FULL THICKNESS WOUND HEALING IN INDUCED DIABETIC MICE USING LOW LEVEL LASER IN COMPARISON WITH ULTRASOUND

*Thanaa I. Shalaby, *Abd El Rahman T.Heraba,*Maisa E.Moustafa and **Amal S. El Sodfy
Corresponding author's name: Thanaa I. Shalaby

*Medical Biophysics Department,**Pathology Department, Medical Research Institute, Alexandria University, Egypt

ABSTRACT

Physical treatments such as therapeutic ultrasound and Laser are cited as important aides in wound management. This work expected to assess the role of ultrasound and laser on excisional wound healing progression in incited diabetic mice. Forty Swiss albino mice were divided into 2 groups; group I (diabetic free, 10mice) was used as a control group, while the other; group II(30 mice) was diabetic induced. Circular wounds were created on the dorsal fur of the animals. The wounds of laser treated group (N=10) were exposed to laser irradiation (830nm, 60mW), 5 minutes daily, 5 days/week until the wounds healed completely. The wounds of ultrasound treated group (N=10) were exposed to pulsed ultrasound (0.5 W/cm^2, 1MHz) 5 minutes daily until the wounds healed completely. The untreated group (N=10) wound was left without treatment. The wounds for control group did not receive any type of treatment. The healing was noticed and assessed during the treatment period. Histological analysis of mice skin was accomplished at days 4, 10 and 15 post- wounding. Taking after healing period and determine the mechanical properties of healed skin. By looking at results acquired from various treatment modalities, we found that the two remedial devices encourage and quicken diabetic injury recuperating and enhancing scar quality yet with various degrees. Lighted injury with laser exhibited a more propelled recuperating process than ultrasound treated gathering. We can conclude that, both low-power laser and ultrasound treatments may quicken cutaneous injury mending in diabetic injury model. This finding may widen current treatment regimens

KEYWORDS:

Laser, Therapeutic ultrasound, Wound healing, Diabetic mice.

1. INTRODUCTION

Diabetes is a syndrome of disordered metabolism characterized by abnormal high sugar levels in blood (hyperglycemia). Diabetic patients have poor circulation, poor resistance to infection, thus their wounds are highly susceptible to infection [1]. Patient with diabetes suffer from many complications in the form of tropical ulcer and vascular abnormality resulting in vascular stasis and poor wound healing [2]

Lasers and therapeutic ultrasound are alternative therapeutic tools to improve diabetic wound healing [3]. Such tools are able to accelerate and facilitate wound healing and increase scar quality [4, 5]. Low Intensity Laser Therapy depends on photons emitted from laser and absorbed by cells to be converted to chemical kinetic energy resulting in changes in membrane

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permeability and increased oxidative metabolism to produce more ATP. Laser irradiation was found to improve cell-to-cell communication through increasing levels of cytokines or growth factors [6].

Ultrasound is a non-invasive therapeutic tool for wound healing. Ultrasound has the ability to penetrate tissue and can be focused locally into a small target to give very high energy. The therapeutic potentials of ultrasound are derived from its thermal and non-thermal effects. At intensities of 1-1.5 Watts/cm^2 the ultrasound has thermal effects. The non-thermal effects of ultrasound are achieved at intensities of <0.3-1W/cm^2. The non-thermal effects cause changes in cell membrane permeability and consequently the diffusion of cellular metabolites [7, 8]. Ultrasound is considered a promising approach for wound healing. Ultrasound has been shown to enhance collagen synthesis and improve tensile strength, angiogenesis and wound contraction and stimulate fibroblast and macrophage proliferation [9, 10].

In this study, we investigated and compared the effects of ultrasound and laser exposures on the wound healing process in excisional wound healing dynamics in diabetic mice through measurement of wound contraction and mechanical properties of the healed skin.

2. MATERIALS AND METHODS

2.1 Diabetic Induced Mice

A total of 40 Swiss albino mice of either sex, 6-8 weeks of age, weighing 25-30 g were acquired from the Animal House, Medical Research Institute. Alexandria University, Egypt. Diabetes was induced in 40 mice using Streptozotocin (STZ) (Sigma). Streptozotocin solution with a dose of (180 mg/kg) was administrated intraperitoneally (i.p.) as a single dose after a fasting period of 12 hours, after 48h blood glucose levels were determined using a blood glucose monitoring system (Free Style Freedom Lite). When blood glucose level reach 300 mg/dl or more, the animal considered to have successful induction of diabetes and was used in the study; all induced mice (30 mice) were treated with insulin intraperitoneally with a dose of 5 IU/kg. All experiments have been approved by the Medical Research Institute animal ethics committee.

2.2 Excision wound and Grouping

2.2.1 Wound surgery

Animals were anaesthetized with intravenous ketamine of 2 mg/kg body weight prior to and during creation of the wounds. The dorsal fur of the animals was shaved using an electric clipper, excess hair was removed by a lotion, and the site was disinfected with an isopropyl alcohol swab, and the anticipated area of the wound to be created was outlined on the back of the animals with methylene blue using a circular stainless steel stencil. A circular wound of 5.6 mm diameter and 2 mm depth was created and left open in all animals. After wound surgery, mice were randomly divided into two main groups (I and II), the treatment was started in each group within 3hours of the surgical procedure.

- Group (I): Diabetic free served as control group (10 mice) their open wounds were left without treatment.
- Group (II): Experimental group (30 mice) diabetic induced, this group was divided into the following subgroups:
- Subgroup (IIa): (10 mice), their open wounds were left without treatment.
- Subgroup (IIb): (10 mice), their wounds exposed to laser (830 nm) with a fluence of 5 J /cm^2 5 minutes daily.
- Subgroup (IIc):(10 mice), their wounds exposed to pulsed ultrasound (0.5 W/cm^2, 1MHz) 5 minutes daily.

2.2.2. Treatment intervention

2.2.2.1 Ultrasound Treatment

An ultrasound stimulator (model Sonopuls 463, Enraf Nonius Co. Netherlands) with a probe 1.9 cm^2 in diameter (Enraf-Nonius Co.) was used. The ultrasonic therapy treatment protocol include: cleaning of wound using alcohol, filled with coupling gel then covered by plastic drape, a layer of coupling gel was put over the plastic drape as impedance matching.

Pulsed ultrasonic therapy (pulsed duty cycle 20% (2 ms on, 8 ms off,and average intensity 0.5 W/cm^2) was applied over plastic drape and moved within the wound boundaries. Moving of ultrasonic applicator was done for 5 minutes per day, till complete healing. Gel and plastic drape were removed and, wounds were cleaned, dried at the end of each session.

2.2.2.2 Laser treatment

Diode laser (830 nm, 60 mW) with a proper driver, beam-delivery fiber and hand piece was used (Kondortech, BioWave LLLT, Brazil). The output power was checked using power meter (Molectron Max 5200). The laser probe was directed over the animal wound with a fluence of 5 J/cm^2 for 5 minute daily and a distance 0.6 cm apart from the wound, till complete healing. The laser beam was adjusted to cover the entire wound area, including the wound margin.

2.3 Wound contraction measurements

The percentage of wound contraction was calculated by measuring the wound area; wound margins were traced every three days using transparent sheet. The area of the wound was measured by measuring the area of traced circle on the sheet.

The wound area on day 0 of each animal was measured at 3 h post-wounding, as the delay of 3 h after the creation of wound for measurement was allowed to accommodate the wound stretching that occurs due to the struggle of the animal during recovery from the anesthesia. Subsequent measurement of wound area was taken every 3 days for 21 day. The values were expressed as percentage values of the day 0 measurements and were calculated by Wilson's formula as follows [11]:

2.4 Histopathological analysis and tensile strength evaluation

For histopathological study, two animals for each group were sacrificed on the day 4, 10 and 15 post wounding. The skin biopsies were carefully collected. All biopsies were immediately fixed in formalin 10% for 24 h. Thereafter, biopsies were included in paraffin and 6 mm thickness transversal sections were obtained. The sections were stained by using the hematoxylin–eosin (H&E) and Masson-Trichrome techniques [12]. Histopathological alterations were observed under Olympus light microscope (BH2, Japan). After complete healing, full-thickness of healed skin was used for mechanical evaluation using tensile testing machine (Force meter BG500, USA Mark-10). A sample was spaced between the jaws of the machine and stretched to failure.

3. RESULTS

3.1 Effect of laser and ultrasound treatment on wound contraction

As shown in Fig. 1, the mean percentage of wound contraction of the laser treated group (5 J/cm^{2}for 5 minutes daily) was significantly higher compared with those treated with pulsed ultrasound (0.5W/cm^2 for 5 minutes daily) on day 6 and this significant difference continued till day 21after wound creation. There was no significant difference between lasers treated group and control (diabetic free) group. The lowest wounds contracted percentage obtained for diabetic mice treated with insulin only as compared to other groups. Data are expressed as means ± SD (n = 5).

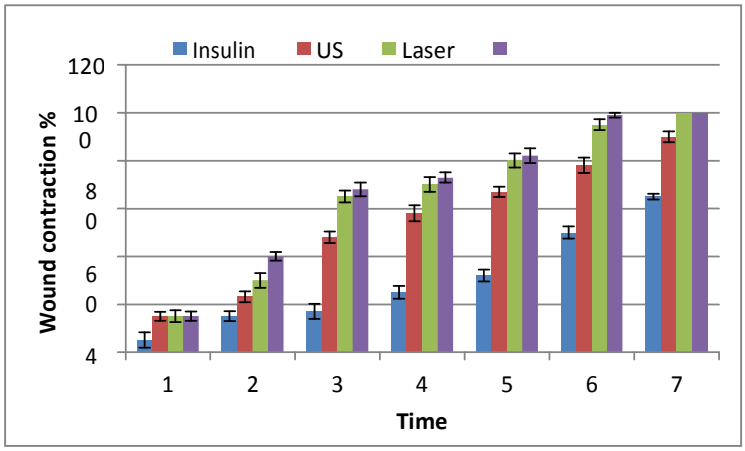

Figure 1.Wound contraction (%) in the control and experimental groups treated with different modalities with time (in day).

3.2 Mechanical properties of skin of diabetic mice

As shown in Fig. 2, improvement in wound strength of skin treated with laser or ultrasound as compared to control group. The improvement in wound strength and healing may be due to increase in formation of collagen fibers and activity of the epithelial covering in mice treated with insulin in combination with laser or ultrasound.

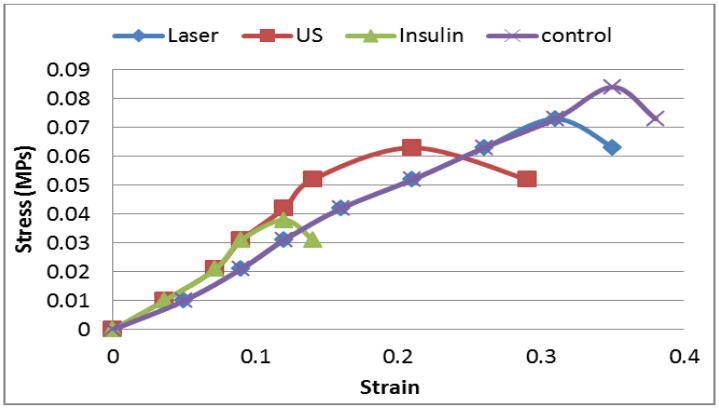

Figure 2. Stress-strain curve of the skin of control and diabetic micetreated with different modalities.

3.3 Histological analysis:

The histopathological analysis of the healed skin treated with different modalities was performed on the day 4, 10 and 15 post wounding (Figures 3-5). At day 4 post wounding, dermis of diabetic mice irradiated with Laser (5J/cm^2) showed complete ulceration of the surface epithelium, moderate inflammatory infiltrate that involves the subcutaneous fat (Fig.3a). Dermis of diabetic mice treatment with pulsed ultrasound (0.5W/cm^2), showed complete ulceration of surface epithelium that covered by fibrinoid material infiltrated by inflammatory cells and moderate collagen deposition (Fig.3b). Dermis of diabetic mice without treatment showed complete ulceration of surface epithelium and inflammatory cells infiltrate (Fig.3c). Control dermis of diabetic free mice showing complete ulceration of surface epithelium and moderate collagen deposition (Fig.3d). At day 10 post wounding, dermis analysis of diabetic induced mice irradiated with Laser (5J/cm^2) showed complete ulceration of the surface epithelium, moderate inflammatory infiltrate that involves the subcutaneous fat (Fig.4a). Dermis of diabetic induced mice treatment with ultrasound (0.5 W/cm^2) showed complete ulceration of surface epithelium that covered by fibrinoid material infiltrated by inflammatory cells and moderate collagen deposition Fig.4b). Dermis of diabetic induced mice without treatment shows complete ulceration of surface epithelium and inflammatory cells infiltrate (Fig.4c). Control dermis at 10th day showed complete ulceration of surface epithelium and moderate collagen deposition(Fig.4d). At day 15post wounding, dermis analysis of diabetic induced mice irradiated with Laser (5J/cm^2) showed intact skin covering with dense collagen deposition (Fig.5a). Dermis of diabetic induced mice treatment with ultrasound (0.5 W/cm^2) showing intact skin with normal maturation sequence of the epithelium, the collagen is abundant with mild fibroblastic proliferation (Fig.5b). Dermis of diabetic induced mice without treatment showing thinning of the epithelium, the collagen is disrupted with edematous areas (Fig.5c). Control dermis (diabetic free mice) at 15th day showing focal ulceration of the epithelium, the collagen is dense in the upper dermis (Fig. 5d).

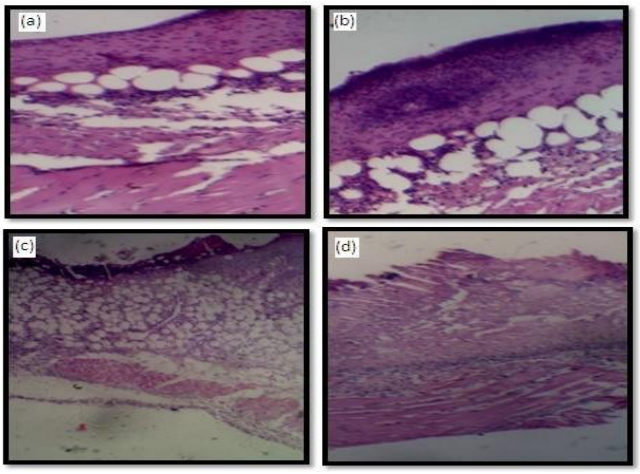

Figure 3(a–d) Photomicrograph of mice skin at 4th day post wounding, (a) dermisirradiated with Laser (5J/cm^2) shows complete ulceration of the surface epithelium, moderate inflammatory infiltrate that involves the subcutaneous fat. (b) dermis treatment with ultrasound (0.5 W/cm^2) showing complete ulceration of surface epithelium that covered by fibrinoid material infiltrated by inflammatory cells and moderate collagen deposition. (c) dermis without treatment showing complete ulceration of surface epithelium and inflammatory cells infiltrate. (d) control dermis at 4th day showing complete ulceration of surface epithelium and moderate collagen deposition. (H&E x40).

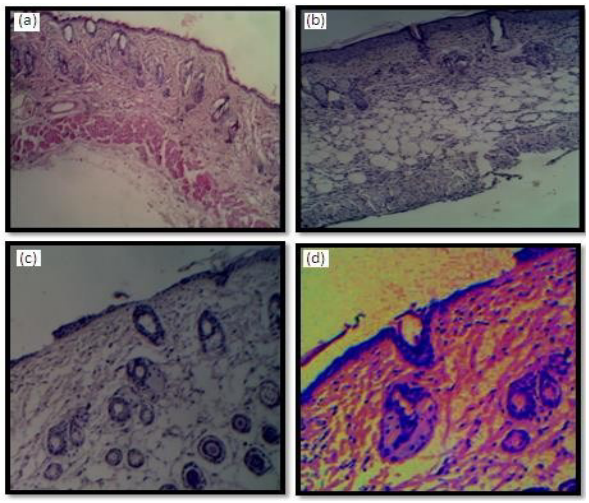

Figure 4(a–d) Photomicrograph of mice skin at 10th day post wounding, (a) dermisirradiated with Laser (5J/cm^2)showing intact thinned epidermis with normal maturation sequence of the epithelium.(b) dermis treatment with ultrasound (0.5 W/cm^2) showing intact skin with normal maturation sequence, the collagen deposition in upper and mid dermis and mild inflammatory cellular infiltrate. (c) dermiswithout treatmentshowingskin with focal epidermal ulceration, thinning and the minimal collagen deposition**.**(d) controldermis (diabetic free mice) at 10th day showing focal ulceration, minimal edema and moderate collagen deposition (H&E x40).

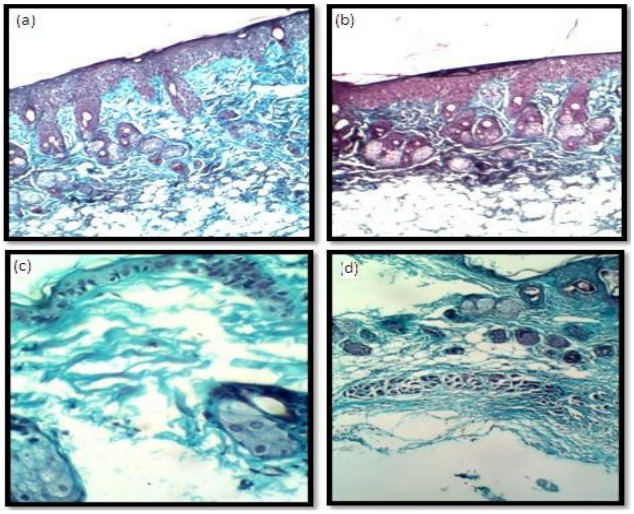

Figure 5(a–d) Photomicrograph of mice skin at 15th day post wounding, (a) dermisirradiated with Laser (5J/cm^2) showing intact skin covering with dense collagen deposition. (b) dermis treatment with ultrasound (0.5 W/cm^2) showing intact skin with normal maturation sequence of the epithelium, the collagen is abundant with mild fibroblastic proliferation. (c) dermis without treatment showing thinning of the epithelium, the collagen is disrupted with edematous areas. (d) control dermis (diabetic free mice) at 15th day showing focal ulceration of the epithelium, the collagen is dense in the upper dermis.(Masson trichromex40).

4. DISCUSSION

The aim of the present study was to compare the effect of laser and ultrasound treatment on the wound healing in induced diabetic mice as a trail to solve wound healing in diabetic patients. The impairment of wound healing in patients with diabetes as reported by researchers is due to alteration in the fibroblasts in the wound area; diabetic process and the wound environment itself cause the fibroblasts to age. Fibroblasts are responsible for the production of components of the extracellular matrix (collagen) and wound contraction by production of granulation tissue. A significant reduction in the numbers of fibroblasts was found in the deep components of the wounds. The overall functional activity of the fibroblast is thus reduced [13, 14]. Diabetic fibroblasts have a decreased proliferation response to growth factors caused by deficiency in growth factor receptor expression, compared with non- diabetic fibroblasts from sibling control [15].

Our results clearly indicate that low energy photons emitted from diode laser (830 nm, 60 mW) with a fluence of 5 J/cm^2 5 minutes daily facilitate diabetic wound healing process as evidenced by measuring wound contraction percentage and the mechanical properties of the healed skin which was confirmed by histolopathological study. It was found that, laser energy at certain frequencies can modulate cell proliferation and stimulate the release of growth factors from fibroblasts which enhance the pro-collagen production, increased cross-linking of existing collagen molecules, acceleration of epithelial repair, and early growth of granulation tissue [16, 17]. Another mechanism of photostimulation was that, the respiratory chain in the mitochondria can absorb light energy, producing changes in the redox status in mitochondria and cytoplasm. The activation of electron transport chain results in an increase in the ATP pool, and activation of nucleic acid synthesis [18].

In our study, ultrasound of a power density 0.5W/cm^2 has proved to be an effective modality supporting the treatment of full thickness wound in diabetic mice. The main effect of ultrasound is probably a mechanical and piezoelectric effect, which is created through the shearing action of stable microbubbles in alternating high and low pressure waves that are generated by the ultrasonic transducer. This phenomenon, called stable cavitation, is most important in making low-dose ultrasound a potential tool for debridement without damaging vital structures within the wound [19].

It has been suggested that the non-thermal effects of ultrasound is the most prominent in wound healing processes than its thermal effects The non-thermal effects of ultrasound (which are achieved at intensities of <0.3-1W/cm^2) produces two effects, cavitation and streaming. These effects cause changes in cell membrane permeability and thus the diffusion of cellular metabolites [20]. It was found that, ultrasound affect cellular recruitment, collagen production, increased collagen tensile strength, angiogenesis, wound contraction, fibroblast and macrophage stimulation, fibrinolysis, and reduction of the inflammatory phase and promotion of the proliferative phase of healing [21]. In the present study, the influence of laser treatment on the healing process was most pronounced in the healing process than ultrasound treatment. The wound contraction percentage of different groups increase with increasing the treatment period as shown in Fig.1, and the highest value obtained for diabetic mice treated with insulin and their wounds treated with laser. Laser treatment improves the mechanical properties of the treated skin of the diabetic mice. Marked improvement in wound strength and healing due to increase in formation of collagen fibers and activity of the epithelial covering in mice receiving STZ and treated with insulin in combination with laser irradiation.

5. CONCLUSION

Abnormal wound healing that can occur during many pathological states like in diabetic patients is a very dangerous and can lead to mortality in some cases, such wounds are resistant to conventional therapies. Experimental studies indicated that both laser and ultrasound treatments can be used successfully for treatment of diabetic wounds. The laser treatment, however, was considered more effective than the ultrasound treatment in wound healing.

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