Archives of Razi Institute, Vol. 76, No. 5 (2021) 1445-1451

DOI: 10.22092/ari.2021.356310.1818

Original Article

Physical, Chemical, and Mechanical Evaluation of Irradiated Xeno-Sheep Bony Implantation by Low-Level Laser Therapy of Long Bones Fractures in Rabbits

Nazht, H. H[1] *, Abduljabbar Imran, H[1], Omar, R. A[2]

1. Surgery and Obstetric Department, Veterinary Medicine College, Baghdad University, Baghdad, Iraq
2. Physiology, Biochemistry, and Pharmacology Department, Veterinary Medicine College, Baghdad University, Baghdad, Iraq

Received 1 October 2021; Accepted 25 October 2021

Corresponding Author: humam_nazhat@yahoo.com

Abstract

Bone grafts/implantation is widely used in veterinary medicine. The present study aimed to evaluate the physical, chemical, and mechanical prosperities of irradiated xeno-sheep bony implantation (X-SBI) by low-level laser therapy (LLLT) implanted in the induced empty defect of the femoral bones in rabbits. A total of 10 adult rabbits were used to create a 1cm length of the femoral gab surgically; thereafter, the empty space was filled with X-SBI and internally fixed by intramedullary pinning with two femoral fragments. The operated rabbits were assigned to the un-irradiated control group 1 which was left without laser irradiation, and irradiated group (group 2) which was irradiated on a daily basis by a continuous diode laser, a single dose at four points at the lateral aspect of the X-SBI for 5 min at a period of 72 intervals for 14th days post-operation with a dosage of 850 nm, 148.4 J/cm^2. The parameters which were used for the evaluation of results after 3rd-month post-operation were physical, chemical, and mechanical examinations. The physical examination revealed high bone density and hardness at the sites of X-SBI of the irradiated group, as compared to the un-irradiated animals. Moreover, the chemical analysis demonstrated an increment in the level of bone calcium and phosphorus elements, as well as a decrease in the level of magnesium, potassium, and sodium in the irradiated group, as compared to the un-irradiated group. The mechanical and fracture tolerance results demonstrated a gradually high resistance level of fracture tolerance of irradiated animals, as compared to un-irradiated rabbits. It can be concluded that the irradiated X-SBI by LLLT could be used strongly and successfully to fill the empty space in the femoral bone, supporting body weight better and faster than the control group, with no complications or body rejection.

Keywords: Xeno-sheep bony implantation, Low-level laser therapy, Intramedullar

---

1. Introduction

Bone graft/implantation is widely used in veterinary medicine, especially as a common procedure for the treatment of various types of fractures characterized by bone loss, such as gunshot fracture, or fracture complications, such as delayed union or nonunion, as well as pathological fractures in bone osteosarcoma (1). Bone grafting \implantation could be used as a framework to provide stability, promote healing, and fill osseous defects or cavities (2, 3). Bone Fracture repair processing must be ended by restoration and proper tissues mineralization with mechanical strength and bone integrity to normalize functionality of the affected bone (4).

For several years, many trials were employed to accelerate bone repair and healing regeneration processing using internal or external fixation devices, as well as different chemical agents, such as prostaglandins (PGF2a or PGE2), to promote fracture healing or bone resorption in the remodeling phase (5, 6) or facilitate fracture healing using Laser (7-9), stem cells, growth factors, extracorporeal shock waves, platelets rich plasma (PRP), or omental graft (10). Thanoon (11) pointed out that fracture repair processing can be stimulated by applying bone marrow and PRP. Moreover, the results of a study conducted by Zebon (12) indicated that using nano crab shell scaffold gave better acceleration repair processing in large bone defects.

In the same context, in their study, Bielby (7) suggested that applying low-level laser therapy (LLLT) lead to a significant increase in the biological effects in the following process: epithelization with fibroblast cells stimulation, new collagen fiber production, positive phagocytosis stimulation, and bone tissue regeneration. Weber, Pinheiro (13) also indicated that laser had a stimulatory effect on bone implantation.

In light of the aforementioned issues, the current study aimed to assess the effect of irradiated xeno-sheep bony implantation by LLLT inserted in the femoral bone defect in rabbits. The evaluation was conducted based on the following parameters: physical, chemical, and mechanical lab analysis.

2. Materials and Methods

2.1. Experimental Animals

A total of 10 apparently healthy adult rabbits of local breed, weighing about 1.25-1.5 kg, were housed in special cages (40×60×70 cm) for 7 days (in the animals' house /veterinary medicine college, university Baghdad) for general observation and body adaptation. They received an antiparasitic drug (0.1 mg/kg Ivermectin). The rabbits were kept in standard cages, one in each, under living conditions of a 12-hour light/12-hour darkness cycle. The room temperature and humidity were maintained at 23°C and 55±10%, respectively.

2.2. Surgical Procedure and Laser Treatment

All the animals were employed to induce two surgical transverse fractures in the femoral diaphysis to remove 1 cm length of the bone (Figure 1), surgically under general anesthesia (14, 15). Thereafter, the femoral gap was filled with a suitable size of xeno- bony implantation (Figure 2) which was prepared from the sheep ribs (the specimens were freshly collected from a slaughterhouse, the soft tissues were removed, and the device was washed and cleaned, followed by deproteinization). Both the xeno-bony device and the two femoral fragments were fixed with intramedullary pinning of 2.5 mm diameter (Figure 3), the operated rabbits were subdivided into two groups (n=5). Group 1, the control group, was followed for normal bone healing processing without laser irradiation, while group 2, the treatment group, applied a daily dose of diode laser (continuous) immediately (k-laser portable) after the operation at four points on the lateral aspect of the femoral bone exactly at the Xeno bony implantation sites for 5 min each 72 h until 14 days post-operation at a dose of 850 nm and 148.4 J/cm^2 (Figure 4). The animals were kept for clinical observation at 3rd-month post-operation. The physical, chemical, and mechanical examinations were carried out in the physical laboratory of Al-nahreen University. All the operated rabbits were euthanized with a high dose of an anesthetic agent; subsequently, the specimen of bone about 1cm length was taken which includes a part of the recipient femoral bone and the xeno-bony implantation which were kept in 10% nutrient formalin.

Figure 1. Exposing the femoral bone for a transverse fracture to remove 1cm of bone

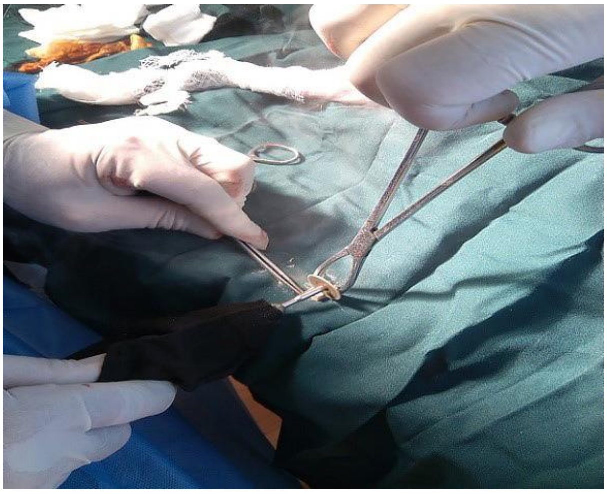

Figure 2. Preparing the satiable size of the sheep Xeno bony implantation

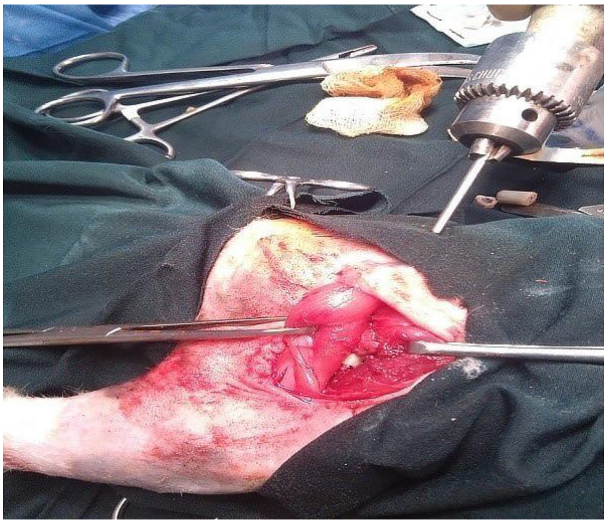

Figure 3. Both the sheep Xeno bony implantation and the two femoral fragments were fixed with intramedullary pinning (yellow arrow)

Figure 4. Laser irradiation at the treatment group 850 nm 148.4 J/cm at four points at the lateral side of the femoral bone for 5 min at 72 intervals for 14 days

2.3. Tensile test

The rabbits were euthanized by an intravenous injection of 1 ml fluanisone/fentanyl (Hypnorms, Janssen, Belgium) and 1 ml/kg body weight intravenous pentobarbital (Mebumals, Rikshospitalets Apotek, Oslo, Norway). Immediately after euthanization, the superficial soft tissues covering the implants were carefully removed. A hole was made in the center of the PTFE cap with a hypodermic needle, and pressurized air was applied to remove the caps. The tibial bone was then fixed in a specially designed jig to stabilize the bone during the tensile test procedure. A threaded pin with a ball-head was then attached to the implant and adjusted perpendicular to the load cell using a level tube. To minimize shear forces, a 300 mm long wire was connected between the load cell and the ball-head. The tensile test was performed with a Lloyds LRX Material testing machine fitted with a calibrated load cell of 100 N. The crosshead speed range was set to 1 mm/ min. Force measuring accuracy was 71% (16).

2.4. Bone Chemical Analysis

Photoacoustic (PA) signal analysis based on ultrasonic wave detection can provide both high-sensitivity optical contrast information and micro-architectural information which is highly related to the chemical composition of tissue. In the present study, the feasibility assessment of bone composition assessment was investigated using the multi-wavelength PA analysis (MWPA) method which could reflect the molecular information. By illuminating a bone specimen using laser light with wavelength over an optical spectrum ranging from 680-950 nm, the optical absorption spectrum of the bone was acquired. Thereafter, with the optical absorption spectra of all optical absorption chemical components in the known bone, a spectral unmixing procedure was performed to quantitatively assess the relative content of each chemical component. The bone composition of the two groups was measured using MWPA at the range of 690-950 nm at 10 nm intervals (17).

2.5. Statistical Analysis

All measurements of data were made blindly without knowing if a treated or untreated specimen was evaluated. Statistical analyses were performed using Student's t-test to compare the data from irradiated and control groups. The results were considered significant when the level of probability was 0.05 or less (16).

3. Results

3.1. Bone Physical Examination

The physical examination in both groups revealed that the treatment group which was exposed to laser irradiation displayed a marked increase in the density and hardness of the bone, as compared to the control group (Figures 5 and 6).

3.2. Bone Chemical Analysis

There was an increase in the levels of calcium and phosphor elements in the laser group, as compared to the un-irradiated group, while the levels of other elements, such as sodium, magnesium, and potassium showed a decrease in the total concentration of the laser group, compared to the un-irradiated group (Figures 7, 8, and Table 1).

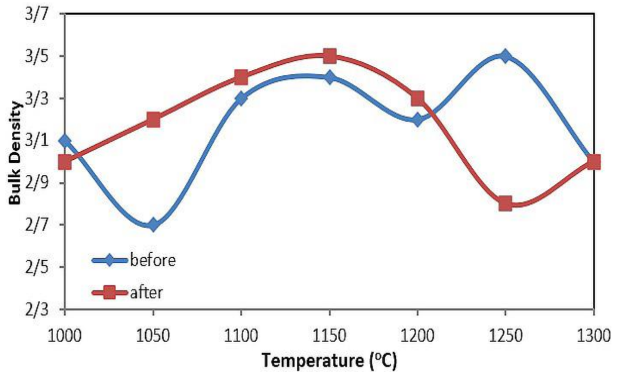

Figure 5. High mineralized bone density of laser group red line, as compared to the low bone density of the control group blue line

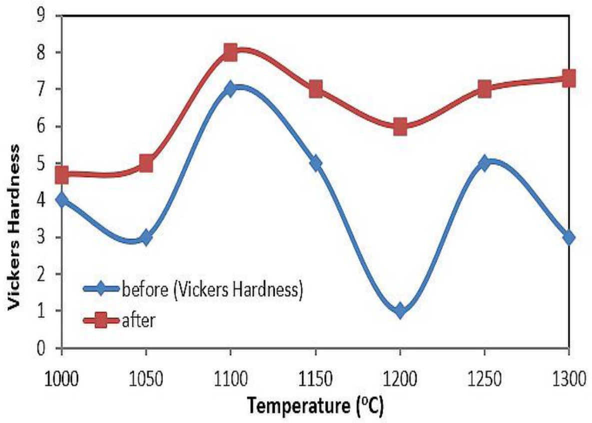

Figure 6. Hardness of the new bone formation, treatment group showing more hard red line and the control group depicting a less hard bone blue line

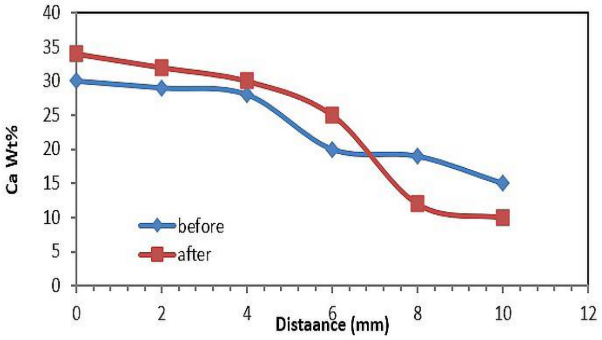

Figure 7. Different calcium levels of high-level treatment group red line and the low-level control group blue line

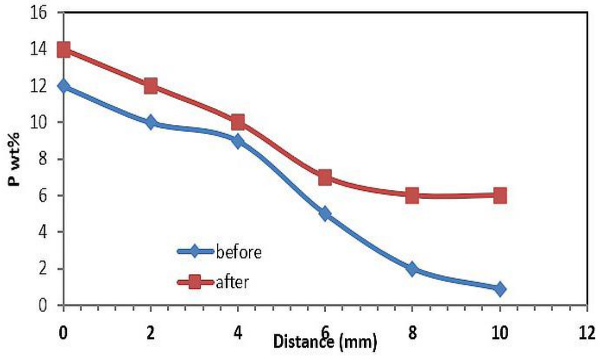

Figure 8. Different phosphor levels between the high-level treatment group red line and the low-level control group blue line

Table 1. Different element levels between the treatment and control groups

Elements	Concentration (wt.%) of irradiated group	Concentration (wt.%) of the control group
Ca	80	75
P	25	20
Na	2	3
Mg	1.5	2
K	0.04	0.05

3.3. Bone Mechanical and Fracture Tolerance Analysis

The treatment group demonstrated more resistance to pressure and was able to tolerate the fracture test more than the control group (Figure 9).

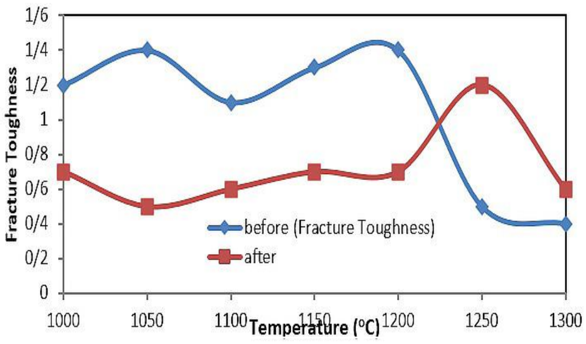

Figure 9. Graphic design displaying a difference in fracture tolerance between the more fracture resistance of treatment group red line and the less fracture resistance of the control group blue line

4. Discussion

The sheep Xeno bony implantation in both groups can be used as a space filler, supporting body weight and promoting fracture healing, without body rejection or complication conducted (18). The use of Xeno bony implantation has low immunogenicity and can promote healing, fill the spaces, and support body weight.

The irradiated group resist fracture tolerance examination, as compared to the un-irradiated group. The mechanical and physical test displayed the hard, high density with an increase in calcium and phosphor levels, as compared to the control group. This statement is in agreement with the finding obtained by Pinheiro, Oliveira (19), and Liu, Lyon (20) who indicated that laser leads to an increase in the volume and opacity of callus formation around the bony device, fixing the Xeno bony implantation more and supporting the body weight.

The LLLT had positive effects on the bone healing leading to more mineral production and deposition lead to increased hardness and density of the new bone formation (21-23) as shown in the results of the treatment group which lead to bear the weight even after removing the intramedullary pin. As mentioned by Pinheiro, Oliveira (19), and Liu, Lyon (20), laser irradiation leads to the increased amount and mineralized bone tissue, with market collagen fiber mineralization and hasten bone cell proliferation onto implant surface, resulting in bony incorporation.

The physical and mechanical test in the laser group seemed more density and hardness than the un-irradiated group and when exposed to the pressure with heat the treatment group can tolerate fracture than the control group because of the high density during mineral deposition (4, 24).

The chemical analysis showed an increase in the calcium and phosphorus levels in the treatment group, compared to the control group. Laser power accelerates bone regeneration, can be reflected by alkaline phosphates activity, and leads to calcium accumulation (22, 23, 25). The LLLT increases bone metabolism and mineralization in early periods of bone regeneration processing (24, 26) referring to bony device changes to the active and alive bone by creeping substitution process through invading active osteocytes and blood vessels from the recipient toward the Xeno bony implantation.

The irradiated Xeno-sheep bony implantation can be used in the treatment of bone defect and fracture healing processing since it supports body weight without body rejection.

Authors' Contribution

Study concept and design: H. H. N.
Acquisition of data: H. H. N.
Analysis and interpretation of data: H. A. I.
Drafting of the manuscript: R. A. O.
Critical revision of the manuscript for important intellectual content: H. H. N., H. A. I. and R. A. O.
Statistical analysis: H. H. N.
Administrative, technical, and material support: H. H. N., H. A. I. and R. A. O.

Ethics

All the procedures were approved by the Ethics Committee at the Baghdad University, Baghdad, Iraq under the project number: 2020-7897-4125.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Schell H, Thompson MS, Bail HJ, Hoffmann J-E, Schill A, Duda GN, et al. Mechanical induction of critically delayed bone healing in sheep: radiological and biomechanical results. J Biomech. 2008;41(14):3066-72.
2. Lohmann C, Andreacchio D, Köster G, Carnes Jr D, Cochran D, Dean D, et al. Tissue response and osteoinduction of human bone grafts in vivo. Arch Orthop Trauma Surg. 2001;121(10):583-90.
3. Myerson MS, Neufeld SK, Uribe J. Fresh-frozen structural allografts in the foot and ankle. J Bone Joint Surg Am. 2005;87(1):113-20.
4. Marsell R, Einhorn TA. The biology of fracture healing. Injury. 2011;42(6):551-5.
5. Nazhat HH. Effects of Prostaglandin E2 on Bone Resorption in the Remodeling Phase of the Femoral Bone Fractures in Rabbits. 2000.
6. Nazht HH, Omara RA, Al Dahhan MR, Khaleefa RK, Kareem SM, Manual ZM, et al. Estimation of alkaline phosphatase enzymes level in the femoral transverse fractures healing in rabbits. Arts, Humanities and Natural Sciences Conferences. 2019.
7. Bielby R, Jones E, McGonagle D. The role of mesenchymal stem cells in maintenance and repair of bone. Injury. 2007;38(1):S26-S32.
8. Eesa M. Histopathological study of the influence of platelet rich-plasma and low level laser therapy on healing of experimentally fractured proximal sesamoid bone in equine. Iraqi J Vet Med. 2017;41(1):160-8.
9. Nazhta HH, Almahmoudb AM, Aboodc DA. Using Xeno-Bovine Bony Implantation as Space Filler in Femoral Defect in Rabbits. Arts, Humanities and Natural Sciences Conferences. 2020.
10. Eesa M, Mohammed H, Nahi H. Role of Auto-Omental Graft on The Fracture Healing After Periosteum Destruction, Radiographic Study. Basra J Vet Res 2013;12(2):301-6.
11. Thanoon M. Histopathological evaluation of the platelets rich fibrin and bone marrow on healing of experimental induced distal radial fracture in local dogs. Iraqi J Vet Med. 2019;43(1):11-20.
12. Zebon SH. Efficacy of Nano Composite Porous 3D Scaffold of Crab Shell and Al-Kharit Histological and Radiological for Bone Repair in Vivo. Iraqi J Vet Med. 2020;44(2):15-24.
13. Weber JBB, Pinheiro ALB, Oliveira MGd, Oliveira FAM, Ramalho LMP. Laser therapy improves healing of bone defects submitted to autologus bone graft. Photomed Laser Surg. 2006;24(1):38-44.
14. Eesa M. Evaluation of general anaesthesia by using Propionylpromazine, Xylazine and Ketamine in rabbits. Iraqi J Vet Med. 2010;34(1):208-17.
15. Dadashpour Davachi N, Bartlewski PM, Masoudi R, Ahmadi B, Didarkhah M. Induction of ovulation after artificial insemination in rabbits: Intramuscular injection of gonadotropin-releasing hormone (GnRH) agonist vs. intravenous administration of mated doe serum. Iran J Vet Med. 2021.
16. Khadra M, Rønold HJ, Lyngstadaas SP, Ellingsen JE, Haanæs HR. Low-level laser therapy stimulates bone–implant interaction: an experimental study in rabbits. Clin Oral Implants Res. 2004;15(3):325-32.
17. Feng T, Xie Y, Xie W, Ta D, Cheng Q. Bone Chemical Composition Analysis Using Photoacoustic Technique. Front Phys. 2020;8(594).
18. Slatter DH. Textbook of small animal surgery: Elsevier Health Sciences; 2003.
19. Pinheiro AL, Oliveira MG, Martins PPM, Ramalho LMP, de Oliveira MAM, Júnior AN, et al. Biomodulatory effects of LLLT on bone regeneration. Laser Ther. 2000;13(1):73-9.
20. Liu X, Lyon R, Meier HT, Thometz J, Haworth ST. Effect of lower-level laser therapy on rabbit tibial fracture. Photomed Laser Surg. 2007;25(6):487-94.
21. Dörtbudak O, Haas R, Mailath- Pokorny G. Effect of low- power laser irradiation on bony implant sites. Clin Oral Implants Res. 2002;13(3):288-92.
22. Otremski I, Irga D, Edelstein S, Ornoy A, Newman R. Does laser irradiation effect fracture healing? Med Laser Appl. 2004;19(3):146-9.
23. Pretel H, Lizarelli RF, Ramalho LT. Effect of low- level laser therapy on bone repair: Histological study

in rats. Lasers Surg Med. 2007;39(10):788-96.

24. Schindeler A, McDonald MM, Bokko P, Little DG, editors. Bone remodeling during fracture repair: The cellular picture. Seminars in cell & developmental biology. 2008.

25. AboElsaad NS, Soory M, Gadalla LM, Ragab LI, Dunne S, Zalata KR, 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. 2009;24(3):387-95.

26. Valiati R, Paes JV, de Moraes AN, Gava A, Agostini M, Masiero AV, et al. Effect of low-level laser therapy on incorporation of block allografts. Int J Med Sci. 2012;9(10):853.