PRAMANA
— journal of
physics

Vol. 75, No. 6
December 2010
pp. 1287–1293

Development and evaluation of an optical fibre-based helium–neon laser irradiation system for tissue regeneration: A pilot study

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Abstract. Low level laser therapy is being extensively used to treat various medical ailments including wound healing. In the present study, an optical fibre-based helium–neon (He–Ne) laser irradiation system was designed, developed and evaluated for optimum tissue repair on mice excision wounds. Circular wounds of 15 mm diameter were created on the dorsum of animals and single exposure of uniformly distributed laser beam was administered at 1, 2 and 3 J/cm^2 to the respective test groups with suitable controls. Progression of healing was monitored by measuring wound contraction and mean healing time. Significant reduction in wound size and mean healing time ($p < 0.001$) were observed in the test groups for the laser dose of 2 J/cm^2 compared to the unilluminated controls, suggesting the suitability of this dose.

Keywords. Therapeutic application; optimization; tissue response; contraction.

PACS Nos 87.50.ct; 87.55.De; 87.55.dh; 87.29.rj

1. Introduction

Wound healing is a complex biological sequence of events in a closely orchestrated cascade to repair damage [1]. This process is divided into four overlapping but well-choreographed phases including haemostasis, inflammatory, proliferative and remodelling and scar formation [2]. For the normal healing process, it is essential to progress thorough sequential events which results in the immediate filling of the gap. Acute wound healing follows a predictable chain of events in a well-organized fashion, whereas chronic wounds will have prolonged inflammatory or proliferative phases resulting in tissue fibrosis and nonhealing ulcers [1]. According to recent medical survey, approximately 86,000 lower limbs are annually amputated due to the diabetic-related wound complications which alarmingly increased the morbidity and severely affected the quality of life [3].

In recent years, phototherapy is widely used as a treatment modality to accelerate wound healing. In light-based therapy, living cells or tissues were exposed to low power of red and infrared light [4] of wavelength ranging from 600 to 1070 nm involving diverse light sources. The red He–Ne laser is considered to have the best therapeutic effect at cellular level [5,6]. Being minimally invasive or noninvasive with no side effects, low level laser therapy (LLLT) produces significant bioeffects at cellular and biochemical levels in a wavelength-dependent manner without significant heating thereby avoiding thermal damage of the target cells/tissues. In LLLT, for every biological tissue/cell type there exists a threshold dose that varies depending on the application and the doses higher or lower than the therapeutic dose certainly lead to undesirable outcome [7]. Although the experiments conducted on various *in vivo* [8,9] and *in vitro* [10,11] models well demonstrated the therapeutic role of LLLT, this method continues to be contentious. This could be attributed to the lack of knowledge in choosing the critical illumination parameters such as light source, wavelength, dose etc. and incomplete understanding of the underlying biochemical mechanism responsible for stimulatory effect [6]. Based on these reports, the present preclinical study aimed to develop a He–Ne laser-based fibre-optic probe to irradiate animal wounds in dose-dependent manner, evaluating optimum dose for tissue regeneration in mice.

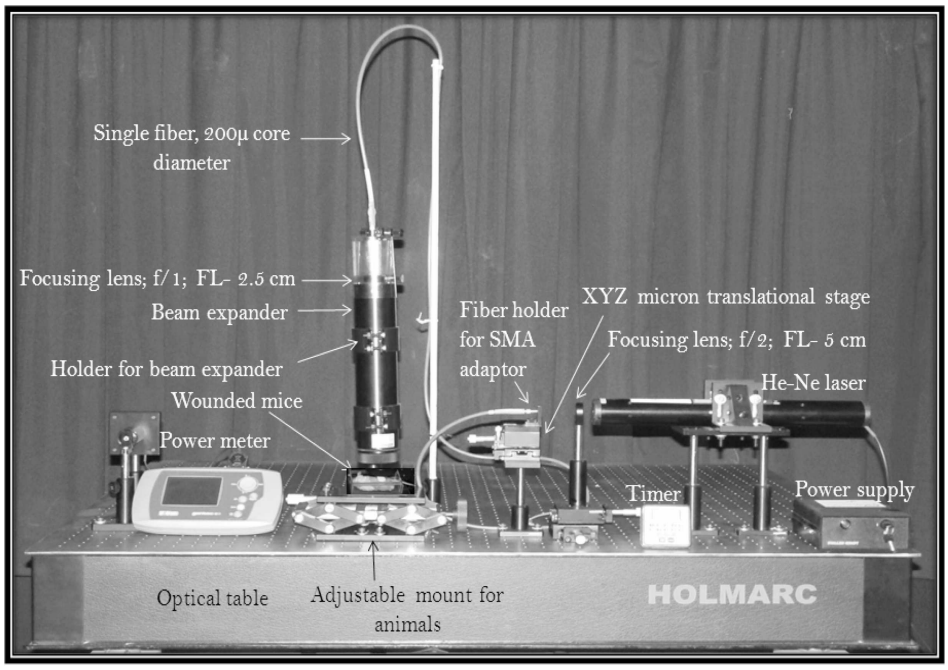

Figure 1. The assembled low level laser therapy system for tissue regeneration.

2. Methods and materials

2.1 *Design and development of laser delivery system*

In the present study, we have designed and fabricated few essential auxiliary components such as, fibre holder, fibre probe, clamps for holding the beam expander etc. locally at the Manipal University, Manipal. The fabricated mechanical components were used to assemble and to develop laser irradiation system (figure 1). He–Ne laser (CVI Melles Griot, USA) was used as the illumination source which was mounted on an optical table. The red light emitting from the laser (632.8 nm) was coupled to the optical fibre (Ocean Optics, USA) having a core diameter of 200 μm with a numerical aperture of 0.22 (transmittance >90%) using a 5 cm focal length ($f/2$) lens. To avoid any ambiguity in this coupling process and to focus the laser beam accurately at the centre of the fibre, the input end of the fibre was rigidly fixed onto the fibre holder mounted on the XYZ precession mount providing necessary freedom required for alignment in three mutually perpendicular directions X, Y and Z. The output from the fibre was focussed onto the entrance slit of the vertically positioned beam expander using 2.5 cm focal length focusing lens ($f/1$). As per requirement, the spot size of the beam expander output was adjusted using the adjustable knobs provided for it. The beam expander output could be made to fall on the wound of the animal placed just below the beam expander on a movable lab jack having provision to increase/decrease the distance between the wound surface and the beam expander.

2.2 *Selection of animals*

We followed the guidelines of World Health Organization and the Indian National Science Academy, New Delhi for animal care and handling. The current study has been approved by the Institutional Animal Ethical Committee (IAEC). Swiss albino mice weighing 25–30 g of either sex were selected from an in-bred colony maintained under the controlled conditions of temperature ($23 \pm 2^{\circ}$C), humidity ($50 \pm 5\%$) and light (14 and 10 h of light and dark respectively). Selected animals were monitored for infections during the experimental period and animals which showed any such signs were excluded from the study.

2.3 *Production of full thickness skin lesion*

Dorsal hair of each animal was cleanly shaved prior to wounding using cordless electric mouse clipper (Philips Electronics, India Ltd). The animals were then anesthetized by administering ketamine (65 mg/kg body weight) and diazepam (8 mg/kg body weight) intraperitonially. The shaved skin portion of the body was sterilized by wiping it with sterillium solution. Following anesthesia, 15 mm circular full thickness wound was created by excising skin in an aseptic environment using sterile scissors and forceps. Individual animal was housed separately in polypropylene cages with wounds left undressed. The animals were randomly distributed into

four groups with six animals in each group, group 1 as control, group 2–4 as laser dose variants (1, 2 and 3 J/cm^2).

2.4 *Laser irradiation*

The animals were irradiated with He–Ne laser (wavelength = 632.8 nm, power = 7 mW) only once immediately after the wounding. Each group was assigned to a particular laser dose of 1, 2 and 3 J/cm^2 obtained by exposing for different time durations (4 min 15 s for 1 J/cm^2, 8 min 32 s for 2 J/cm^2 and 12 min 47 s for 3 J/cm^2). For each irradiation, uniform exposure of the laser beam to the entire wound site was ensured by maintaining 20 mm distance between wound site and the beam expander. To provide optimal laser penetration and to minimize any reflection loss from the skin while irradiation, the laser beam was allowed to incident normally on the wound surface. A sensitive laser power meter (Gentec, Canada) was used to measure the laser power before and after each exposure, which would ensure the proper energy delivery to the wounded site. To determine the effect of single exposure of each dose on wounded mice, all irradiation experiments were performed in duplicate. Control animals were not exposed to laser irradiation.

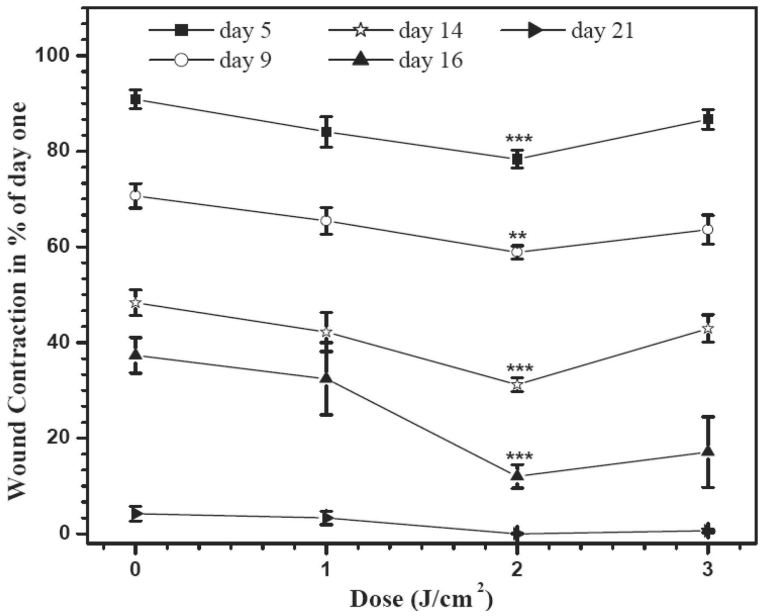

Figure 2. Changes in wound contraction as percentage of day 1 after exposure to various laser doses assessed at different post-wounding days. The significant levels are $^{***}p < 0.001$ and $^{**}p < 0.01$ and no symbol means nonsignificant compared to control.

Light-based tissue regeneration set-up

2.5 *Measurement of lesion contraction and determination of mean healing time*

Video image of each full thickness wound was captured using a CCD camera to monitor the lesion contraction. The first wound image was captured one day after its creation for all the groups. This process of capturing the wound images was continued at alternate post-wounding days till the complete healing. Auto CAD R14 software (Autodesk, San Rafael, CA) was used to calculate the precise wound area using the wound images. The mean healing time was determined for controls as well as for the test groups, which were expressed in days.

2.6 *Analysis of data*

Statistical significance among the treatment and control groups was determined using One-Way ANOVA with Bonferroni's posthock test using GraphPAD Prism 4 (GraphPad Software, Inc, USA). All the data were expressed as mean $\pm$ SEM and $p < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1 *Power stability*

Once the experimental set-up was ready, the laser power stability studies were conducted. He–Ne laser power stability was monitored as a function of time by measuring it at the output end of the fibre at an interval of 1 min for 30 min. First reading was recorded immediately after the laser was switched on considering it as zero time and the recording was continued till 30 min. Repeated power measurements indicated the stable and accurate output power after 15 min of laser warm-up with less than 2% fluctuations over a period of 5–6 h. Therefore, all the laser irradiation studies were carried out after 15 min of laser warming up time avoiding possible error in the laser power delivery.

3.2 *Wound contraction and mean healing time*

Healing status of the excision wound could be assessed by the periodic measurement of the wound contraction. The area of each wound at a specific time was expressed as the percentage of its original size on day 1. The mean relative area of each group was plotted as function of days after wounding. Wound contraction is the process that occurs throughout the healing process, results in shrinkage and ultimately closure of the wounds. A steady decrease in wound area was observed in test as well as the control groups with time (table 1). The maximum contraction was observed at days 9–16 post-irradiation in 2 J/cm^2 treated group compared to unirradiated controls and other test groups. Single exposure of the laser dose at 2 J/cm^2 showed significant reduction in wound size at day 5 (p <0.001), day 9 (p <0.01), day

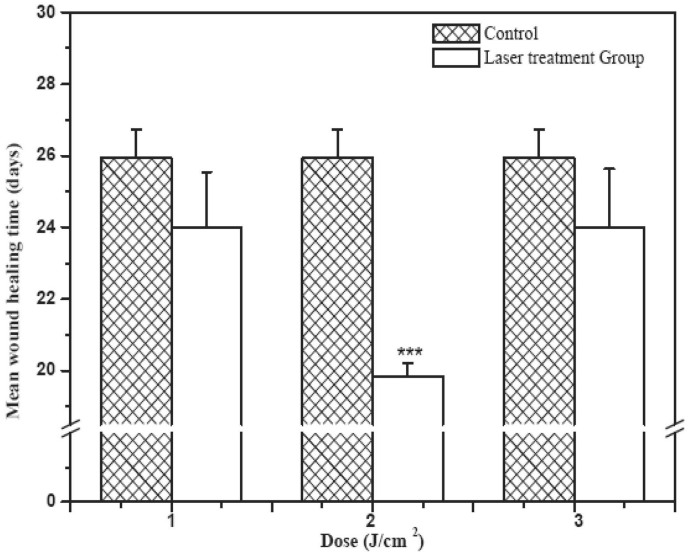

Figure 3. Mean wound healing time in days after exposure to various laser doses in LLLT. The significant levels are $^{***}p < 0.001$ and $^{**}p < 0.01$ and no symbol means nonsignificant compared to control.

Table 1. Wound contraction of the test and control groups. Rate of wound contraction in different laser dose treated as well as unirradiated controls on different post-wounding days. Data expressed as mean ± SEM.

	Wound contraction in percentage of day 1 in experimental groups			
Days post-wounding	Control	1 J/cm^2	2 J/cm^2	3 J/cm^2
1	100	100	100	100
3	93.36 ± 1.70	89.82 ± 2.40	87.74 ± 1.59	94.78 ± 1.92
5	90.88 ± 1.97	84.04 ± 3.12	78.34 ± 1.79	86.68 ± 2.04
7	79.86 ± 1.97	73.92 ± 1.37	72.92 ± 3.10	78.14 ± 1.81
9	70.68 ± 2.58	65.42 ± 2.75	58.88 ± 1.37	63.62 ± 3.02
12	56.07 ± 2.50	52.72 ± 4.32	41.50 ± 1.88	52.02 ± 2.82
14	48.31 ± 2.67	42.17 ± 4.05	31.18 ± 1.48	42.95 ± 2.88
16	37.32 ± 3.73	32.42 ± 7.55	12.02 ± 2.50	17.10 ± 7.38
19	5.66 ± 1.57	3.54 ± 1.05	0.60 ± 0.27	1.60 ± 0.43
21	4.24 ± 1.54	3.30 ± 1.36	0.02	0.64 ± 0.40
23	2.60 ± 1.37	0.40 ± 0.33	0	0.38 ± 0.28
26	0.92 ± 0.51	0.33 ± 0.26	0	0.19 ± 0.14
28	0.17 ± 0.13	0.26 ± 0.20	0	0.04
30	0	0	0	0

14 ($p < 0.001$) and day 16 ($p < 0.001$) compared to the unilluminated controls (figure 2). The healing time of the entire experimental group was calculated and expressed as mean healing time. Complete closure of the artificially created wound in unirradiated control was observed on day 25.92 ± 0.8 of post-wounding (figure 3). Treatment with laser at 2 J/cm^2 resulted in significant decrease in the healing time (day 19.83 ± 0.38 post-wounding). Mean healing times of 24.0 ± 1.54 and 24.0 ± 1.63 days post-wounding was observed for doses 1 and 3 J/cm^2 respectively. The reduction of mean healing time at 2 J/cm^2 treated group was found to be statistically significant ($p < 0.001$) compared to control.

4. Conclusion

In conclusion, He–Ne laser-based LLLT system designed and developed, demonstrated tissue regenerative capability, reducing mean healing time by promoting wound contraction, decreasing its size. These findings could justify the inclusions of lasers in the management of wound healing.

Acknowledgement

The present study is being carried out under project, no. DLS/81/48222/LSRB-164/BDB/2008, DRDO, G.O.I. entitled, 'Development of fibre-optic probe-based low level laser therapy (LLLT) for tissue regeneration'.

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