Effects of photobiomodulation therapy with various laser power densities on wound healing

Daing Hanum Farhana Abdul Munap, Pik Suan Lau, Noriah Bidin, Hazri Bakhtiar and Ganesan Krishnan

1 LaserCentre, IbnuSina Institute for Scientific and Industrial Research (ISI-SIR), UniversitiTeknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia
2 Department of Physics, Faculty of Science, UniversitiTeknologi Malaysia, 81310 UTM Johor, Bahru, Johor, Malaysia.

Email: kganesan@utm.my

Abstract. Photobiomodulation therapy (PBMT) on wound using infrared laser accelerates the wound healing process. The enhancement effects of different power densities of laser irradiation to achieve optimal result were not focused in previous works. In this study, we evaluated the effect dose–response relationship of power density below 100mW/cm² in PBMT with 808 nm laser. Twenty Sprague Dawley rats were divided into 6 groups; control group (GC) was a non-treated group and 5 laser treated groups with different power densities in the range of 20-100mW/cm². The wound was created on the dorsal region of the rats and irradiated with 808 nm laser with different power densities for 9 consecutive days except for the control group. Our results showed that the dose of 40-80mW/cm² power densities had beneficial effects on wound healing and 40mW/cm² attained maximum rate of wound healing. We conclude that power densities in PBMT effectively influenced the rate of wound healing process.

1. Introduction

Wound healing is a dynamic and complex biological process, which commences immediately after an injury occurs on skin tissue. The healing process of wound involves regeneration and tissue repair process comprehends different stages of molecular and cellular events in order to restore damaged tissue [1]. Cells, growth factor and Cytokines participate in the tissue repair leads to signalling pathway that regulate the sequential phases of wound healing; inflammatory, proliferative and remodelling stages. Severe complications such as prolonged inflammation, oxidative stress, free radical-induced damage and delayed granulation tissue formation during tissue repair process may arise from wound with massive destruction on the skin tissue hence slows down the healing stimulation [2]. Furthermore, delayed wound healing and wound infection can cause serious health risk and higher cost of treatment for patients.

Photobiomodulation therapy (PBMT) is a treatment using low power of light mainly from laser source to initiate light stimulation and interaction in living cells which resulted in effective biological effects such as promote tissue repair, decrease inflammation, and producing analgesic [3]. PBMT has gained growing interest among researchers in biomedical field due to the friendly, safe and non-invasive application. Numerous studies indicated that wavelength and doses of laser light are fundamental parameters in PBMT and strongly influenced the effectiveness of the treatment [5]. In
PBMT, photon of laser absorbed by intracellular chromophores such as mitochondrial and membranal cytochromes to be converted into chemical kinetic energy in order to change the activity in permeability membrane and oxidative metabolism in cell [4][6]. Near infrared light is exclusively used in PBMT due to the “therapeutic window” is in the range of 600-950 nm wavelength region, as supported by the first law of photobiology which requires specific absorption bands of some molecular chromophore to be absorb the photons from PBMT light source [7]. Stimulation of PBMT in near infrared region and dose of laser light gives beneficial clinical effects on cutaneous wounds in rat models such as the increased of fibroblasts and proliferation and stimulation of angiogenesis [8]. According to previous studies [9], 5 J/cm² of 808 nm laser was found to be the most predominant value of laser fluence to accelerate healing process, and the wound contraction rate was two times faster than non-treated wound. Nevertheless, most of researchers have high attention on energy-doses studies and often omitted the power density studies in wound healing. In fact, power density plays a key role in treatment, which decide the types of interaction between laser and tissue [10]. Low power density of below 0.2 W/cm² in PBMT can trigger infiltration of inflammatory cells, blood vessels formation, activate cell migration, and enhance collagen deposition and epithelization for wound healing [11]. However, lack of clinical report to evaluated power density-dose relationship in wound healing.

Therefore, this aim of this study is to investigate the effect dose–response relationship of power density below 100mW/cm² in PBMT with 808 nm laser diode at 5J/cm² laser fluence to establish the optimal healing parameter.

2. Methodology

Twenty Sprague Dawley rats were used in this work. The rats were anesthetized by 10mg kg⁻¹of KTX (2.5ml Ketamine, 12.5ml Xylazine, 250mg Zoletil-50) before creating the wounds on the rats. Four circular wounds were created on the dorsal region with 6 mm diameter biopsy punch. All procedures were carried out in accordance with the policy for care and use of experimental animals as approved by Animal Ethics Committee, at the Universiti Kebangsaan Malaysia (approval project code: UTM/2015/NORIAH/29-JULY/694-JULY-2016-JULY-2018).

The rats were divided into 6 groups, which consist of 1 control group (GC) and 5 laser treated groups. The laser treated groups were treated with power densities of 20 mW/cm² (G1), 40 mW/cm² (G2), 60 mW/cm² (G3), 80 mW/cm² (G4) and 100 mW/cm² (G5). The laser irradiation area for the laser treated groups was fixed at 1 cm². Also, the fluence for every laser treated groups was kept constant at 5J/cm² by varying the exposure time. The exposure time for the laser treated groups are listed in the Table 1. After the induction of wounds, the laser treated groups were treated once a day. For both the control and laser treated groups, observation and record of the wound areas were taken once a day for 9 days after the wounding process.

| Group Label | Power density (mW/cm²) | Exposure time (s) |
|-------------|------------------------|------------------|
| GC (control)| 0                      | 0                |
| G1          | 20                     | 250              |
| G2          | 40                     | 125              |
| G3          | 60                     | 83               |
| G4          | 80                     | 63               |
| G5          | 100                    | 50               |

The photographs of wound areas were recorded using a digital camera. The area of wounds from photographs were measured using the Image J software. The percentage of wound closure (PWC) was calculated using following formula:
\[ PW = \frac{A_0 - A_p}{A_0} \times 100\% \]  

(1)

Where \( A_0 \) is the initial area of wound at day zero and \( A_p \) is the progressive area of wound. One-way ANOVA was used as the statistical analysis to gauge the significance of experimental data.

3. Results and Discussion

Figure 1 shows the percentage of wound closure over a period of nine days for the control group and the laser treated groups. On the ninth day of study, the groups showed various percentages of wound closure. The GC had the lowest percentage of wound closure, which was 70.0 %. G2 and G3 showed almost complete wound healing process. Interestingly, the lowest (G1) and higher (G5) power density groups were less performing in wound closure that were 74.3% and 79.5 % respectively.

![Figure 1. Percentage of wound closure for the period of 9 days. Data shown are the mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.0001.](image)

Based on results of figure 1, the effective slopes of wound closure were determined by fitting a linear trendline to the percentage of wound closure against time graph and it is plotted in figure 2. In figure 2, the healing rate of GC is located at power density of 0 mW/cm² and the healing rate was 8.74 %/day. The minimal healing rate among the laser treated groups was shows by the lowest power density group (G1), which was 8.79 %/day. The healing rate increased gradually while the power density increased to 40 mW/cm² (G2). The maximum rate of healing was 12.44 %/day at 40 mW/cm². When the power density increased beyond 80 mW/cm², the healing rate starts to decrease gradually till 9.7 %/day at 100 mW/cm² (G5). A bell-shape curve of healing rate was obtained in Figure 2, it shows that the healing rate achieves the maximum effect at 40mW/cm² and begin to drop after 80mW/cm², which describing a dose-response relationship that involving low dose stimulation and high dose inhibition. This phenomenon is known as Arndt-Schulz La [12]. The lowest power density group (G1) does not provide desirable biological effects in wound healing. This minimal effect might due to 20 mW/cm² is below treatment threshold. Arndt-Schulz curve in laser treatment has been reported in in vivo or in vitro studies [13] D Carroll [14] stated that biological effects are sensitive to power or
fluency. Low dose of light stimulates physiologic activity leading to activate signalling pathways and up regulation of transcription factor, while higher doses of light decreases the enzyme's activity results in inhibition of cellular [14]. Huang et al., [15] evaluated that low fluence of light produce small amount of reactive oxygen species (ROS), which initiates beneficial in cell signalling. In contrast, high amount of ROS induced by high fluence of light that induces apoptosis and destructs mitochondrial membrane potential (MMP).

Figure 2. Wound healing rates of control and laser treated groups

4. Conclusion
In summary, the present data contribute to support that the healing rate of a wound was significantly affected by power density. Overall, the beneficial effect of PBMT is in range of 40-80mW/cm². The power density of 40mW/cm² at 5 J/cm² induces the maximal healing rate in a cutaneous wound. However, further studies are needed to identify the possible biological effects occur at various values of power density.

Acknowledgement
Authors are thankful to Malaysian Ministry of Education and Universiti Teknologi Malaysia for the financial assistance through GUP Tier 1 15H47. Also, authors would like to extend their gratitude to the research management centre (RMC) of Universiti Teknologi Malaysia.

References
[1] Gonzalez AC de O, Costa TF, Andrade Z de A and Medrado ARAP 2016 Wound healing - A literature review Anais Brasileiros De Dermatologia 91 614-20
[2] Yadav A, Gupta A, Keshri GK, Verma S, Sharma SK and Singh SB 2016 Photobiomodulatory effects of superpulsed 904nm laser therapy on bioenergetics status in burn wound healing J Photochem Photobiol B 162 77-85
[3] Chung H, Dai T, Sharma SK, Huang YY, Carroll JD and Hamblin MR 2012 The nuts and bolts of low-level laser (light) therapy Ann Biomed Eng 40 516-33
[4] Lubart R, Friedmann H and Onit R 2000 Photostimulation as a function of different wavelengths Laser Therapy 12 38-41
[5] De Freitas LF and Hamblin MR 2016 Proposed Mechanisms of Photobiomodulation or Low-Level Light Therapy IEEE J Sel Top Quantum Electron 22 7000417
[6] Shalaby TI, Abd El Rahman TH, Moustafa ME, El Sodfy AS 2017 Full thickness wound healing in induced diabetic mice using low level laser in comparison with ultrasound International journal of Biomedical Engineering and Science 4 01-09
[7] Hamblin M and Demidova-Rice TN 2007 Cellular chromophores and signaling in LLLT
[8] Chaves MED, De Araujo AR, Piancastelli ACC and Pinotti M 2014 Effects of low-power light therapy on wound healing: LASER x LED Anais Brasileiros De Dermatologia 89 616-3
[9] Lau P S, Bidin N, Krishnan G, AnaybBaleg S M, Marsin F M, Sum M B M, Baktiar H, Nassir Z, Chong P L and Hamid A 2015 Wound treatment on a diabetic rat model by a 808 nm diode laser Laser Phys. 25 075601
[10] Lau P, Bidin N, Krishnan G, AnaybBaleg S M, Sum M B M, Bakhtiar H, Nassir Z and Hamid A 2015 Photobiostimulation effect on diabetic wound at different power density of near infrared laser J. Photochem. Photobiol. B 151 201–7
[11] Lau P S, Bidin N, Islam S, Musa N, Zakaria N and Krishnan G 2017 Influence of low power density on wound healing in streptozotocin-induced diabetic rats Laser Phys. 27 055604
[12] Calabrese EJ and Baldwin LA 2001 U-shaped dose-response in biology, toxicology, and public health Annual review of public health 22 15-33
[13] Huang YY, Sharma SK, Carroll Jand Hamblin MR 2011 Biphasic dose response in low level light therapy - an update Dose Response 9 602-18
[14] D Carroll J 2008 A 3D dose model for low level laser / led therapy biostimulation and bioinhibition SPIE - The International Society for Optical Engineering Proc., 6846
[15] Huang YY, Chen ACH, D Carroll JD and Hamblin MR 2009 Biphasic dose response in low level light therapy Dose-Response 7 09-27