

Low-level laser therapy with pulsed infrared laser accelerates third-degree burn healing process in rats

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Abstract—This study investigated the influence of pulsed low-level laser therapy (LLLT) on the healing of a third-degree burn in a rat model. Two third-degree burns (distal and proximal) were made in the skin of 74 rats. Rats were divided into four groups. In group 1, the distal burn received LLLT with laser switched off; in groups 2 and 3, distal burns were treated with a 3,000 Hz-pulsed infrared diode laser with 2.3 and 11.7 J/cm² energy densities, respectively. In group 4, the distal burns were treated topically with 0.2% nitrofurazone. The proximal burn of all groups was considered a control burn. We assessed the response to treatment both microbiologically and macroscopically. The chi-square test showed that the incidence of *Staphylococcus epidermidis*, *Lactobacillus*, and diphtheria decreased significantly in laser-treated groups compared with other groups. Independent sample *t*-test showed that LLLT with 11.7 J/cm² energy density significantly increased wound-closure rate at 3 and 4 weeks after burning compared with their relevant control burns ($p = 0.018$ and $p = 0.01$, respectively). Pulsed LLLT with 11.7 J/cm²/890 nm of a third-degree burn in a rat model significantly increased wound-closure rate compared with control burns.

Key words: basic science, burn, infrared diode laser, in vivo, low-level laser therapy, microbiology, rat, third-degree burn, wound contraction, wound healing.

INTRODUCTION

Burns are among the most devastating of all injuries, with outcomes spanning the spectrum from physical

impairments and disabilities to emotional and mental consequences [1–2]. In the United States, approximately 2.4 million burn injuries are reported each year. Nearly 650,000 persons with these injuries are treated by medical professionals through outpatient care and 750,000 through inpatient or hospital care. Of those persons hospitalized, 20,000 have major burns involving at least 25 percent of their total body surface. Between 8,000 and 12,000 of patients with burns die and approximately 1 million will sustain permanent disabilities resulting from burn wounds [3]. Third-degree or *full-thickness* burns involve the entire epidermis and dermis and may appear as white, thick brown, or tan and have a leathery texture [4].

Low-level laser therapy (LLLT) has been used clinically since the first successful cases reported by Professor Mester and colleagues [5–6]. Cameron et al. reported that the frequency of the laser light, as well as the type of tissue being irradiated, determines the depth to which light penetrates [7]. Laser light with a wavelength

Abbreviations: ANOVA = analysis of variance, CFU = colony-forming units, CW = continuous wave, GaAlAs = gallium aluminum arsenide, GaAs = gallium arsenide, LLLT = low-level laser therapy, LSD = least significant difference.

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between 600 and 1,300 nm optimizes the depth of penetration in human tissue at 1 to 4 mm and is therefore most frequently used in the clinical setting. Laser light with a longer wavelength, such as the (infrared) diode produced by the gallium arsenide (GaAs) or gallium aluminum arsenide (GaAlAs) laser, penetrates deeper [8], whereas laser light with a shorter wavelength, such as red light produced by the helium-neon laser, penetrates human skin very superficially [7]. Research findings have shown that 99 percent of low-level laser is absorbed in the superficial 3.6 mm of human skin [7].

Studies on the influence of continuous-wave (CW) diode lasers on burn healing were few and have shown inconsistent results [8–12]. While Cambier et al. [8], Schlager et al. [9–10], and Al-Watban and Delgado [11] reported irradiation of burns with different wavelengths, powers and energy densities produced no beneficial effects on the wound-healing process. Meireles et al. in a recent study indicate that a 660 nm laser effectively improved the healing of third-degree burns in diabetic rats [12]. Cambier et al. inflicted two burns on each rat: one was left untreated and the other was treated with a continuous GaAs diode laser with 0.210 J/cm^2 energy density [8]. Treatment frequency was 5 times a week over 6 weeks. No major stimulating effect was observed based on the size of index. Cambier et al. reported that type of burn or protocol parameters could be responsible for this lack of effect [8]. Schlager et al. investigated the effect of a CW low-power diode laser with a wavelength of 670 nm on the healing of burn wounds in rats [9]. The animals were burned on each flank. One of the burns was treated by laser irradiation, whereas the other burn received no treatment. Laser irradiation was performed daily with a 2 J/cm^2 energy density (dose). Neither macroscopic nor histological examination of the irradiated wound showed accelerated wound healing when compared with the control wound [9].

In another study, Schlager et al. investigated the effects of two different low-power diode laser lights on the healing process of rats [10]. The animals were burned on each flank and allocated to one of three groups. In group A, both wounds remained untreated. In groups B and C, one wound was irradiated with a CW low-power diode laser at a 690 nm wavelength and the other wound a 635 nm wavelength, respectively. Laser irradiation in both groups was performed daily with an energy density of 1.5 J/cm^2 at each treatment. Schlager et al. found that between and within each group, diameter, redness, and

edema of the wound were similar throughout the entire observation period [10]. Schlager et al. mentioned that the reason such differences were obtained on the use of low-power laser light in the burn healing is unknown [9–10]. Al-Watban and Delgado initiated a study using a diode laser at varied doses on burn healing to determine optimum energy density and treatment schedule [11]. Burns on both flanks of rats were created and measured daily with a caliper. The right-side burns were irradiated. Slopes from the actual burn areas were obtained and compared with the control group, with the healing rats calculated and expressed in percent. Al-Watban and Delgado reported that with reference to the control group, they observed no significant difference in the healing process [11]. They also reported that in younger rats, they observed accelerated healing with the highest rates in the lower range of doses (1 and 5 J/cm^2), 12.4 and 11.6 percent, respectively. They concluded that their study affirms that the beneficial effect of laser on burn healing in rats is indeed affected by interplay of several factors [11].

Meireles et al. made a third-degree burn in the 55 diabetic rats [12]. They were divided into three groups that were or were not treated with LLLT (wavelength = 660 nm or wavelength = 780 nm, 35 mW; laser beam diameter = 2 mm, 20 J/cm^2). They found that the healing in animals receiving 660 nm laser energy was more apparent at early stages, with positive effects on inflammation, the amount and quality of granulation tissue, fibroblast proliferation, and collagen deposition and organization [12]. The studies on the influence of CW diode lasers on burn healing have apparently shown inconsistent results.

Baxter reported that although a large percentage of the diode low-level laser instruments used in clinical practice are CW output, most instruments now available in the United Kingdom have pulsed output [13]. The application of frequency is growing rapidly. In this regard, results of several cellular studies [14–16], an *in vitro* model of a fetal mouse limb growth [17], and three clinical trials [18–20] suggest that the frequency parameter is critical to at least some biological and medical effects of this parameter. Thawer and Houghton investigated the effects of a 904 nm GaAs laser on the growth and development of fetal limb tissue [17]. Organ culture dishes that contained ipsilateral forelimbs and hind limbs were exposed to laser irradiation. The limbs were assigned to receive energy densities of 0 (control), 0.23, 1.37, 2.75, 3.66, or 4.58 J/cm^2 , with frequencies of 0 (control), 500,

3,000, 6,000, 8,000, and 10,000 Hz, respectively. Thawer and Houghton found that the dermal cell number and collagen fiber thickness increased after lower frequencies of laser (500 and 3,000 Hz) [17]. These laser frequencies also produced a greater amount of dermal collagen [17]. In another study, Karu et al. investigated the effects of 1,300 nm CW diode laser and 950 nm modulated super-luminous diode laser, which had frequencies of 2, 26, 700, 1,000, and 5,000 Hz [21]. The effects of both diodes on the rate of *Escherichia coli* WP2 division were examined [21]. The radiation of CW mode of 1,300 nm laser increased the division of *Escherichia coli* in the dose range of 0.9 to 9.0 J/cm². The 950 nm-pulsed irradiation inhibited the division rate of bacteria at frequencies of 1,000 and 5,000 Hz. Karu et al. mentioned that their results indicate that one of the critical parameters of laser irradiation when acting on living cells is the pulse duration and/or frequency [21].

Review of the literature has revealed that no studies have been done regarding the effect of pulsed LLLT on burn healing. On the other hand, a number of studies have reported the effects of pulsed diode lasers on skin wound healing [22–24]. Al-Watban and Zhang evaluated the effects of pulsed CW and the role of wound healing in rats by using both pulsed and CW LLLTs [22]. An elliptic wound was made on the back of rats after anesthesia. The study was performed with the use of a pulsed laser at a wavelength of 635 nm. Pulse frequencies of 100, 200, 300, 400, and 500 Hz in CW were used in the study. Every rat in the treatment group was irradiated with a laser at a 0.89 mW/cm² power density for 18.7 minutes with a 1.0 J/cm² incident dose or energy density. They reported the percentage of relative wound healing was 4.32 in 100 Hz, 3.21 in 200 Hz, 3.83 in 300 Hz, 2.22 in 400 Hz, 1.73 in 500 Hz, and 4.81 in CW. Al-Watban and Zhang concluded that LLLT using pulsed CW laser at the appropriate dosimetry and frequency can accelerate wound healing in rats [22]. The 100 Hz frequency had a better effect than other pulse frequencies used in the study. The effects of CW laser treatment were higher than pulse frequency. The frequency of pulsed CW laser was not found to increase wound healing in rats compared with normal (not pulsed) CW laser [22].

Recently, Demir et al. investigated the effects of electrical stimulation and laser treatment on wound healing in rats [23]. They made a 6 cm linear incision at the dorsal skin of rats. Group 1 was given a constant direct current of 300 μ A a day. Group 3 was treated with a GaAs laser device, delivering a 904 nm wavelength, 6 mW average

power, 1 J/cm² dose, with a maximum frequency of 128 Hz. This dose was delivered continuously for 10 minutes each day for 10 days. Additional specifications of the laser device were an infrared GaAs laser tube, 6 mW mean and 27 mW maximum power, 15° emission angle, continuous and modulated output type, and 1 to 128 Hz frequency. Groups 2 and 4 were considered the control groups and received sham treatment. Demir et al. concluded that electrical current and laser treatment both benefited healing during the inflammation, proliferation, and maturation phases of a wound [23]. More recently, Matic et al. made a rectangular defect of all skin layers at the dorsal part of the rat neck under general anesthesia [24]. They used an 890 nm wavelength of a pulsed semiconductor laser, with a frequency of 1,500 Hz, impulse duration of 300 ns, maximum strength output of 36 mW, and medium strength of 15.4 mW. The exposure lasted for 5 minutes every day for 21 days. The control group was not exposed to any irradiation. Matic et al. found that the average surface area of the wounds in the laser-treated group decreased significantly more than that of the control group [24].

However, the benefits of pulsed diode lasers in the wound healing process are still controversial and many other investigators found no improvement in the wound-healing process [25–26]. Because of these contradictory results, still no consensus of the effects of LLLT in the wound-healing process exists. Recent studies of skin wound-healing and burn-healing processes have used various diode lasers with different wavelengths, laser power, and stimulation doses. Concerning the type of laser and sufficiency of wavelength, no clear recommendation can be made yet. On the other hand, low-level-pulsed diode laser has not been examined in burn-healing treatment yet. The recent investigations contain no burn healing for an 890 nm infrared diode laser with a 3,000 Hz frequency. Therefore, the present study aimed to examine the influence of LLLT using a 3,000 Hz-pulsed infrared diode on the healing of third-degree burns in rats. Infection is a major cause of morbidity and mortality in burns [27–28], so we also examined microbial flora of the burn.

MATERIALS AND METHODS

Animals and Study Design

We used 74 adult male Wistar rats, 4 months old and weighing 250 \pm 30 g, in this study. (Values throughout the article are expressed as mean \pm standard deviation.)

Rats were divided into groups 1 to 4. They were provided food and water ad libitum. Two third-degree burns were made at the dorsal proximal and distal regions of the thoracic region of each rat (**Figure 1**). Distal (experimental) burn of group 1 was treated with LLLT with the radiating head without the laser switched on and was considered the placebo group. Distal burns of groups 2 and 3 were treated with two different energy densities of infrared diode laser (experimental), so groups 2 and 3 and group 1 had no differences except LLLT. Distal burn of group 4 was treated three times a week with topical application of 0.2 percent nitrofurazone (Iran Nago Pharmaceutical Co; Tehran, Iran) during the study. Treatment was started in all groups immediately after burns were made. Proximal burn of all rats was considered as their relevant control burn. All burn wounds were examined macroscopically and microbiologically. Six rats of each group were randomly selected for day 7 (group A), six rats of each group were randomly selected for day 15 (group B), and remaining six rats of each group were selected for day 28 (group C). Two groups of microbiological examination had 7 rats. Groups A and B were used for microbiological examination, and group C was used for clinical examination. **Table 1** gives the distribution of groups 1 to 4 by examination of study treatment.

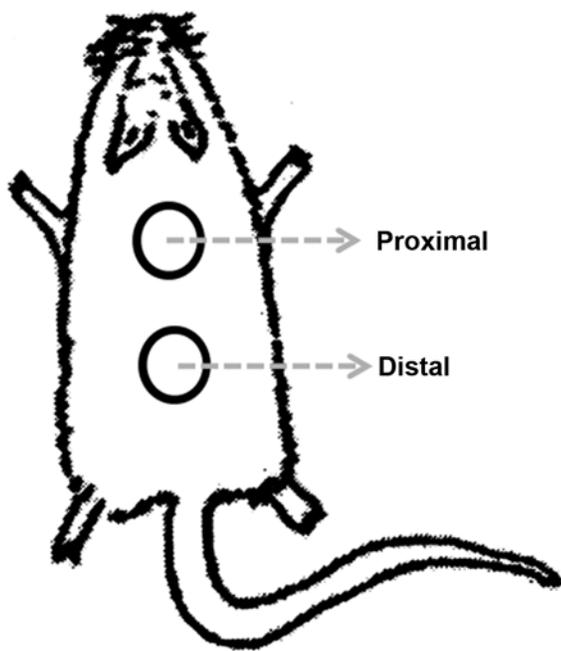


Figure 1.
Diagram of location of burns in rat model.

Burning of Animals

On day 0, all rats were anesthetized by 50 mg/kg ketamine hydrochloride injected intramuscularly along with 5 mg/kg diazepam. The dorsal hair of the rats' thoracic region was shaved and cleaned with povidone-iodine. Each rat was kept in a special box that had a 3×3 cm hole. At first, each rat's proximal and then distal part of thoracic region were exposed separately to the external tip of a 5 cm-long cylinder, 22 mm in diameter, and connected to a source (5 L kettle) of boiling water for 7 s (**Figure 1**). A pilot study was performed at the beginning of the current study and also during our previous study using histological examination that revealed that the epidermis and the whole thickness of the dermis were burned [29]. The burned area of the skin was 3.8 cm^2 [29]. The Medical Ethics Committee of Shahid Beheshti University, MC, approved all procedures.

Low-Level Laser Therapy

Distal burns of groups 2 and 3 were exposed to a pulsed infrared laser (MUSTANG 2000 with L 07 radiating head made by Technica Co; Moscow, Russia):

- Average power output: 70 W.
- Wavelength: 890 nm.
- Pulse frequency: 3,000 Hz.
- Spot size: 1 cm^2 .
- Pulse duration: 180 μs .
- Duration of exposure for group 2: 62 s $3 \times / \text{wk}$.
- Duration of exposure for group 3: 310 s $3 \times / \text{wk}$.
- Energy density for group 2: 2.3 J/cm^2 .
- Energy density for group 3: 11.7 J/cm^2 .

LLLT was begun immediately after skin was burned. To administer laser irradiation, we divided the burned area and normal surrounding skin into eight equal squares ($1 \times 1 \text{ cm}$). Next, we held the tip of the laser source about 5 mm above the skin center of each square and directed it perpendicularly to the target tissue for the designated time just mentioned, i.e., 62 s for group 2 and 310 s for group 3 [30]. Note that LLLT was restricted to three times a week; duration of LLLT was calculated for 1 J/cm^2 energy density each day for group 2 and 5 J/cm^2 energy density each day for group 3 of each point (center of square) for 7 days, and then the time was divided by three. So energy density for groups 2 and 3 was 2.3 and 11.7 J/cm^2 , respectively.

Microbiological Examination

On days 7 and 15, we took microbiological samples from the burned skin of groups A and B rats. Swabs were taken from burns under anesthesia. We cultured and tested the samples to identify *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Bacillus subtilis*, *Lactobacillus*, diphtheria, and *Pseudomonas aeruginosa* using the routine methods of microbiology originally described by Fingold and Martin [31], Baron and Fingold [32], and Brooks et al. [33]. The number of rats in each microbiological group was six plus one additional rat on days 7 and 15. The data for each bacterium were compared between each group's distal burns and also between each group's proximal and distal burns with use of the χ^2 test. Also between study groups, we further compared bacteria assumed to be non-pathogenic (class 1: *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Bacillus subtilis*, *Lactobacillus*, and diphtheria) and organisms assumed to be pathogenic (class 2: *Staphylococcus aureus* and *Pseudomonas aeruginosa*). We statistically compared the data of classes 1 and 2 using the χ^2 test. Colony-forming units (CFU) of each sample were counted semiquantitatively. We compared the data of distal burn and proximal burn of rats and the data of distal burn of groups using independent sample Student *t*-test. Values of $p < 0.05$ were considered statistically significant.

Clinical Examination of Burn Size

The burn area of group C rats was photographed with a digital camera (5-megapixel Canon PowerShot G6; Ohta-ku, Tokyo, Japan), and the surface was measured with Adobe Photoshop CS3 (version 10; San Jose, California) extended image. Each rat was photographed five

times on days 0, 7, 14, 21, and 28. To measure the burn area, we placed the photographed images on a grid, equally dividing each into four regions (Nos. 1, 2, 3, and 4). The holes of all regions completely occupied by the burn were counted. The holes of number 1 and holes of number 3 regions partially occupied by the burn were counted, too. The holes of numbers 2 and 4 regions partially occupied by the burn were not counted.

We calculated the percentage wound size using

$$S_n / S_0 \times 100(\%) , \quad (1)$$

where S_0 is the surface area of the wound on day 0 and S_n is the surface area of the wound on the indicated day [34].

We compared the surface area of the two burns in each rat of all groups using an independent sample Student *t*-test. The surface area of placebo, laser-treated burn, and nitrofurazone-treated burn study groups was analyzed with analysis of variance (ANOVA) in each week and between each group. Statistical significance was set at $p < 0.05$.

RESULTS

Microbiological Examination

Statistical analysis of the incidence of microbial flora is shown in **Table 2**. Significant differences were found between study groups: The incidence of *Staphylococcus epidermidis* and also *Lactobacillus* decreased significantly in group 3 compared with group 1 on day 7 (both $p = 0.046$). The incidence of diphtheria increased significantly in group 2 compared with group 4 on day 15 ($p = 0.018$).

Table 1.

Distribution of rats in study periods and groups.

Day	Group 1: Placebo	Group 2: 2.3 J/cm ² LLLT	Group 3: 11.7 J/cm ² LLLT	Group 4: Nitrofurazone
7	Microbiological examination	Microbiological examination	Microbiological examination	Microbiological examination
15	Microbiological examination	Microbiological examination	Microbiological examination	Microbiological examination
28	Clinical examination	Clinical examination	Clinical examination	Clinical examination

LLLT = low-level laser therapy.

Table 2.

Number of rats from which bacteria were cultured by study group.

Day	Bacteria	Group 1: Placebo (n = 6)	Group 2: 2.3 J/cm ² LLLT (n = 6)	Group 3: 11.7 J/cm ² LLLT (n = 6)	Group 4: Nitrofurazone (n = 7)
7	<i>S. epidermidis</i>	6	5	5	7
	<i>Lactobacillus</i>	2	2	2	2
	<i>Bacillus subtilis</i>	0	0	0	0
	<i>S. saprophyticus</i>	0	0	0	0
	Diphtheria	2	3	3	0
	<i>S. aureus</i>	1	0	1	1
	<i>P. aeruginosa</i>	0	1	0	0
15	<i>S. epidermidis</i>	6	5	3	7
	<i>Lactobacillus</i>	3	2	0	3
	<i>Bacillus subtilis</i>	0	0	0	0
	<i>S. saprophyticus</i>	0	0	0	0
	Diphtheria	0	0	0	0
	<i>S. aureus</i>	0	2	0	1
	<i>P. aeruginosa</i>	0	0	0	0

LLLT = low-level laser therapy, *P.* = *Pseudomonas*, *S.* = *Staphylococcus*.

The χ^2 test of *Staphylococcus epidermidis* and also *Lactobacillus* differed significantly between groups 1 and 3 (both $p = 0.046$). Also, a significant difference of diphtheria was found between groups 2 and 4 ($p = 0.018$).

Colony-Forming Units Count

Day 15

Statistical analysis of the incidence of CFU count of flora is shown in **Table 3**. Student *t*-test showed that CFU count of *Staphylococcus epidermidis* of nitrofurazone-treated burns was significantly lower than that of control burns ($p = 0.025$). Student *t*-test also showed CFU count of *Staphylococcus epidermidis* differed significantly between group 4 and its control burn ($p = 0.025$).

Day 7

Student *t*-test showed that CFU count of *Lactobacillus* in group 3 was significantly lower than that of group 1 ($p = 0.041$). *Staphylococcus epidermidis* in group 4 was significantly lower than that of group 1 ($p = 0.017$).

Independent sample Student *t*-test of CFU count of *Lactobacillus* differed significantly between groups 3 and 1 ($p = 0.025$).

Clinical Examination

Between Groups

Statistical analysis of the wound-closure examination for weeks 1 to 4 is shown in **Figures 2 to 5** and **Table 4**.

In week 1, no significant differences were found between groups. In week 2, independent sample Student *t*-test indicated that the wound-closure rate of experimental (laser-treated) burns was significantly higher than that of the relevant control burn in group 2 ($p = 0.028$). In weeks 3 and 4 after burning, the experimental wound-closure rate compared with its relevant control burn rate increased significantly in group 3 ($p = 0.018$ and $p = 0.01$, respectively). In week 4 alone, the rate also increased significantly in group 4 ($p = 0.005$) compared with that of its relevant control burn.

Comparing experimental burns in group 4 with placebo burns of group 1, we found that the ANOVA test increased significantly in wound-closure rate of 3 weeks after burning (least significant difference [LSD] test, $p = 0.013$). In addition, in groups 3 and 4, the statistical analysis showed a significant increase in wound-closure rate of experimental burns 4 weeks after burning compared with that of group 1 (ANOVA test: $p = 0.005$; LSD tests: $p = 0.028$ and $p = 0.007$, respectively). Significant increase of wound-closure rate was also found in experimental burn of groups 3 and 4 compared with that of group 2 (ANOVA test: $p = 0.005$; LSD tests: $p = 0.028$ and $p = 0.007$, respectively).

Within Groups

ANOVA test differed significantly within each group between sequential intervals in most cases (ANOVA test:

Table 3.Mean \pm standard deviation of colony-forming units of study groups at days 7 and 15.

Day	Bacteria	Group 1 (n = 6)		Group 2 (n = 6)		Group 3 (n = 6)		Group 4 (n = 7)	
		Placebo	Control	2.3 J/cm ² LLLT	Control	11.7 J/cm ² LLLT	Control	Nitrofurazone	Control
7	<i>S. epidermidis</i>	383.3 \pm 312.5	333.3 \pm 150.5	233.3 \pm 296.0	583.3 \pm 780.0	66.6 \pm 81.6	366.0 \pm 492.0	250.0 \pm 4.0	121.4 \pm 107.0
	<i>Lactobacillus</i>	16.7 \pm 40.8	100.0 \pm 89.4	25.0 \pm 41.0	33.3 \pm 51.6	60.0 \pm 0.0	25.0 \pm 41.8	28.6 \pm 39.3	35.7 \pm 47.6
	<i>Bacillus subtilis</i>	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	8.3 \pm 20.4	40.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	<i>S. saprophyticus</i>	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	Diphtheria	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	14.3 \pm 37.8	14.2 \pm 37.8
	<i>S. aureus</i>	0.0 \pm 0.0	0.0 \pm 0.0	175.0 \pm 304.0	343.0 \pm 812.0	16.7 \pm 40.8	16.7 \pm 40.8	14.3 \pm 37.8	14.3 \pm 3.8
	<i>P. aeruginosa</i>	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
15	<i>S. epidermidis</i>	1,038.3 \pm 491.6	1,083.0 \pm 491.6	660.0 \pm 466.6	1,040.0 \pm 638.0	400.0 \pm 344.4	783.3 \pm 676.0	642.0 \pm 350.0	1,142.0 \pm 378.6
	<i>Lactobacillus</i>	75.0 \pm 75.8	25.0 \pm 41.8	30.0 \pm 44.7	20.0 \pm 44.7	50.0 \pm 83.6	366.6 \pm 804.0	428.6 \pm 48.8	57.1 \pm 97.6
	<i>Bacillus subtilis</i>	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	10.0 \pm 22.4	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	<i>S. saprophyticus</i>	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
	Diphtheria	133.3 \pm 196.6	100.0 \pm 200.0	110.0 \pm 134.3	310.0 \pm 439.3	333.3 \pm 51.6	66.7 \pm 103.0	0.0 \pm 0.0	14.3 \pm 37.8
	<i>S. aureus</i>	166.7 \pm 408.0	166.7 \pm 408.0	0.0 \pm 0.0	0.0 \pm 0.0	8.3 \pm 20.4	8.3 \pm 20.4	14.3 \pm 37.8	28.6 \pm 75.6
	<i>P. aeruginosa</i>	0.0 \pm 0.0	0.0 \pm 0.0	100.0 \pm 223.6	20.0 \pm 44.7	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0

LLLT = low-level laser therapy, *P.* = *Pseudomonas*, *S.* = *Staphylococcus*.

$p = 0.001$). However, no significant differences were found in—

- Group 1: 2 and 3 weeks after burning.
- Group 2: 1 and 2 weeks after burning.
- Group 3: 0 and 1 day and 2 weeks after burning.
- Group 4: 0 days and 1 week after burning.

DISCUSSION

Despite the failure of some studies [8–11] to show beneficial effect of CW low-level diode lasers on burn healing in healthy animals, the present study for the first time demonstrated that pulsed LLLT can significantly accelerate the wound-closure rate of a third-degree burn model in healthy rats.

The biostimulatory effect of pulsed LLLT in the current study is demonstrated by the significant increase of the wound-closure rate of laser-treated burns compared with the placebo group 1, 3, and 4 weeks after burning, while nitrofurazone-treated burns significantly increased the wound-closure rate compared with placebo burns only 4 weeks after burning. Apparently, LLLT was more effective than nitrofurazone ointment in healing a third-degree burn model. LLLT, when used appropriately, can stimulate the healing of injured tissue such as those of dermis [35]. Investigations into the mechanisms involved

have shown that many of the cell types whose interactions repair the dermis can be therapeutically stimulated by treatment with LLLT both in vitro and in vivo. Mast cells and macrophages can be stimulated to release growth factors and other substances, whereas the proliferation of fibroblasts, endothelial cells, and keratinocytes maintained during adverse conditions can also be stimulated. The development of granulation tissue is mainly controlled by growth factors released from macrophages [35].

In the present investigation, we found that the effects of 2.3 J/cm² LLLT of third-degree burns are more evident only at the early stage of the burn-healing process; however, we cannot find a significant effect of 2.3 J/cm² LLLT at the late stage of burn healing compared with its control burns. One proposed mechanism by which LLLT stimulates the wound-healing process is light energy absorbed by mitochondria, which increases cell energy and stimulates the release of chemical mediators [36–38]. Apparently, such a mechanism did not occur in the 2.3 J/cm² laser-treated burns of the present study, as well as of the Cambier et al., Schlager et al., and Al-Watban and Delgado studies [8–11]. This finding may be due to insufficient light energy reaching the cells. Allendorf et al. have suggested that laser light penetrations of tissue and eschar debridement are involved in wound healing [39]. Wounds that are not debrided, such as wounds in the current study, may not allow the maximum amount of light

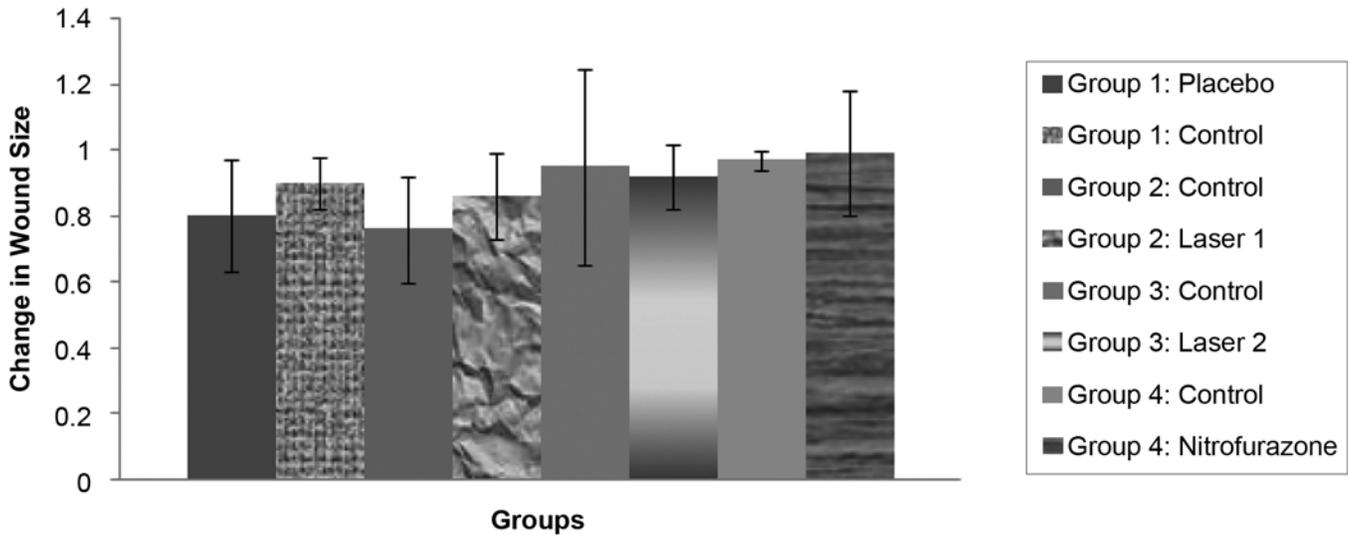


Figure 2.

Wound-closure rate represented as percentage of wound size after burn induction at week 1 between groups. No significant differences were found between groups.

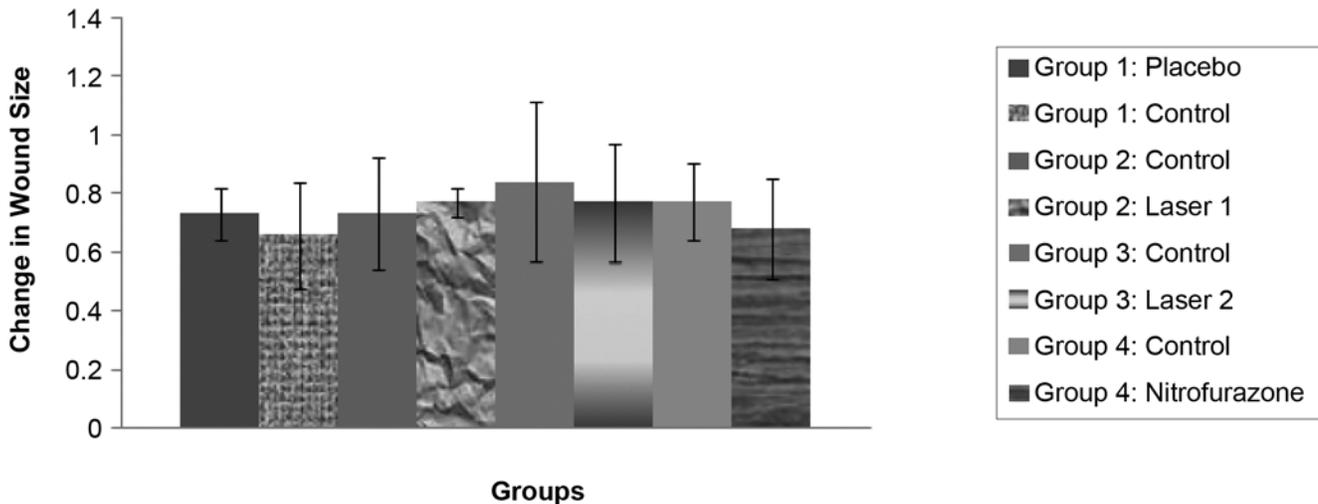


Figure 3.

Wound-closure rate represented as percentage of wound size after burn induction at week 2 between groups: independent sample Student *t*-test showed significant differences between control and experimental (laser-treated) burns of group 2 ($p = 0.028$).

to reach the tissue. Our results suggest that pulsed LLLT at a 11.7 J/cm^2 dose significantly increases the wound-closure rate. Our results also confirm Matic et al.'s findings that pulsed LLLT significantly accelerates the wound-closure rate of a surgically induced cutaneous wound [24]. Other studies failed to show positive effect of pulsed LLLT on the impaired wound-healing process

[25–26], whereas the results of the present study and of Matic et al.'s study confirmed positive effect of pulsed LLLT on burn and acute skin wound. Using a GaAlAs 890 nm multidiode ($n = 60$) array unit (270 Hz; maximum rated output 300 mW), Lowe et al. examined wound healing in mice that had been exposed to X-ray irradiation [25]. They found that although wounds treated

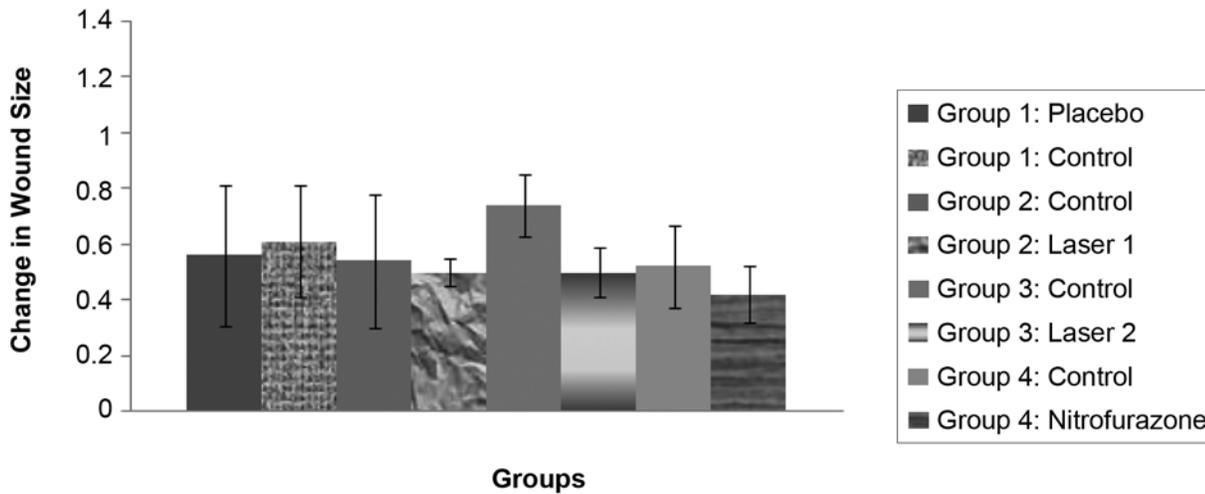


Figure 4.

Wound-closure rate represented as percentage of wound size after burn induction at week 3 between groups. Significant differences were found between control and experimental (laser-treated) burns of group 3 at week 3 after burning ($p = 0.018$, $p = 0.01$, respectively).

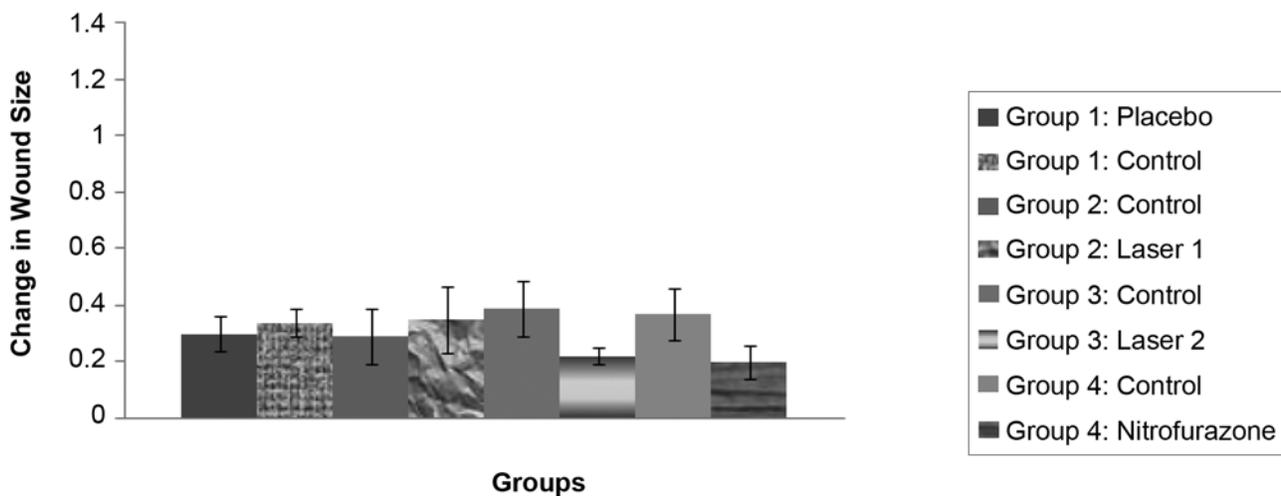


Figure 5.

Wound-closure rate represented as percentage of wound size after burn induction at week 4 between groups. Significant differences were found between control and experimental (nitrofurazone-treated) burns of group 4 after burning ($p = 0.003$). Analysis of variance (ANOVA) test showed significant differences between experimental burns of groups 3 and 4 and that of group 1 after burning (ANOVA test: $p = 0.005$, least significant difference (LSD) test: $p = 0.028$ and $p = 0.007$, respectively). Significant differences were also found between groups 3 and 4 and group 2 (ANOVA test: $p = 0.005$, LSD test: $p = 0.028$ and $p = 0.007$, respectively).

with X-ray irradiation showed delayed wound-healing treatment with 890 nm, light therapy did not significantly affect wound closure at doses of 0.18 and 0.54 J/cm² and only further delayed wound healing at a dose of 1.54 J/cm² [26]. Using a similar animal model of radiation-impaired wound healing in mice, Walker and colleagues found no hastening in wound healing with 660 nm GaAlAs laser

(5 kHz; 15 mW; 0.5, 1.5, and 4.0 J/cm² for three groups) [26].

The statistically significant difference found in wound-closure rate of burns between laser-treated (distal) and control (proximal) burns in group 3 of the current study clearly rejects the probable systemic effect of LLLT. Rochkind et al. reported that irradiation of low-power

Table 4.

Mean \pm standard deviation of wound-closure rate represented as percentage of wound size after burn induction at weeks 1, 2, 3, and 4 within each group. ANOVA test ($p = 0.001$) also showed significant differences within each group between sequential intervals in most cases.*

Weeks After Burning	Group 1 (n = 6)		Group 2 (n = 7)		Group 3 (n = 6)		Group 4 (n = 6)	
	Placebo	Control	2.3 J/cm ² LLLT	Control	11.7 J/cm ² LLLT	Control	Nitrofurazone	Control
1	0.90 \pm 0.08	0.8 \pm 0.17	0.86 \pm 0.13	0.76 \pm 0.16	0.92 \pm 0.10	0.95 \pm 0.30	0.99 \pm 0.19	0.97 \pm 0.03
2	0.66 \pm 0.18	0.73 \pm 0.09	0.77 \pm 0.05	0.73 \pm 0.19	0.77 \pm 0.21	0.84 \pm 0.27	0.68 \pm 0.17	0.77 \pm 0.13
3	0.61 \pm 0.20	0.56 \pm 0.25	0.50 \pm 0.05	0.54 \pm 0.24	0.50 \pm 0.09	0.74 \pm 0.11	0.42 \pm 0.10	0.52 \pm 0.15
4	0.34 \pm 0.05	0.30 \pm 0.06	0.35 \pm 0.12	0.29 \pm 0.10	0.22 \pm 0.03	0.39 \pm 0.10	0.2 \pm 0.06	0.37 \pm 0.09

*No significant differences were found in group 1, between 2 and 3 weeks after burning; group 2, between 1 and 2 weeks after burning; group 3, between day 0 and 1 week and 2 weeks after burning; and group 4, day 0 and 1 week after burning.

ANOVA = analysis of variance, LLLT = low-level laser therapy.

laser on a crushed injured sciatic nerve in a right leg of a bilaterally inflicted crush injury significantly increased the compound action potential in the left nonirradiated leg as well [40].

Microbiological examination showed that the control burns had few pathogen microorganisms; however, pulsed LLLT significantly decreased incidences of *Staphylococcus epidermidis* and *Lactobacillus* compared with group 1 (control burns), incidence of diphtheria compared with nitrofurazone-treated burns, and CFU of *Lactobacillus* compared with placebo burns. The current results provide little evidence of inhibitory effect of pulsed LLLT on microbial flora of a third-degree burn model.

Examining the burns using a histological method may help detect differences between study groups at the cellular level; therefore, further histological studies are suggested.

CONCLUSIONS

We conclude that irradiation of a third-degree burn model with an 11.7 J/cm²/890 nm-pulsed low-level laser in rats significantly increased wound-closure rate compared with control burns. In addition, the inhibitory effect of the LLLT on microbial flora of the burn was minimal.

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