-Whitney test to compare periods. The analysis was based on the median and interquartile range (IQR), with a significance level set at $P<0.05$.

Results

Clinical results

The exclusion of a limited number of cases involving post-surgical limping, most animals exhibited favorable tolerance towards the surgical procedure, and the surgical sites exhibited uneventful healing. In control, the significant difference in densitometric means was shown between four and eight weeks 137.20 and 144.80, respectively (Table 1). A significant difference in densitometric means was also shown in the study group between four and eight weeks 156.00 and 172.60, respectively (Table 2). Comparison between groups: After four weeks, there was a significant difference between the control and study groups in densitometric means of 137.20 and 156.00, respectively (Table 3). In the eight-week interval, a higher mean value with a significant difference was also disclosed in the test group 172.60 compared to the control group 144.80, as shown in (Table 3).

Table 1: Changes in densitometric in BIC in control group

Interval	Number	Mean±SD	Sig
Four weeks	10	137.20±5.49	0.05*
Eight weeks	10	144.80±4.91	

SD: Standard Deviation / Sig: *Significance at P≤0.05.

Table 2: Changes in densitometric in BIC in study group

Interval	Number	Mean±SD	Sig
Four weeks	10	156.00±3.46	0.02*
Eight weeks	10	172.60±2.88	

SD: Standard Deviation / Sig: *Significance at P≤0.05.

Table 3: Comparison of changes in densitometric in BIC between control and study groups

Interval	Groups	Mean±SD	Sig
Four weeks	Control	137.20±5.49	0.03*
	Study	156.00±3.46	
Eight weeks	Control	144.80±4.91	0.01*
	Study	172.60±2.88	

SD: Standard Deviation / Sig: *Significance at P≤0.05. n=10.

Alkaline phosphatase

In control group, the median and IQR at four weeks were 1(0), while at eight weeks, they were 2(1.75). Thus, there were significant differences in scores of the immunohistochemistry expression of the alkaline phosphatase throughout the two scheduled intervals (Figure 3). While in study group, there were no significant differences between the two periods of time in scores of the immunohistochemistry expression of the Alkaline phosphatase. However, at four weeks, the median and IQR

were 3(0.25), and at eight weeks, the median and IQR were 3(3) (Figure 4). In the current study, the median and IQR at four weeks for control group and study group was 1(0) and 3(0.25), respectively, so there were highly significant differences in scores of the immunohistochemistry expression of the Alkaline phosphatase between two groups (Figures 5 and 6). while the median and IQR at eight weeks for control group was 2(1.75). The study group was 3(3); therefore, there was a significant difference between the control group and the study group (Table 4).

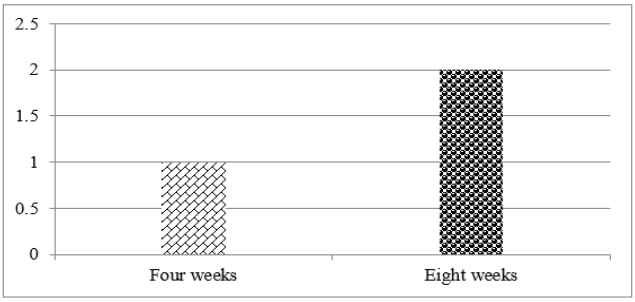

Figure 3: Scores of the immunohistochemistry expression of the Alkaline phosphatase throughout the two scheduled intervals for the control group.

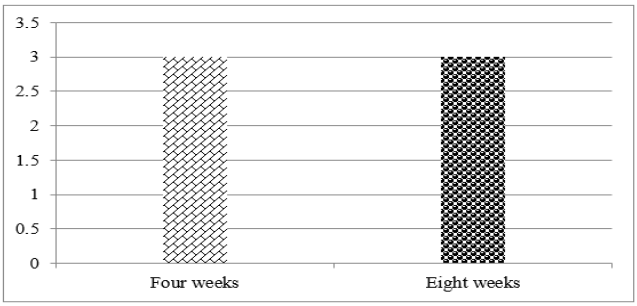

Figure 4: Scores of the immunohistochemistry expression of the Alkaline phosphatase throughout the two scheduled intervals for a study group.

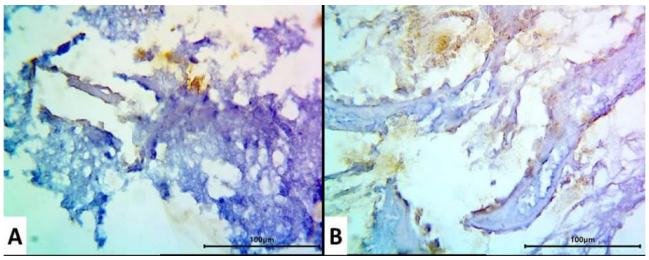

Figure 5: Immunohistochemical expression of Alkaline phosphatase after four weeks. (A): control group reveals weak positive reaction (1+). (B): study group reveals intense positive reaction (3+). (Dark brown is a positive reaction). 400X.

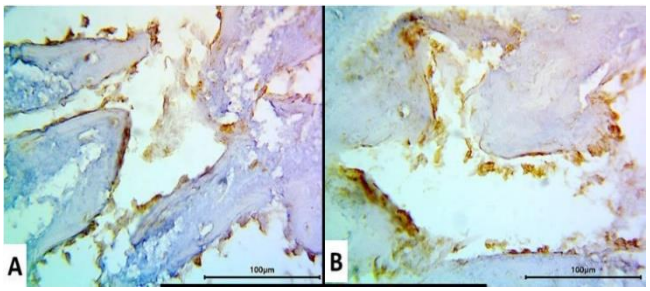

Figure 6: Immunohistochemical expression of Alkaline phosphatase after eight weeks. (A): control group reveals moderate positive reaction (2+). (B): study group reveals intense positive reaction (3+). (Dark brown is a positive reaction). 400X.

Table 4: Scores of the immunohistochemistry expression of the Alkaline phosphatase between control and study group protocols throughout the two scheduled intervals

Groups	Four weeks	Eight weeks	P value
Control	1 (0) B b	2 (1.75) B a	0.006
Study	3 (0.25) An a	3 (3) An a	0.651
P value	<0.001	0.002	

Data expressed as Median & IQR ((Inter-Quartile-Range) (N= 6 animals). Different capital letters among material groups in rows mean a significant difference at P≤0.05. Different small letters among periods in column groups mean there is a significant difference at P≤0.05.

Histopathological assessment

In control group, histological section of sheep tibia bone at four weeks showing the implant space area surrounded by high connective tissue, bone tissue, and few new bone formations with a low number of osteoblasts and osteocytes while at eight weeks showing the implant space area surrounded by new bone formation with a good number of osteoblasts and osteocytes (Figures 7 and 8). In study group, a histological section of sheep tibia bone at four weeks shows the implant space area surrounded by bone tissue, high new bone formation, and a good number of osteoblasts and osteocytes, while at eight weeks, the implant space area is surrounded by very well-developed new bone formation with a high number of osteoblasts and osteocytes (Figures 9 and 10).

The mean value of the thickness of new bone formation for the control group at four weeks was 112.60, and for the study group, it was 231.40, so there were significant differences between the two groups. After eight weeks, the mean value of the thickness of new bone formation of the control group was 181.20, and for the study group, it was 538.50. Therefore, significant differences existed between the control group and study group (Table 5).

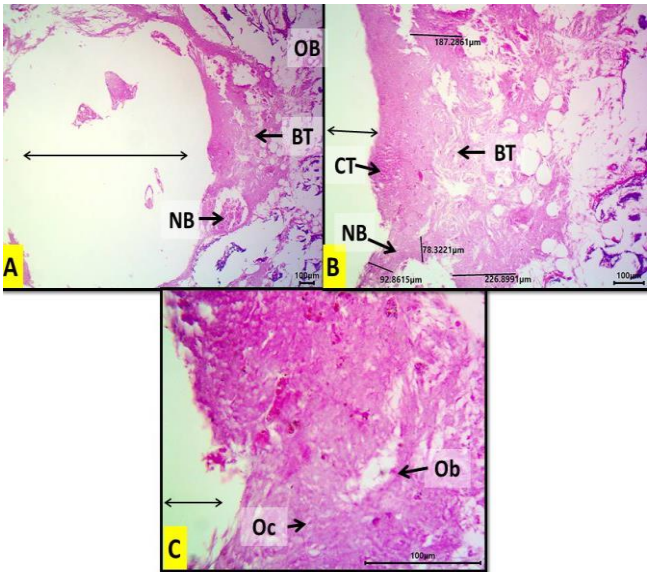

Figure 7: Histological section of sheep tibia bone of the control group at four weeks showing the implant space area (↔) surrounding by high connective tissue (CT), bone tissue (BT), few new bone formations (NB) with a low number of osteoblasts (OB) and osteocytes (OC), and old tibia bone (OB),). H&E stain, (A: 40X; B: 100X; C: 400X), Scale bar=100µm.

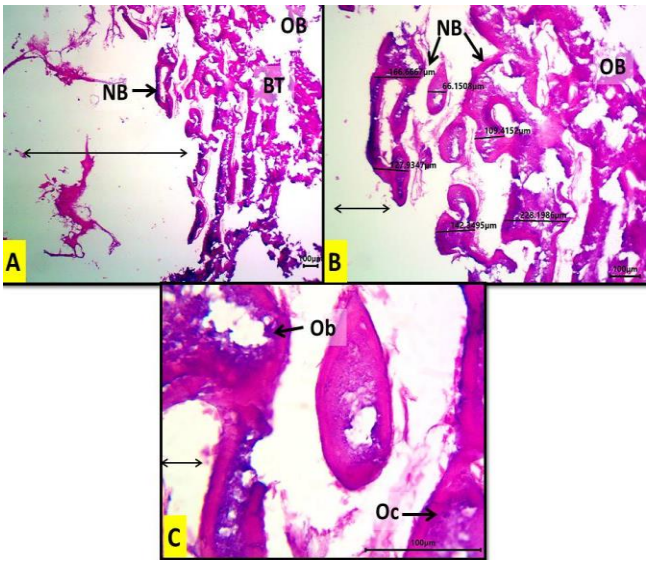

Figure 8: Histological section of sheep tibia bone of the control group at eight weeks showing the implant space area (↔) surrounded by new bone formation (NB) with a good number of osteoblasts (OB) and osteocytes (OC) and old tibia bone (OB),). H&E stain, (A: 40X; B: 100X; C: 400X), Scale bar=100µm.

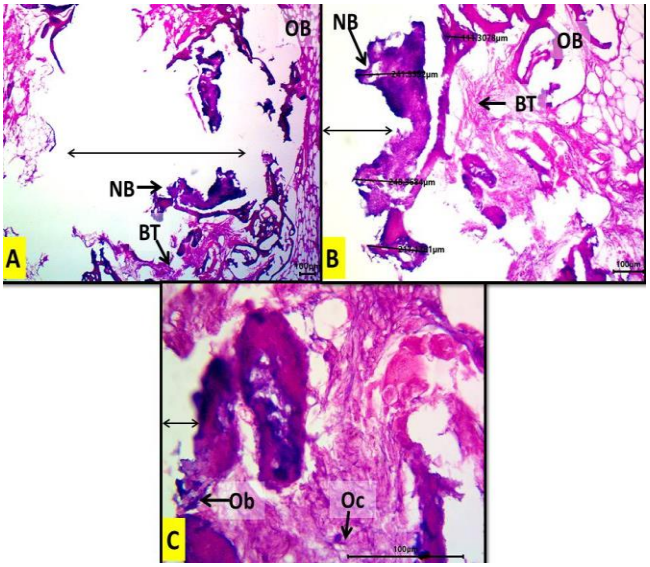

Figure 9: Histological section of sheep tibia bone of the study group at four weeks showing the implant space area (↔) surrounded by bone tissue (BT), high new bone formation (NB) with several osteoblasts (OB), and osteocytes (OC), and old tibia bone (OB),). H&E stain, (A: 40X; B: 100X; C: 400X), Scale bar=100µm.

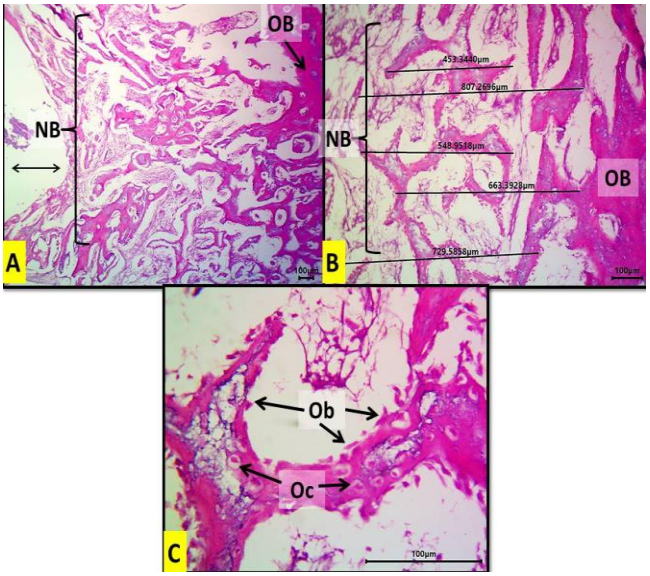

Figure 10: Histological section of sheep tibia bone of the study group at eight weeks showing the implant space area (↔) surrounded by very well-developed new bone formation (NB) with a high number of osteoblasts (OB) and osteocytes (OC) and old tibia bone (OB),). H&E stain, (A: 40X; B: 100X; C: 400X), Scale bar=100µm.

Table 5: The thickness of the new bone formation NB (micrometer µm) between control and study group protocols throughout the two scheduled intervals

Groups	Four weeks	Eight weeks	P value
Control	112.6±17.3 B b	181.2±21.6 B a	0.024
Study	231.4±15.3 A b	538.5±46.8 A a	<0.001
P value	0.001	<0.001	

Data expressed as Mean±stander error (N= 10 specimens). Different capital letters among material groups in rows mean a significant difference at P≤0.05. Different small letters among periods in column groups mean there is a significant difference at P≤0.05.

In the current study, the median and IQR for counts of osteoblast at four weeks for the control group was 3(3). The study group was 10.5(7.75); therefore, there was a significant difference between the control group and the study group, the median and IQR at eight weeks for the control group. The study group was 12.5(11.75) and 26(24.75), respectively, for counts of osteoblast, so there were highly significant differences between the two groups (Table 6).

The median and Inter-Quarter-Range for counts of osteocytes at four weeks for the control group was 4(0.5), and for the study group was 13(11.5); therefore, there was a significant difference between the control group and the study group, the median and Inter-Quarter-Range at eight weeks for control group and study group was 10.5(9), 25(22.5) respectively for counts of osteocytes so there were highly significant differences between two groups (Table 7).

Table 6: The counts of osteoblasts in control and study group protocols throughout the two scheduled intervals

Groups	Four weeks	Eight weeks	P value
Control	3 (3) B b	12.5 (11.75) B a	<0.001
Study	10.5 (7.75) A b	26 (24.75) A a	<0.001
P value	0.001	<0.001	

Data expressed as Median & IQR ((Inter-Quartile-Range) (N= 6 animals). Different capital letters among material groups in rows mean a significant difference at P≤0.05. Different small letters among periods in column groups mean there is a significant difference at P≤0.05.

Table 7: The counts of osteocytes in control and study group protocols throughout the two scheduled intervals

Groups	Four weeks	Eight weeks	P value
Control	4 (0.5) B b	10.5 (9) B a	<0.001
Study	13 (11.5) A b	25 (22.5) A a	<0.001
P value	0.001	<0.001	

Data expressed as Median & IQR ((Inter-Quartile-Range) (N= 6 animals). Different capital letters among material groups in rows mean a significant difference at P≤0.05. Different small letters among periods in column groups mean there is a significant difference at P≤0.05.

Discussion

The process of osseointegration is widely recognized as a fundamental requirement for the successful long-term clinical outcome and functional loading of endosseous dental implants. It plays a critical role in ensuring the stability of the implants. The phenomenon can be characterized as a direct anatomical and physiological connection between well-structured, viable bone tissue and the outer surface of an implant designed to endure mechanical loads (11). Platelet-rich fibrin is widely recognized for its potential as a potent inducer of bone formation and regeneration. This is attributed to its ability to enhance the proliferation of osteogenic cells, expedite the healing process of both soft and hard tissues, mitigate inflammation, suppress the formation of osteoclasts, and upregulate the expression of diverse growth factors in mesenchymal cells (12).

The contemporary utilization of platelet-rich fibrin presents many advantages. These include the facilitation of vascularization owing to its high concentration of growth factors, the absence of allergic reactions when obtained from the patient's own body (autologously), straightforward preparation within a brief timeframe, the elimination of potential risks associated with disease transmission, and the regulation of inflammation and infection through leukocytes and the cytokines they release (13,14).

Platelet-rich fibrin has the potential to enhance the process of bone repair (15-17). The osteopromoting matrix of the substance facilitates the controlled release of growth factors, which play a crucial role in regulating and facilitating the proliferation, migration, and adhesion of osteoblasts. These growth factors also modify the synthesis of collagen proteins (18). Low-level laser therapy demonstrates therapeutic effects in promoting the biostimulation of bone tissue and facilitating bone healing (19,20). Additionally, they enhance the viability of osteoblasts by exerting an osteogenic bio-stimulatory impact on cells resembling osteoblasts. This, in turn, facilitates the progression of linear bone formation, ultimately improving the healing capacity of the periodontal osseous defect (21,22). Additionally, it facilitates cellular proliferation and promotes the structure of proteins and collagen (23).

The process of wound healing differentiates osteoblasts and chondrocytes and cell regeneration (24-27). Furthermore, low-level laser therapy has been shown to stimulate blood circulation, promote rejuvenation, decrease susceptibility to infection, elevate metabolic activity, and facilitate the mending of damaged tissues (28). The rationale for utilizing Low-Level Laser Therapy is its ability to enhance cellular-level mechanisms that promote biochemical and molecular processes involved in tissue regeneration. Numerous in vivo and in vitro investigations have demonstrated the beneficial effects of low-level laser therapy on tissue healing processes (29).

Preclinical investigations have demonstrated the beneficial effects of low-level laser therapy on tissue healing, specifically in bone maturation and enhanced bone-implant contact. These studies have observed increased bone maturation and improved bone-implant contact in bone subjected to LLL irradiation, as compared to the control group (30); numerous studies have been undertaken to enhance bone healing in the vicinity of dental implants through investigations into implant designs, preservation of the host site, modification of surgical procedures or implant surfaces, duration of loading, and incorporation of bioactive materials into the prepared osteotomy site before dental implant fixture placement (31-34).

There were significant differences in alkaline phosphatase expression between the control and study groups at both four and eight weeks, indicating that the interventions in the study group had a significant and lasting effect on this biochemical marker.

The results of the current study showed that the combined use of low-level laser therapy and platelet-rich fibrin has a synergistic effect in promoting bone healing around dental implants (35). Several studies have shown a positive effect of such a combination in several treatment modalities. El-Hayes *et al*. (36) showed that the combined use of low-level laser therapy and platelet-rich fibrin had a more significant potential for inducing bone formation in bone defects compared to the use of LLLT or PRF alone (37). Thalaimalai *et al*. (38) found that when LLLT is utilized in conjunction with PRF in treating intra-bony defects, there is an observed improvement in both clinical and radiographic outcomes. Cevizcioğlu *et al*. demonstrated that combined utilization of platelet-rich fibrin and low-level laser therapy had greater efficacy in promoting bone healing than their application, resulting in an augmented volume of regenerated bone (39).

Arakeeb *et al*. (40) evaluated the concurrent utilization of platelet-rich fibrin and low-level laser therapy as having the potential to improve the process of osseointegration in dental implants, albeit to a lesser degree than the administration of PRF alone. Furthermore, the concurrent utilization of platelet-rich fibrin and low-level laser therapy has been found to have a more significant impact on the osseointegration of dental implants than the application of LLLT alone (41).

Conclusion

The study showed that bone healing around dental implants could be increased using concentrated growth factor with low-level laser therapy biostimulation. Such a combination will positively accelerate bone healing and the formation of new bone around the implant site.

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Conflicted interest

None.

References

1. Zhu Y, Zhang K, Zhao R, Ye X, Chen X, Xiao Z, Yang X, Zhu X, Zhang K, Fan Y, Zhang X. Bone regeneration with micro/nano hybrid-structured biphasic calcium phosphate bioceramics at segmental bone defect and the induced immunoregulation of MSCs. Biomater. 2017;147:133-44. DOI: 10.1016/j.biomaterials.2017.09.018
2. Mavrogenis AF, Dimitriou R, Parvizi J, Babis GC. Biology of implant osseointegration. J Musculoskelet Neuronal Interact. 2009;9(2):61-71. [available at]
3. Kwiatek J, Jaroń A, Trybek G. Impact of the 25-hydroxycholecalciferol concentration and vitamin D deficiency treatment on changes in the bone level at the implant site during the process of osseointegration: A prospective, randomized, controlled clinical trial. J Clin Med. 2021;10(3):526. DOI: 10.3390/jcm10030526
4. Thalaimalai DB, Victor DJ, Prakash PS, Subramaniam S, Cholan PK. Effect of low-level laser therapy and platelet-rich fibrin on treating intra-bony defects. J Las Med Sci. 2020;11(4):456. DOI: 10.34172/jlms.2020.71
5. Atiyah AG, Alkattan LM, Shareef AM. The radiological study of using fabricated calcium hydroxide from quail eggshell and plasma-rich fibrin to reconstitute a mandibular bone gap in dogs. Iraqi J Vet Sci. 2024;38(1):55-62. DOI: 10.33899/ijvs.2023.139898.2998
6. Mijiritsky E, Assaf HD, Peleg O, Shacham M, Cerroni L, Mangani L. Use of PRP, PRF, and CGF in periodontal regeneration and facial rejuvenation—a narrative review. Biol. 2021;10(4):317. DOI: 10.3390/biology10040317
7. Zedan IA, Alkattan LM, Al-Mahmood SS. Histopathological and immunohistochemical assessment of the using platelets rich fibrin to reinforce ventral hernioplasty in the sheep model. Iraqi J Vet Sci. 2023;37(4):821-829. DOI: 10.33899/ijvs.2023.139183.2900
8. Karaca IR, Ergun GU, Ozturk DN. Is low-level laser therapy and gaseous ozone application effective on osseointegration of immediately loaded implants?. Nigerian J Clin Pract. 2018;21(6):703-10. DOI: 10.4103/njcp.njcp_82_17
9. Yammine S, Jabbour E, El Toum S, Cassia A. Histological Study of induced incisions on rabbits' tongues with three diode lasers with different wavelengths in continuous mode. Sci. 2018:2691942. DOI: 10.1155/2018/2691942
10. Azevedo LH, Trevisan MS, de Souza AM. Lasers in soft tissues surgeries. In: de Freitas PM, Simões A, editors. Lasers in Dentistry. USA: Wiley; 2015. 173-178 p. DOI: 10.1002/9781118987742.ch23
11. Parithimarkalaignan S, Padmanabhan TV. Osseointegration: An update. J Indian Prosthodont Soc. 2013;13(1):2-6. DOI: 10.1007/s13191-013-0252-z
12. Kargarpour Z, Nasirzade J, Strauss FJ, Di Summa F, Hasannia S, Müller HD, Gruber R. Platelet-rich fibrin suppresses in vitro osteoclastogenesis. J Periodont. 2020;91(3):413-21. DOI: 10.1002/JPER.19-0109
13. Del Fabbro M, Bucchi C, Lolato A, Corbella S, Testori T, Taschieri S. Healing of postextraction sockets preserved with autologous platelet concentrates. A systematic review and meta-analysis. J Oral Maxillofac Surg. 2017;75(8):1601-15. DOI: 10.1016/j.joms.2017.02.009
14. Dragonas P, Katsaros T, Avila-Ortiz G, Chambrone L, Schiavo JH, Palaiologou A. Effects of leukocyte–platelet-rich fibrin (L-PRF) in

different intraoral bone grafting procedures: a systematic review. J Oral Maxillofac Surg. 2019;48(2):250-62. DOI: 10.1016/j.ijom.2018.06.003
15. Hamed R. Comparison of platelet-rich fibrin using three centrifuges and its impact on bone [Ph.D. dissertation]. Mosul: College of Dentistry/ University of Mosul; 2016.
16. Caruana A, Savina D, Macedo JP, Soares SC. From platelet-rich plasma to advanced platelet-rich fibrin: Biological achievements and clinical advances in modern surgery. Eur J Dent. 2019;13(02):280-6. DOI: 10.1055/s-0039-1696585
17. Lokwani BV, Gupta D, Agrawal RS, Mehta S, Nirmal NJ. The use of concentrated growth factor in dental implantology: A systematic review. J Indian Prosthodont Soc. 2020;20(1):3. DOI: 10.4103/jips.jips_375_19
18. Schär MO, Diaz-Romero J, Kohl S, Zumstein MA, Nesic D. Platelet-rich concentrates differentially release growth factors and induce cell migration in vitro. Clin Orthop Relat Res. 2015;473:1635-43. DOI: 10.1007/s11999-015-4192-2
19. Çırak E, Özyurt A, Peker T, Ömeroğlu S, Güngör MN. Comparative evaluation of various low-level laser therapies on bone healing following tooth extraction: An experimental animal study. J Craniomaxillofac Surg. 2018;46(7):1147-52. DOI: 10.1016/j.jcms.2018.05.012
20. Noor SK, Munahi AK, Allban AA, Hussain MH. Study the beneficial activity of low-level laser in experimentally induced pain in mice. Iraqi J Vet Sci. 2021;35(3):569-573. DOI: 10.33899/ijvs.2020.127228.1487
21. Hamajima S, Hiratsuka K, Kiyama-Kishikawa M, Tagawa T, Kawahara M, Ohta M, Sasahara H, Abiko Y. Effect of low-level laser irradiation on osteoglycin gene expression in osteoblasts. Lasers Med Sci. 2003;18:78-82. DOI: 10.1007/s10103-003-0255-9
22. Cortellini P, Tonetti MS. Minimally invasive surgical technique and enamel matrix derivative in intra-bony defects. I: Clinical outcomes and morbidity. J Clin Periodont. 2007;34(12):1082-8. DOI: 10.1111/j.1600-051X.2007. 01144.x
23. Rola P, Włodarczak S, Lesiak M, Doroszko A, Włodarczak A. Changes in cell biology under the influence of low-level laser therapy. InPhotonics. 2022;9(7):502. DOI: 10.3390/photonics9070502
24. Abdulmawjood YF, Thanoon MG. A comparative study of nano magnesium oxide versus platelets rich fibrin to repair the induced radial fracture in dogs. Iraqi J Vet Sci. 2022;36(2):451-458. DOI: 10.33899/ijvs.2021.130500.1836
25. Yang TS, Nguyen LT, Hsiao YC, Pan LC, Chang CJ. Biophotonic effects of low-level laser therapy at different wavelengths for potential wound healing. InPhotonics. 2022;9(8):591. DOI: 10.3390/photonics9080591
26. Shaikh-Kader A, Houreld NN. Photobiomodulation, Cells of connective tissue and repair processes: A look at in vivo and in vitro studies on bone, cartilage and tendon cells. InPhotonics. 2022;9(9):618. DOI: 10.3390/photonics9090618
27. Babtan AM, Ilea A, Feurdean CN, Ceci S, Pula B, Candrea S, Azzollini D, Piras F, Curatoli L, Corriero A, Patano A. Biostimulation with low-level laser therapy and its effects on soft and hard tissue regeneration. Literature review. J Mind Med Sci. 2022;9(1):28-37. DOI: 10.22543/7674.91.P2837
28. Torkzaban P, Kasraei S, Torabi S, Farhadian M. Low-level laser therapy with 940 nm diode laser on stability of dental implants: A randomized controlled clinical trial. Laser Med Sci. 2018;33:287-93. DOI: 10.1007/s10103-017-2365-9
29. Goymen M, Isman E, Taner L, Kurkcu M. Histomorphometric evaluation of the effects of various diode lasers and force levels on orthodontic mini screw stability. Photomed Laser Surg. 2015;33(1):29-34. DOI: 10.1089/pho.2014.3833
30. Gomes FV, Mayer L, Massotti FP, Baraldi CE, Ponzoni D, Webber JB, De Oliveira MG. Low-level laser therapy improves peri-implant bone formation: Resonance frequency, electron microscopy, and stereology findings in a rabbit model. J Oral Maxillofac Surg. 2015;44(2):245-51. DOI: 10.1016/j.ijom.2014.09.010
31. Smeets R, Stadlinger B, Schwarz F, Beck-Broichsitter B, Jung O, Precht C, Kloss F, Gröbe A, Heiland M, Ebker T. Impact of dental implant surface modifications on osseointegration. BioMed Res Int. 2016;2016. DOI: 10.1155/2016/6285620
32. Allawi AH, Saeed MG. Effect of homologous platelet rich fibrin matrix and injectable platelet rich fibrin on total thickness skin autograft healing in dogs. Iraqi J Vet Sci. 2023;37(I-IV):55-64. DOI: 10.33899/ijvs.2022.1371380.2643
33. Souza JC, Sordi MB, Kanazawa M, Ravindran S, Henriques B, Silva FS, Aparicio C, Cooper LF. Nano-scale modification of titanium implant surfaces to enhance osseointegration. Acta Biomater. 2019;94:112-31. DOI: 10.1016/j.actbio.2019.05.045
34. El-shafey SA, El-Mezyen AF, Behery AS, Abd El Raouf M. Comparing efficacy of the platelet rich plasma and advanced platelet rich fibrin on tibial bone defect regeneration in dogs. Iraqi J Vet Sci. 2022;36(4):973-980. DOI: 10.33899/ijvs.2022.132765.2129
35. Chen CH, Coyac BR, Arioka M, Leahy B, Tulu US, Aghvami M, Holst S, Hoffmann W, Quarry A, Bahat O, Salmon B. A novel osteotomy preparation technique to preserve implant site viability and enhance osteogenesis. J Clin Med. 2019;8(2):170. DOI: 10.3390/jcm8020170
36. El-Hayes KA, Zaky AA, Ibrahim ZA, Allam GF, Allam MF. Low-level laser biostimulation and platelet-rich fibrin use in bone healing: Experimental study. Dent Med Probl. 2016;53(3):338-44. DOI: 10.17219/dmp/62973
37. Raffea NM, Allawi AH. Effect of autologous peritoneum and platelet-rich fibrin graft on healing of intestinal anastomosis in dogs. Iraqi J Vet Sci. 2022;36(2):459-470. DOI: 10.33899/ijvs.2021.130529.1840
38. Thalaimalai DB, Victor DJ, Prakash PS, Subramaniam S, Cholan PK. Effect of low-level laser therapy and platelet-rich fibrin on treating intra-bony defects. J Laser Med Sci. 2020;11(4):456. DOI: 10.34172/jlms.2020.71
39. Mahmood S, Abdul Razak IB, Yaseen M, Yusof LM, Jeber ZK, Gimba F, Zakaria MZ. 3-D nanocomposite scaffolds: Tissue engineering for bone reconstruction. Iraqi J Vet Sci. 2022;36(2):433-444. DOI: 10.33899/ijvs.2021.130481.1831
40. Cevizcioğlu Ö, Şençimen M, Özkan A, Bariş E, Çelik D, Samur Ergüven SA. Evaluating the effect of platelet-rich fibrin and low-level laser therapy on new bone formation after tooth extraction. Dent Oral Maxillofac Res. 2021;7. DOI: 10.15761/domr.1000392
41. Arakeeb MA, Zaky AA, Harhash TA, Salem WS, El-Mofty M. Effect of combined application of growth factors and diode laser bio-stimulation on the osseointegration of dental implants. Open Access Maced J Med Sci. 2019;7(15):2520. DOI: 10.3889/oamjms.2019.672

تأثير العلاج بعامل النمو المركز والليزر منخفض المستوى على التكامل العظمي بزرعات الأسنان: دراسة تجريبية في الأغنام

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الخلاصة

تهدف الدراسة الحالية لتقييم تأثير عامل النمو المركز مع التحفيز الحيوي للعلاج بالليزر منخفض المستوى على شفاء العظام حول زراعة الأسنان في نموذج حيواني. تم استخدام ٤ ذكور من الأغنام بصحة جيدة للدراسة. تم تقسيم الأغنام إلى مجموعتين، كل نموذج بمثابة مجموعتين فرعيتين للمراقبة. تم إخضاع ساق كل خروف لإجراءات جراحية عشوائية، مع وجود فجوة شهر وشهرين بين كل عملية. تم زراعة ما مجموعه ٤٠ زراعة أسنان، حيث تلقت كل خروف عشر زرعات (خمس زرعات في كل ساق). تم تقسيم المجموعات على النحو التالي: المجموعة الأولى (السيطرة) حيث تم وضع عشر زرعات في كل خروف (خمس زرعات أسنان في كل قصبة)، والمجموعة الثانية (مجموعة الدراسة حيث تم وضع عشر زرعات في كل خروف (خمس زرعات أسنان في كل قصبة) باستخدام غشاء ساكو (عامل النمو المركز) والتحفيز الحيوي للعلاج بالليزر منخفض المستوى. تم استخدام تحليل قياس الكثافة باستخدام التصوير الشعاعي الرقمي والتقييم النسيجي الكيميائي المناعي والتشريح المرضي. كشفت نتائج البحث الحالي عن تباين ذو دلالة إحصائية في مدى نمو العظام المحيطة بزراعة الأسنان عند أربعة وثمانية أسابيع، حيث أظهرت مجموعة الدراسة نتائج أكثر إيجابية من السيطرة. في الختام، لوحظ أن استخدام عامل النمو المركز بالتزامن مع التحفيز الحيوي للعلاج بالليزر منخفض المستوى يساهم في تكوين عظام جديدة وإنشاء التكامل العظمي.