Photomedicine and Laser Surgery
Volume 25, Number 2, 2007

Pp. 72–77
DOI: 10.1089/pho.2006.1094

Low-Level Laser Therapy Enhances Wound Healing in Diabetic Rats: A Comparison of Different Lasers

FAROUK A.H. AL-WATBAN, M.Sc., Ph.D, XING YANG ZHANG, M.D., and BERNARD L. ANDRES, MT(AMT)

ABSTRACT

Objective: **The effects of wound healing acceleration on diabetic rats were determined and compared using different laser wavelengths and incident doses.** ***Background Data:*** **Many studies have demonstrated that low-level laser therapy (LLLT) can promote the wound healing on non-diabetic animals.** ***Methods:*** **Male Sprague-Dawley rats were used. Streptozotocin (70 mg/kg) was applied for diabetes induction. An oval full-thickness skin wound was created aseptically with a scalpel in 51 diabetic rats and six non-diabetic rats on the shaved back of the animals. The study was performed using 532, 633, 810, and 980 nm diode lasers. Incident doses of 5, 10, 20, and 30 J/cm^2 and treatment schedule of 3 times/week were used in the experiments. The area of wound on all rats was measured and plotted on a slope chart. The slope values (mm^2/day), the percentage of relative wound healing, and the percentage of wound healing acceleration were computed in the study.** ***Results:*** **Mean slope values were 6.0871 in non-diabetic control and 3.636 in diabetic control rats ($p < 0.005$). The percentages of wound healing acceleration were 15.23, 18.06, 19.54, and 20.39 with 532-nm laser, 33.53, 38.44, 32.05, and 16.45 with 633-nm laser, 15.72, 14.94, 9.62, and 7.76 with 810-nm laser, and 12.80, 16.32, 13.79, and 7.74 with 980-nm laser, using incident doses of 5, 10, 20, and 30 J/cm^2, respectively. There were significant differences ($p < 0.001$) in the mean slope value of wound healing on diabetic rats between control groups and treatment groups in 532, 633, 810, and 980 nm lasers.** ***Conclusion:*** **The wound healing on control rats with diabetes was slower than on control rats without diabetes. LLLT at appropriate treatment parameters can enhance the wound healing on diabetic rats. The optimum wavelength was 633 nm, and the optimum incident dose was 10 J/cm^2 in our study.**

INTRODUCTION

DIABETES IS BELIEVED to affect 2–4% of the general population, and its incidence is increasing. It is predicted that it will affect 239 million people worldwide by 2010. As many as 15% of people with diabetes will develop foot ulceration and wounds, and 3% will have a lower limb amputation.[1] Diabetic wounds are complex microcosms of multiple pathophysiologic processes. The wounds are predominantly characterized by polymicrobial infection, peripheral neuropathy, structural deformity, altered immune function or increased susceptibility to infection, decreased wound nitric oxide (NO) production, and often hypoxia/ischemia.[2,3] Diabetic wounds present a major problem for modern health care. Although the beneficial effects of low-level laser therapy (LLLT) on the normal tissue repair process or wound healing have been established,[4–8] the acceleration of diabetic wound healing in animals using laser photostimulation has not yet been investigated extensively. The purpose of this study is to determine and compare the effects of wound healing acceleration on diabetic rats using different laser wavelengths and incident doses.

Laser Research Section, Biological and Medical Research Department, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia.

METHODS

Animals

A number of male Sprague-Dawley rats were used in this study. The animals weighed 299–490 (368.42 ± 43.70) g and were 21–22 weeks old at the start of the experiment. The rats were originally imported from Charles River Co. (Margate, Kent, UK) in 1984 and, at the time of this study, were bred at the animal facility of King Faisal Specialist Hospital and Research Centre (KFSH&RC). Animal protocols were reviewed and approved by the Animal Care and Use Committee (ACUC). During the study, the rats were housed one per cage, maintained under controlled environmental conditions (12-h light/dark cycle, temperature ~23°C), and provided with standard laboratory food and water *ad libitum*.

Chemical induction of diabetes

Diabetes was induced in the rats by an intraperitoneal injection of the pancreatic beta-cell toxin Streptozotocin (freshly dissolved in 0.9% sterile saline; Sigma, St. Louis, MO) at a dose of 70 mg/kg body weight. Diabetes was assessed by estimating hyperglycemia and glycosuria. Animals were rejected from the study when blood and urine glucose did not reach 200 mg/dL and four pluses (≥111 mmol/L or ≥20 g/L), respectively, after 24-h post-induction, and their body weight increased consistently during the first 3 days of induction. Hyperglycemia, glycosuria, and rat weight were determined and monitored on schedule as described previously.[9]

Wound infliction

Non-diabetic rats were anesthetized with 50 mg/kg ketamine and 20 mg/kg xylocaine, while the diabetic rats were given a 30% lower dosage. The surgical site was shaved using an electric clipper, excess hair was removed by a lotion, and the site was disinfected with an isopropyl alcohol swab. Oval-full-thickness wounds of 78.51–88.38 (82.88 ± 3.99) mm^2 area were created aseptically with a scalpel in 51 diabetic rats and six non-diabetic rats on the shaved back of the animal in the gluteus maximums region. All diabetic rats with wounds were divided randomly into control (n = 3) and treatment groups (n = 48) on the basis of the experimental process designated. Six non-diabetic rats as control were used for comparison with diabetic control rats.

Laser system and treatment parameters

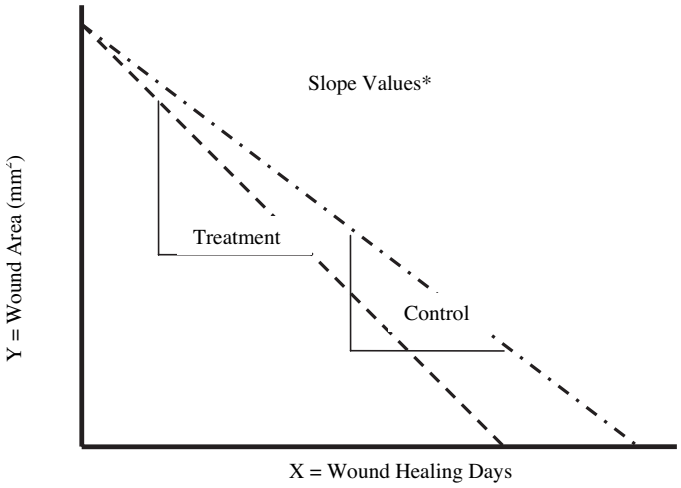
The study was performed using a 532-nm green laser system (GSF32-150P; Intelite, Inc.), with 633-nm, 810-nm, and 980-nm diode laser (Biophoton, St. Alban, France). The output power was measured using a laser power meter (Molectron Max 5200). The laser treatment parameters are listed in Table 1. The laser beam was aligned to cover the entire wound area, including the boundaries. The rats treated were restrained in a Plexiglas cage without anesthesia during the laser irradiation period. The control group also received the same manipulation, excluding the laser exposure.

Data analysis

The areas of wound on all rats were measured using a caliper daily for 5 days/week and plotted on a slope chart of wound healing for 3 weeks (Fig. 1). A trend-line was applied on the slope chart, and the slope value (mm^2/day) of the wound healing in all rats was computed using the linear type and set intercept option. Mean slope value of wound healing was computed in

TABLE 1. LASER TREATMENT PARAMETERS

Laser (nm)	*Power (mW)*	*Spot size (cm^2)*	*Power density (mW/cm^2)*	*Irradiation time (min)*	*Incident dose (J/cm^2)*	*Treatment schedule (times/week)*
Diode 532	143	7	20.4	4.1	5	3
				8.2	10	
				16.3	20	
				24.5	30	
Diode 633	140	9	15.56	5.4	5	3
				10.7	10	
				21.4	20	
				32.1	30	
Diode 810	200	9	22.22	3.8	5	3
				7.5	10	
				15.0	20	
				22.5	30	
Diode 980	200	9	22.22	3.8	5	3
				7.5	10	
				15.0	20	
				22.5	30	



% Relative W.H. = (Slope in Treatment – Slope in Control) / Slope in Control × 100 (%)

*Slope Values means closure of wound area (mm^2) per day

FIG. 1. Calculation of slope values and percentage of relative wound healing on rats.

every group. The percentage of relative wound healing (RWH) was calculated as follows:

$$\% \text{ of RWH} = (\text{slope value in diabetic control or treatment} - \text{slope value in non-diabetic control}) / \text{slope value in non-diabetic control} \times 100\ (\%)$$

The percentage of wound healing acceleration (WHA) was calculated as follows:

$$\% \text{ of WHA} = \%\text{RWH in diabetic treatment group} - \%\text{RWH in diabetic control group}$$

Statistical analysis

Statistical analyses were performed using the Student's *t*-test for comparison of data from the study. Differences were considered statistically significant when the *p* value was <0.05.

RESULTS

Fifty-one diabetic rats were induced successfully by streptozotocin injection and used in the study. Mean slope values of wound healing were 6.0871 mm^2/day on non-diabetic control rats and 3.636 mm^2/day on diabetic control rats. There was a significant difference ($p < 0.005$) in the mean slope values of wound healing between non-diabetic control rats and diabetic control rats (Fig. 2). Mean slope values of wound healing on diabetic rats in control and treatment group using different laser doses and wavelengths are shown in Figure 3. The percentage relative wound healing on diabetic control rats and diabetic treatment rats is shown in Figure 4. The percentage of wound healing acceleration in diabetic rats after laser therapy is shown in Figure 5. The results of statistical analysis using Student's *t*-test on the mean slope value of wound healing

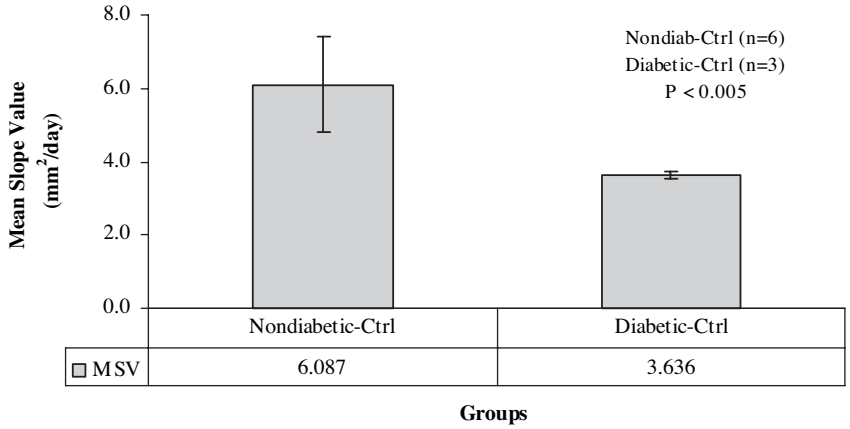
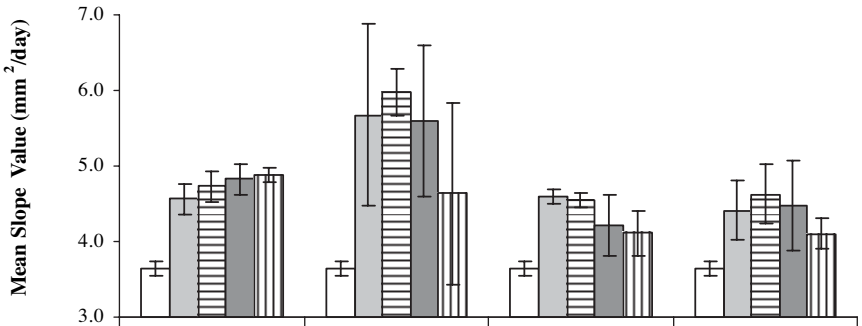


FIG. 2. Mean slope values of wound healing on non-diabetic control rats and diabetic control rats. Results are presented as mean ± SD.



	532 nm	633 nm	810 nm	980 nm
D-Ctrl	3.636	3.636	3.636	3.636
D-5J	4.563	5.6767	4.5926	4.415
D-10J	4.735	5.9756	4.5449	4.629
D-20J	4.825	5.5866	4.2212	4.475
D-30J	4.877	4.637	4.1081	4.107

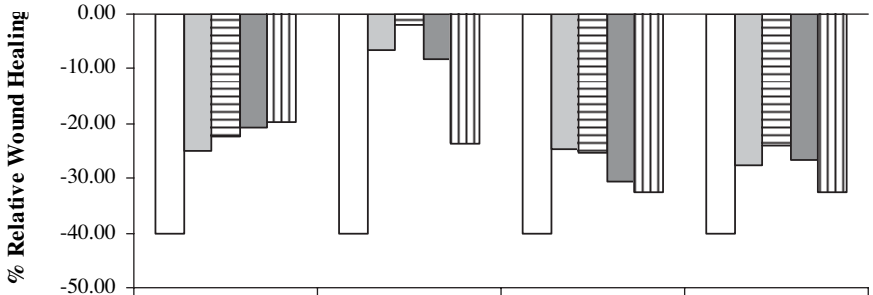
Laser Wavelength

FIG. 3. Mean slope values of wound healing on diabetic rats in control group and treatment groups using different laser doses and wavelengths. Results are presented as mean ± SD.

between diabetic control and diabetic treatment groups using different wavelengths are shown in Table 2.

DISCUSSION

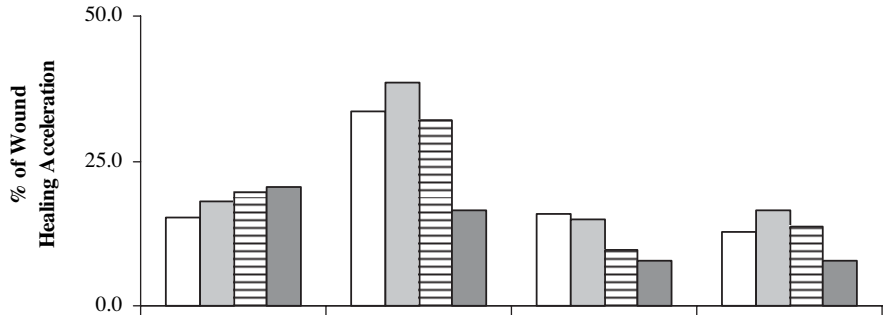
A total of 51 Sprague-Dawley rats with diabetes induced by Streptozotocin were used in the study. Additionally, six non-diabetic rats as control were used for comparison with diabetic control rats. Our results showed that wound healing on control rats with diabetes was slower than on control rats without diabetes. There was a significant difference ($p < 0.005$) in the mean slope values of wound healing between diabetic control rats and non-diabetic control rats (Fig. 2). Impairment of diabetic wound healing is well recognized. However, the exact mechanism is still not completely known.[10] The course of diabetic wound healing appears to be hindered by many factors, including specific metabolic deficiencies and impaired physiological responses—for example, the altered metabolism of carbohydrates, fats and proteins resulting from the absence or deficiency of insulin, hyperglycemia leading to non-enzymatic glycosylation, osmotic diuresis leading to decreased perfusion and oxygenation, and free radical production.[11] Olerud et al.[12] observed the wound healing in normal elderly



	532 nm	633 nm	810 nm	980 nm
D-Ctrl	-40.27	-40.27	-40.27	-40.27
D-5J	-25.04	-6.74	-24.55	-27.47
D-10J	-22.21	-1.83	-25.33	-23.95
D-20J	-20.73	-8.22	-30.65	-26.48
D-30J	-19.88	-23.82	-32.51	-32.53

Laser Wavelength

FIG. 4. The percentage of relative wound healing (RWH) in the diabetic rats after laser therapy compared with non-diabetic control. % of RWH = (slope value in diabetic control or treatment − slope value in non-diabetic control)/slope value in non-diabetic control × 100 (%).



	532 nm	633 nm	810 nm	980 nm
D-5J	15.23	33.53	15.72	12.80
D-10J	18.06	38.44	14.94	16.32
D-20J	19.54	32.05	9.62	13.79
D-30J	20.39	16.45	7.76	7.74

Laser Wavelength

FIG. 5. The percentage of wound healing acceleration (WHA) in the diabetic rats after laser therapy using different laser doses and wavelengths. %WHA = %RWH in diabetic treatment group − %RWH in diabetic control group.

adults and patients with diabetes or peripheral vascular disease. Their results showed that wound healing was impaired in patients with diabetes, with a significant reduction in the numbers of fibroblasts found in the deep components of the wounds. On our animal study, the wound healing was delayed in diabetic rats. Our results are similar to their findings. A study by Loots et al.[13] demonstrated that fibroblasts from patients with type 2 diabetes showed a significantly lower proliferation rate and an altered morphology *in vitro* compared with non-lesion and age-matched control fibroblasts from patients without diabetes. The researchers conclude that the diabetic process and the wound environment itself cause the fibroblasts to age. The overall functional activity of the fibroblast is thus reduced. The main functions of the fibroblast are the production of components of the extracellular matrix and wound contraction.

On entering the wound space, the fibroblasts become involved in the formation of granulation tissue. Within the wound, the fibroblasts produce collagen, which is the most abundant protein within the extracellular matrix and contributes to the tensile strength of wounds.[14] A more recent study, involving non-lesion fibroblasts, showed that diabetic fibroblasts have a decreased proliferation response to growth factors, caused by a deficiency in growth factor receptor expression, compared with non-diabetic fibroblasts from sibling control.[10] Research has shown that the defects that occur in diabetic wound healing may be caused by altered collagen metabolism and abnormal granulation tissue formation.[15] Diabetes can result in the development of several complications, including diabetic foot wounds that can potentially lead to lower limb amputation. The poor wound healing in diabetes is a barrier in medicine. Our study indicated that the irradiation of low-power lasers at appropriate treatment parameters can accelerate the wound healing on diabetic rats. Mean slope values of wound healing on diabetic rats in treatment groups using different laser doses and wavelengths were bigger than control groups (Fig. 3). There were significant differences ($p < 0.001$) in the mean slope value of wound healing on diabetic rats between control and treatment groups using several wavelengths (Table 2). The percentage of relative wound healing and the percentage of wound healing acceleration after laser therapy showed that the optimum wavelength and incident dose was 633 nm and 10 J/cm^2 in the study (Figs. 4 and 5). The research revealed also that the irradiation of visible laser light was better than invisible laser light in the treatment of wound healing

TABLE 2. RESULTS OF STATISTICS ANALYSIS USING STUDENT'S *T*=TEST ON MEAN SLOPE VALUE OF WOUND HEALING ON DIABETIC RATS BETWEEN CONTROL AND TREATMENT GROUPS

	Mean slope value (mm^2/day)			
Laser (nm)	*Control*	*Treatment*[a]	*n*	*p-value*
Diode (532)	3.636 ± 0.1	4.7501 ± 0.2	15	<0.001[b]
Diode (633)	3.636 ± 0.1	5.4960 ± 1.0	15	<0.001[b]
Diode (810)	3.636 ± 0.1	4.3667 ± 0.3	15	<0.001[b]
Diode (980)	3.636 ± 0.1	4.4064 ± 0.4	15	<0.001[b]

[a]Every treatment group included doses of 5, 10, 20, and 30 J/cm^2.
[b]There was a significant difference in the mean slope value of wound healing between treatment and control.

on diabetic rats. The reason for the effective acceleration of wound healing on diabetic rats using low-power lasers was that perhaps the absorption of laser light with specific wavelength by target tissue resulted in the enhancement of fibroblast proliferation and the promotion of collagen metabolism and granulation tissue formation in the diabetic wound.

The healing process of diabetic wound is a complicated one and is initiated by a complex series of events that include chemoattraction, growth factor pathways, complement generation, and the energy-poor environments created by low oxygen tensions, low PH, and high lactate concentrations.[16] Macrophages attracted to such environments release lactate aerobically, as well as anaerobically, and generate potent growth factors, resulting in brisk angiogenesis and multiplication of fibroblasts at wound margins. These events can take place in a low-oxygen environment. However, the modification of collagen by fibroblasts so that it can be polymerized and secreted into the extracellular space can be accomplished only when oxygen is present at high partial pressures. Further studies are necessary to use more efficient laser light sources, adjust the laser treatment parameters, and improve the supply of energy and oxygen in diabetic wound.

CONCLUSION

Our study indicated that wound healing on control diabetic rats was slower than on control non-diabetic rats. We concluded that irradiation of low-power lasers at appropriate treatment parameters can accelerate wound healing on diabetic rats. The optimum wavelength and incident dose was 633 nm and 10 J/cm^2 in the study.

ACKNOWLEDGMENTS

We would like to thank the Animal Facility and KFSH&RC for their assistance in the study. Also, we would like to thank Maria Victoria D. Gonzaga and Mary Jane S. Anonuevo for their support in completing the experiment and the manuscript. We extend our gratitude to King Abdulaziz City for Science and Technology (KACST) for financial support.

REFERENCES

1. Mandrup-Poulson, T. (1998). Clinical review: diabetes. Br. Med. J. 316, 1221–1225.
2. Schaffer, M.R., Tantry, U., van Wesep, R.A., et al. (1997). Nitric oxide metabolism in wounds. J. Surg. Res. 71, 25–31.
3. Reiber, G.E., Pecoraro, R.E., and Keopsell, T.D. (1992). Risk factors for amputation in patients with diabetes mellitus: a case control study. Ann. Intern. Med. 117, 97–105.
4. Al-Watban, F.A.H., and Zhang, X.Y. (1994). The effect of low-power He-Ne and He-Cd laser therapy on wound healing on rats. J. Clin. Laser Med. Surg. 12, 327–329.
5. Al-Watban, F.A.H., and Zhang, X.Y. (1995). Stimulative and inhibitory effects of low incident levels of argon laser energy on wound healing. Laser Ther. 7, 11–18.
6. Al-Watban, F.A.H., and Zhang, X.Y. (1996). Comparison of the effects of laser therapy on wound healing using different laser wavelength. Laser Ther. 8, 127–135.
7. Al-Watban, F.A.H. (1996). Therapeutic laser's effectiveness and dosimetry, in: *Biomedical Optical Instrumentation and Laser-Assisted Biotechnology*. A.M. Verga Scheggi, et al. (eds.). Amsterdam: Kluwer Academic Publishers, pp. 171–183.
8. Reddy, G.K., Stehno-Bittel, L., and Enwemeka, C.S. (2001). Laser photostimulation accelerates wound healing in diabetic rats. Wound Repair Regen. 9, 248–255.
9. Al-Watban, F.A.H., and Andres, B.L. (2003). Polychromatic LED therapy in burn healing of non-diabetic and diabetic rats. J. Clin. Laser Med. Surg. 21, 249–258.
10. Reenstra, W.R., Veves, A., Orlow, D., et al. (2001). Decreased proliferation and cellular signaling in primary dermal fibroblasts derived from diabetics versus non-diabetic sibling controls. Acad. Emerg. Med. 8, 519.
11. Pecoraro, R.E., Ahroni, J.H., Boyko, E.J., et al. (1991). Chronology and extremities of tissue repair in diabetic lower-extremity ulcers. Diabetes 40, 1305–1313.
12. Olerud, J.E., Odland, G.F., Burgess, E.M., et al. (1995). A model for the study of wound in normal elderly adults and patients with peripheral vascular disease or diabetes mellitus. J. Surg. Res. 59, 349–360.
13. Loots, M.A.M., Lamme, E.N., Mekkes, J.R., et al. (1999). Cultured fibroblasts from chronic diabetic wounds on the lower extremity show disturbed proliferation. Arch. Dermatol. Res. 291, 93–99.
14. Martens, M.F.W.C., Huyben, C.M.L.C., and Hendriks, T.H. (1992). Collagen synthesis in fibroblasts from human colon: regulatory aspects and differences with skin fibroblasts. Gut 33, 1664–1670.
15. Spanheimer, R.G. (1988). Direct inhibition of collagen production *in vitro* by diabetic rats serum. Metabolism 37, 479–485.
16. Knighton, D.R., Fylling, C.P., Fiegel, V.D., et al. (1990). Amputation prevention in an independently reviewed at-risk diabetic population using a comprehensive wound care protocol. Am. J. Surg. 160, 466–471.

Address reprint requests to:
Dr. Farouk A.H. Al-Watban
Laser Research Section
Biological and Medical Research Department
King Faisal Specialist Hospital & Research Centre
MBC-03, P.O. Box 3354
Riyadh 11211, Kingdom of Saudi Arabia

*E-mail:*watban@kfshrc.edu.sa