ISSN 2664-4169 (Print) & ISSN 2664-7923 (Online)
South Asian Research Journal of Biology and Applied Biosciences
Abbreviated Key Title: South Asian Res J Bio Appl Biosci

| Volume-6 | Issue-1 | Jan-Feb -2024 | **DOI: 10.36346/sarjbab.2024.v06i01.003**

Original Research Article

Histological Assessment of the Effect Laser Irradiation on Full-Thickness Skin Wound Healing in Rabbits Models

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Article History
Received: 19.12.2023
Accepted: 24.01.2024
Published: 27.01.2024

Abstract: Since there is currently no ideal treatment, wound healing impairment is a significant clinical challenge that is the subject of ongoing research into effective wound healing strategies. As a result, it has been demonstrated that low level laser therapy (LLLT), which is used for wound healing, can effectively control both the local and the systemic response. This study was conducted on 24 adult, clinically healthy male rabbits for this purpose. On each animal's back, full-thickness excisional wounds measuring 2 cm x 2 cm were created following sterile skin preparation. The animals were randomly split into two equal groups, each with twelve rabbits. In the group that acts as a control; 5 milliliters of phosphate-buffered saline was used to treat wounds. In the group treated; for the first seven days, the wounds were only treated with (LLLT) at a rate of 5/5 second at 8j/cm2. At the seventh, fourteenth, and 35th days of healing, histopathological biopsies were performed. The control group's histopathology revealed hemorrhage with inflammatory cell infiltration, primarily neutrophils, and congested blood vessels in the gap seven days after surgery. Necrotized neutrophils, hemolysis, and granulation tissue under the dermis were present in the gap at fourteen days. Between the muscle fibers, there was hemolysis. At 35 days, there was irregular proliferation of fibrous connective tissue, and congested blood vessels with mononuclear cell infiltration could be seen in the gap. At seven days, the treated group has completely sloughed the epidermis and a thin crust on the skin's surface with a small incision. In the dermis, there is a lot of infiltration of inflammatory cells, mostly lymphocytes and macrophages. There is also a lot of granulation tissue and a lot of adipose tissue with a lot of thick collagen network. At fourteen days, the epidermis' epithelial layers have healed completely and a keratinized layer is present. In the dermis, a proliferating and thick collagen network, scattered inflammatory cells, and regular fibrosis with hemorrhage and the development of a new B.V. At day 35, look for narrow scar tissue with complete epithelization, a keratinized layer above the epidermis, new vascularization with a lot of regular fibrosis and few regular collagen, new hair follicles, new blood vessels, and scattered macrophages. We concluded that full-thickness wound healing is positively influenced by the use of low level laser therapy (LLLT), which creates a microenvironment favorable to cell growth and differentiation.

Keywords: Low level laser therapy, cutaneous wounds, rabbit, wounds healing.

INTRODUCTION

Materials that have the potential to improve the environment of the wound bed have been developed as a result of advancements in tissue repair and the treatment of problematic wounds. Various treatment modalities are used to improve the wound bed as part of proper wound care [1]. The goal of wound care is to speed up wound healing while minimizing pain, discomfort, and scarring for the patient [2]. This must take place in a physiologic environment that encourages tissue repair and regeneration. The return of tissue functions and integrity is the result of a highly organized and coordinated sequence of processes during cutaneous wound healing. Non-healing chronic wounds may develop as a result of a disorder in the normal wound healing process. Diabetes, renal disease, trauma, venous or arterial insufficiency, and old age are all potential causes of a delay in wound healing [3]. In addition to local variables like; ischemia, tissue hypoxia, foreign bodies, exudates, infection, tissue maceration, distraction of the inflammatory process's regulation, and systemic factors like; Immune deficiencies and inadequate nutrition can all hinder healing [4, 5]. Laser therapy has the potential to boost the

number of vessels, boost the number of fibroblasts, and encourage the collagenization process [6]. The anti-inflammatory effect of laser irradiation may be one of the most important therapeutic effects on many injuries and disorders, including wound healing [7], despite the fact that the molecular mechanism of LLLT has not been fully elucidated. It is thought that photons primarily strike mitochondria. Since they contain cytochrome c, cytochrome c oxidase is a component of the cellular respiratory chain. It is a light receptor or photo acceptor that, upon photon absorption, initiates a series of cellular respiratory effects [8]. As a result, the purpose of this study was to use a low-level laser to speed up wound reconstruction and reduce open wound scar formation in a rabbit model, both morphologically and histologically.

MATERIALS AND METHODS

Experimental Animals

Al-Qasim Green University College of Veterinary Medicine's Animal Experimentation Ethics Committee's ethical guidelines were followed during the animal testing process. For this study, twenty adult male local breed rabbits weighing between 1 and 2 kg who appeared to be in good health were recruited. Before beginning the experiments, twenty male health rabbits were clinically and physically examined. During the course of the experiment, the animals were housed in metal cages (30 x 70 x 60 cm) in an air-conditioned room in the animal house of the Veterinary Medicine College, University of Al-Qasim Green. They had free access to food and water (Pellets). The animals were given an oral anti-coccidiosis drug (Amprolium 50%—Kepro Company—Holland) for four weeks to adjust to the experimental environment.

In Vivo Animal Study

Technical of the Injury

The animals were first tranquilized with Diazepam at a dose of 1 mg/kg, followed by an intramuscular injection of a mixture of 5 mg/kg of xylazine hydrochloride and 35 mg/kg of ketamine hydrochloride. After that, an iodine solution of povidone (10 percent dermal beta-dine) was applied to the skin to prepare it for the aseptic surgical procedure. On one side of the back, two (2X2) centimeter-square, full-thickness cutaneous wounds were made. The caudal wound served as the treated wound, and the cranial wound served as the control (untreated) wound, with a space of 5-7 cm between each one. Randomly, the animals were split into two equal groups of ten rabbits each.

Group of Control: Saline solution (phosphate buffered saline) was administered to the animals.
Group of Treated: Treated with low-level laser therapy (LLLT), which was performed spot-on to cover the entire wound area with an energy density of 8 J/cm2 and a five-second exposure time. Irradiation was administered once daily for seven days following the surgical procedure. All of the animals underwent a comprehensive clinical examination, including measurements of their behavior, temperature, and respiratory activity. Throughout the entirety of the experiment, a daily macroscopic follow-up was performed within the wounds. The animals were given a combination of penicillin and streptomycin at a dose of 10.000 IU and 10mg/kg B.W. daily intramuscularly for the first five days after the implant.

Histopathological Observation

The observation was carried out on the days (7, 14, and 35) following treatment, and the control group was observed during the same time frame. Full-thickness incisional biopsy specimens (5-6) mm wide included approximately (3-4) mm of unwounded skin on both sides of the wound. These specimens were embedded in paraffin, fixed in a neutral formalin solution (10%), sectioned to 5-7 microns on a rotary microtome, and stained with hematoxylin-eosin [9].

Statistical Analysis

The Statistical Analysis System-SAS (2004) was used to effect on different factors (treatment & days) in study parameters (percentage). The least significant difference (LSD) test was used to comparative between percentages in this study.

RESULTS

The main differences between treated and untreated wounds began on day 7 of wound healing, according to histopathological examination of wound biopsies from the periphery and beds. Infiltration of neutrophils and mononuclear cells in the wound site's immature granulation tissue by the control group at 7 days (see Fig. 2 below) immature granulation tissues with moderate aggregation of mononuclear cells around blood vessels was found 14 days after surgery. In addition, another section of the wound revealed immature granulation tissue with moderate aggregation of mononuclear cells around blood vessels (see Fig. 3 below). In the 35th day after surgery, there was evidence of debris material surrounded by a thick layer of connective tissue and dense collage. In the dermis, however, there was less cellular fibrous connective tissue covered by a thick epidermal layer, and in another section, there was vascular granulation connective tissue made up of congested blood vessels and collagen fibers (Fig. 4 below). In this group, the treated wound's histopathological section shows that the wound was completely closed, that a skin layer had formed, and that the wound's size had decreased by up to two centimeters. The wound was not yet completely closed, and there was evidence of scar formation when compared to the control group. The wound did not appear to have shrunk in size. At day 14 after treatment, thickened layers of

epithelial cells were formed that extended over granulation tissue and under cellular debris and were infiltrated by a small number of mononuclear cells (Fig. 6 below). Additionally, at day 7 after treatment, dense thick mature granulation tissue was observed, and stained collagen fibers were observed in the wound site on a special stain. On day 35 after treatment, a histological section revealed the development of an epidermal layer over mature granulation tissue and the formation of an epidermal layer with a rete ridge over mature granulation tissue (see Fig. 7 below). On the opposite section, a thickened epidermal layer over dense collagen fibers and dense collagen fibers were visible (see Fig. 8 below). In the injury site, a newly formed hair follicle and sebaceous gland were observed in another section (see Fig. 9 below).

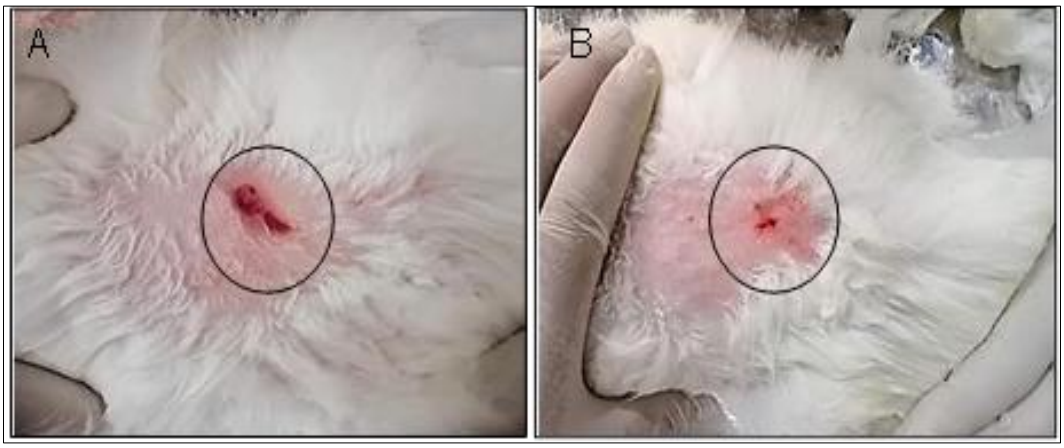

Fig. 1: Representative pictures of the treatment side (A) and the control side (B) on the same rabbit at day 35.

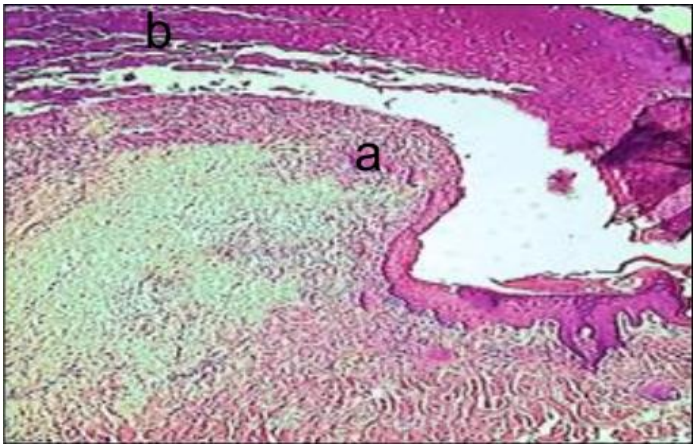

Fig. 2: Histological section of control group, at 7 days PO, showed neutrophils and mononuclear cells infiltration (A) immature granulation tissue (B) (H& E stain 100X).

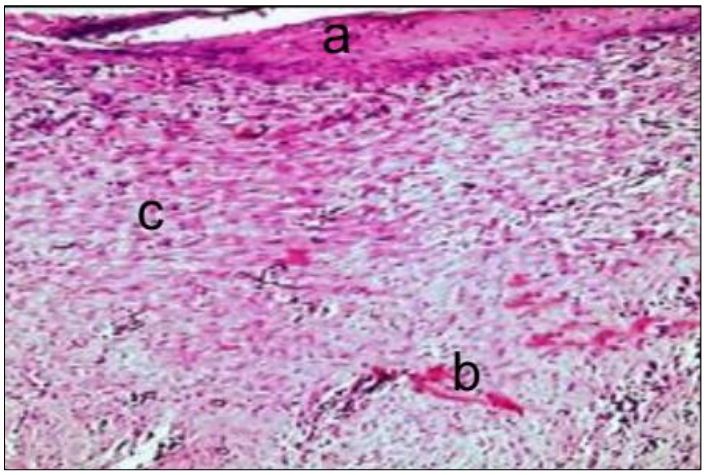

Fig. 3: Histological section of control group, at 14 days PO, showed mild hyperplasia of stratum basale and few granulation tissue (A) new blood vessels (B) Thin, coarse and interlaced collagen fibers in the dermis(C). (H& E stain 100 X).

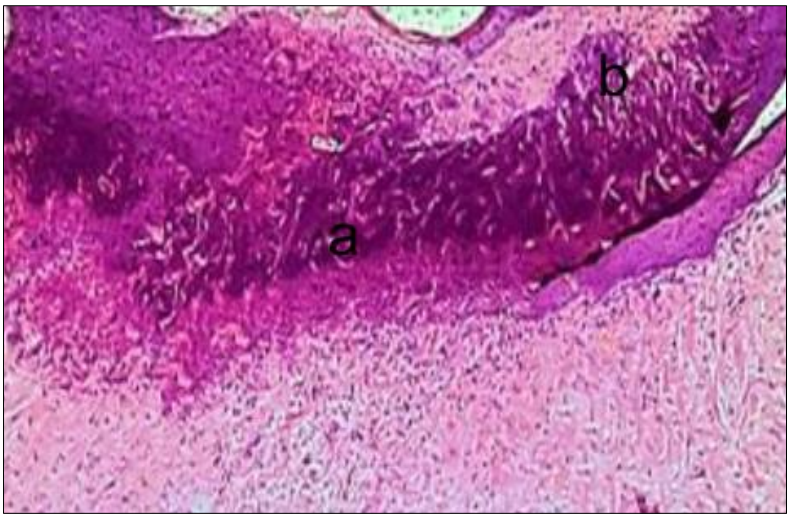

Fig. 4: Histological section of control group, at 35 days PO, showed presence of dense collagen fibers in the wound site (A) with mononuclear cells aggregation around Bv, in the dermal layer (B) (H& E stain 100X).

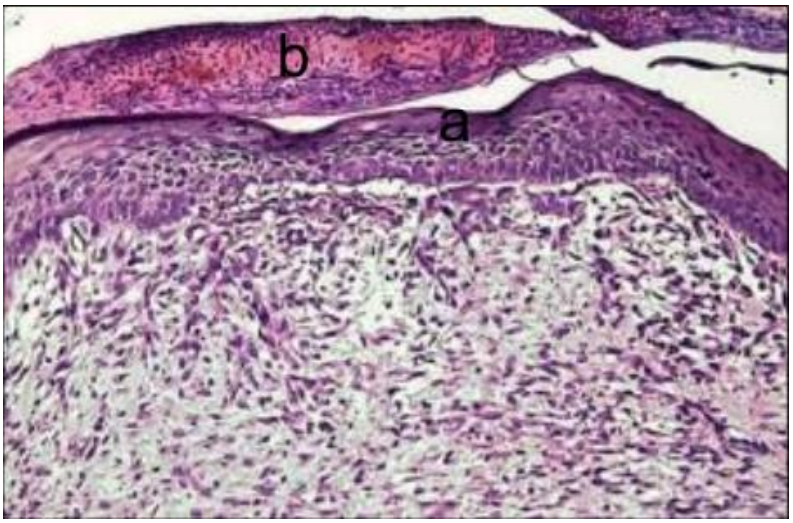

Fig. 5: Histological section of treated wounds, at 7 days PO, showed granulation tissue in the dermis (A) with complete epidermal layer (B) (H& E stain 100X)

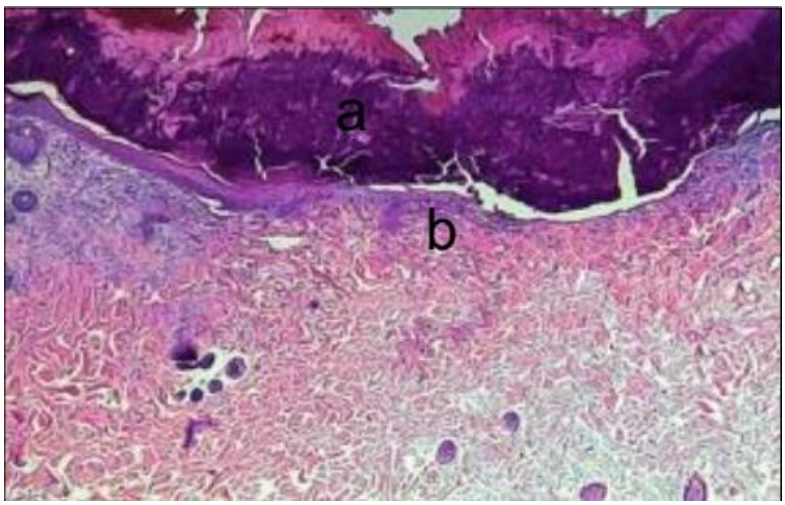

Fig. 6: Histological section treated wounds, at 14 days post treatment showed thickened layer of epithelial cells (A) extended over mature granulation tissue and under cellular debris (B) (H& E stain 200X)

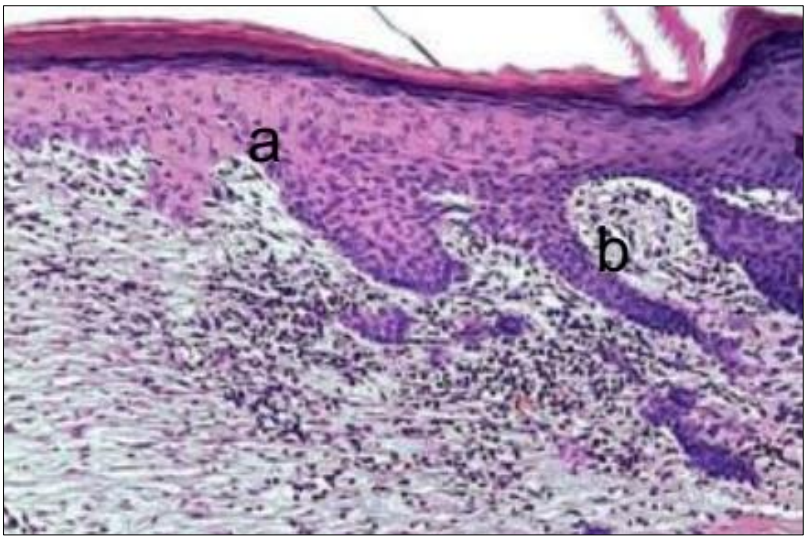

Fig. 7: Histological section of treated wounds, at 35 days PO, showed complete layer of epidermis (A) with rete ridge over mature granulation tissue (B) (H& stain 200X).

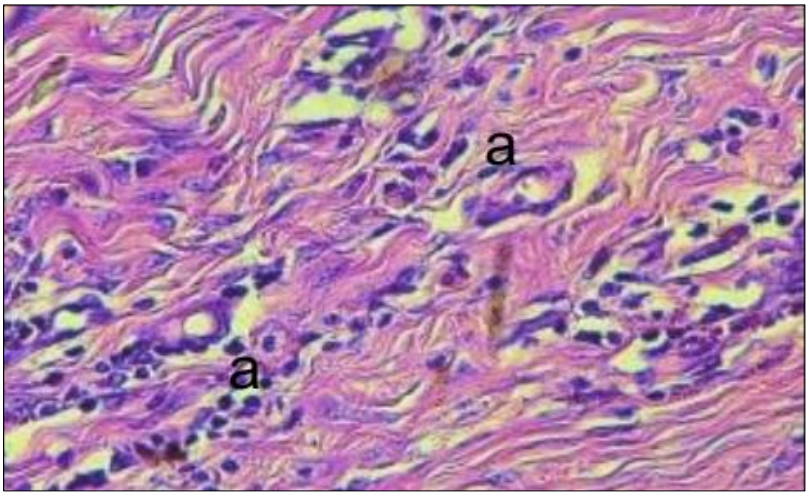

Fig. 8: Histological section of treated wounds, at 35 days PO, showed presence of fibroplasia in the dense fibrous connective tissue (H& stain 200X).

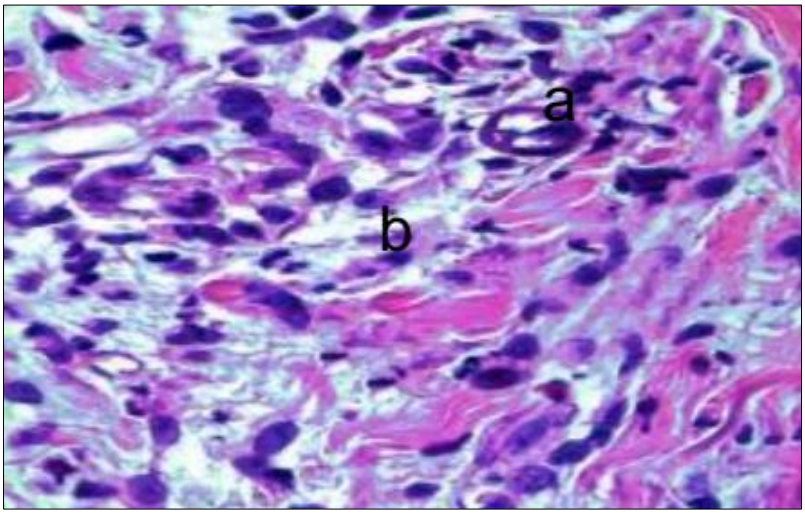

Fig. 9: Histological section of treated wounds, at 35 days PO, showed newly formed hair follicle (A) and sebaceous gland in the injury site (B) (H& stain 400X).

DISCUSSION

The purpose of this study was to determine how the LLLT affects how rabbit skin heals from wounds. It was demonstrated in this study by the histological results that we obtained, which determined its ability to be resorbed in vivo in a rabbit full-thickness wound model and assessed its effects on proliferation, migration, and angiogenesis in vitro. Anyway the outcomes in treatment bunch showed the injury constriction expanded all the more much or decrease in size of the injury since laser impact contrasted and control bunch. Previous research supported this observation, Chaves *et al.*, [10], who demonstrated that a wound repair involves a variety of vascular and cellular changes, epithelial and fibroblast multiplication, the production of collagen and elastin, revascularization, wound contraction, and the accumulation of collagen and elastin. It is also noteworthy that the trophic regeneration, anti-inflammatory facts, and analgesic properties continue to be crucial for completing the healing process [11]. The treated group had more fibroblasts proliferating in the early stages than the untreated group. The findings of a previous study, which suggested a possibility of laser-induced fibroblast proliferation during the healing process, are supported by these observations. The maintenance of the fibroblast's high mitotic activity during the later healing period is how laser stimulation of fibroblasts affects wound regeneration [12]. It has been demonstrated that LLLT preferentially stimulates resting cells rather than proliferating ones. Wardlaw *et al.*, [13], demonstrated that using a laser to speed up the healing process of Dachshund dogs that had had incisions made indicated restoring epithelization by day seven and continuing to improve the healing process until the end of the experiment. Histopathological examination of treated wound sections revealed a high prevalence of mature granulation tissue, myofibroblasts, and new blood vessels, in contrast to the control wound sections, which revealed few myofibroblasts dispersed throughout fibrous connective tissue and congested blood vessels. The effect of the effect laser, which may have a significant impact on the enhancement and acceleration of cutaneous wound healing, may be related to the findings of this investigation, this conclusion is consistent with the findings of numerous other studies that utilized the LLLT to directly repair tissue defects. Suiter *et al.*, research [14], found that LLLT increases fibroblast motility and myofibroblast differentiation, which in turn stimulates wound contraction. By contracting cells, myofibroblasts, which are the result of fibroblast development, bind the wound's margins together. This plays a crucial role in transporting blood vessels that carry nutrients and oxygen to the area and rapidly regenerate under the influence of angiogenetic substances released by fibroblasts. The increased wound contraction observed in this study could be explained by a number of different hypotheses. LLLT therapy may facilitate fibroplasia during the repair phase of tissue healing, as evidenced by in vitro studies showing an increase in fibroblast proliferation following irradiation [15-18]. The LLLT is an efficient treatment for increasing partial thickness wound contraction. Additionally, it aids in wound contraction in untreated wounds, indicating an indirect effect on the tissues surrounding the wound. We believe that despite the fact that our data focused on increased contraction of superficial wounds, this is the first step in drawing meaningful conclusions about LLLT [19]. Aoki *et al.*, [20], who also discovered, on day 28 of their experiment, a focal pattern of epithelial proliferation over granulation tissue; however, these changes were less pronounced in the laser group, with healed epidermal tissue covering granulation tissue that was highly developed. Fibers high in collagen that are arranged parallel to the surface of the wound .

CONCLUSION

That utilizing of Laser irradiation on full-thickness skin improves and enhanced skin wounds healing into enhances cell migration, makes a prior differentiation of fibroblasts to myofibroblasts and hence allows earlier wound remodeling and improves the healing woundcompared to the control group.

ACKNOWLEDGEMENT

The author would like to express thanks to College of Biotechnology, Al-Nahrain University, to support current experimental study.

Conflict of Interest: The authors declare that no conflict of interest exists.

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