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GAVIN PUBLISHERS

Journal of Yoga, Physical Therapy and Rehabilitation

Research Article

Yeong EK, et al. Yoga Phys Ther Rehabil YPTR-162.

DOI: 10.29011/ ISSN: 2577-0756. 000062

Light Emitting Diode 630nm Improves Wound Healing in Induced Diabetic Rats

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Citation: Yeong EK, Wu MZ, Hsieh JH (2018) Light Emitting Diode 630nm Improves Wound Healing in Induced Diabetic Rats. Yoga Phys Ther Rehabil YPTR-162. DOI: 10.29011/ ISSN: 2577-0756. 000062

Received Date: 03 July, 2018; **Accepted Date:** 12 July, 2018; **Published Date:** 20 July, 2018

Abstract

Various methods have been used to promote spontaneous healing in full thickness, deep dermal or diabetic burns, but none has become a standard clinical tool due to debatable study results. As phototherapy has been proved to increase cell migration and proliferation in diabetic wound healing, we aimed to investigate the effect of light emitting diode at wavelength 630nm, power 14 mW/cm^2 and energy density 4 J/cm^2 on full thickness burns of diabetic rats. This was a case control animal study. Rats were divided into normal rats without light irradiation (Group 1); diabetic rats without light irradiation (Group 2); diabetic rats with light irradiation (Group 3). Each group had 4 rats with 4 wounds in each. Each wound was numbered according to the day of burn (post-burn day 1,3,5,7,9,11,13,15,17,19,21,23 and 25) wound area was measured and excision biopsy was taken. Phototherapy was taken on alternate day and a total of 12 light treatments were given to each rat. The study ended on day 25 post-burn and was repeated to achieve 12 rats in each group (N=3). Unpaired Student t test was used in statistical analysis. On day 19, 100% wound closure was noted in Group 1, 80% in Group 3 and 40% in Group 2 ($p<0.01$). On day 7, the mean area of re-epithelialization was 23.33 ± 5.86 mm^2 in Group 3, compared to 10.33 ± 2.89 mm^2 in Group 2 ($p <0.05$). No significant differences in Poly Morphonuclear Leucocyte (PMN), macrophage and fibroblast cell counts were noted. In conclusion, light emitting diode at wavelength 630nm improved wound contraction and re-epithelialization in full thickness burns of diabetic rats. Wavelength > 630nm is suggested for the induction of significant cell proliferation in animal model.

Keywords: Light emitting diode 630nm; Re-Epithelialization; Wound Contraction

Introduction

Spontaneous healing in full thickness or deep dermal diabetic burns is always challenging because of minimal or absence of skin appendages remain in the wound bed after burn injuries. Healing is further aggravated in diabetic patients as there are defects in leukocyte chemotaxis, phagocytosis, bactericidal capacity and dysfunctions of fibroblasts and epidermal cells [1]. Various method such as phototherapy [2], vacuum assisted therapy [3], hyperbaric oxygen [4] and growth factors [5] have been used in difficult wounds, but none of them become a standard treatment tool in burn management due to the lack of Level One clinical evidence. This study evaluates the effect of phototherapy in diabetic healing using an animal model.

In fact, previous studies have shown that low power laser increases fibroblast proliferation [6,7], promotes the release of cytokines [8,9], increases collagen production [10-12], stimulates ATP production by mitochondria activation [13-17], improves

wound tensile strength [18,19] and increases the rate of re-epithelialization [20,21]. However, its clinical use is limited due to high cost, inconvenient to use, small wound treatment size, cell damaging heat production and the pin-point laser beam that may damage the eye. As there are side effects of phototherapy using low power laser, Light Emitting Diode (LED) which provides several advantages over low power laser is another phototherapy option in promoting wound healing [22,23].

Light emitting diode is a semiconductor device that emits visible light when an electric current passes through. The emitted light is mostly monochromatic, but the output may range from red (at a wavelength of approximately 700 nanometers) to blue-violet (about 400 nanometers). These diodes can be configured to produce multiple wavelengths and can be arranged in large flat arrays, allowing the treatment of large wounds with minimal thermal effect. Its clinical application is more versatile, efficient and cheaper than that of low power laser. However, the clinical efficacy of LED remains controversial as there are doubts on the optimal irradiation parameters, effective energy delivery and energy absorption in wound environment.

Based on the assumption that LED improved wound healing, we investigate the effect of LED at wavelength 630nm with power 14 mW/cm^2 and energy 4 J/cm^2 (LED_{630}) on full thickness or deep dermal diabetic burns.

Methods

Animals

Thirty-six age-matched, male Wistar rats, weighing 180-200g, were used in the study. The animals were bred and provided by the Animal Facility in National Taiwan University Hospital and Research Center. All animal protocols were reviewed and approved by the Animal Care Committee. Figure 1 shows the experimental designs of the study. The burn rats were divided into three groups: normal rats without LED irradiation (Group 1); diabetic rats without LED irradiation (Group 2); diabetic rats with LED irradiation (Group 3). A total of 12 light treatments were given on alternate days and the ending point of the study was day 25 after burn. Each group had 4 rats with 4 wounds in each. Each of the 16 wounds were numbered according to the day of burn (post-burn day 1,3,5,7,9,11,13,15,17,19,21,23 and 25) wound area was measured and excisional biopsy was taken (Figure 2). Rats were sacrificed at the end of the study. The study was repeated to achieve a total of 12 rats in each group (N=3). We assured humane treatment of the animals were achieved throughout the study.

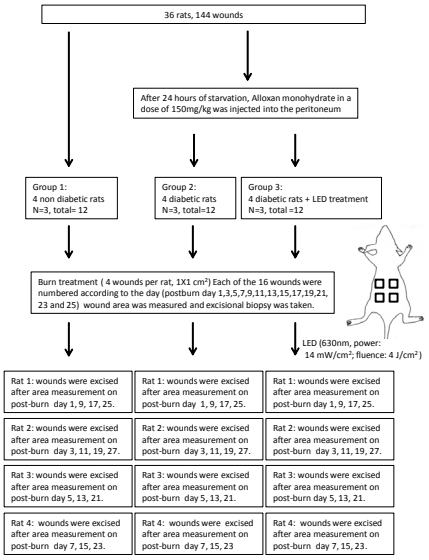

Figure 1: A synopsis of the Material and Methods.

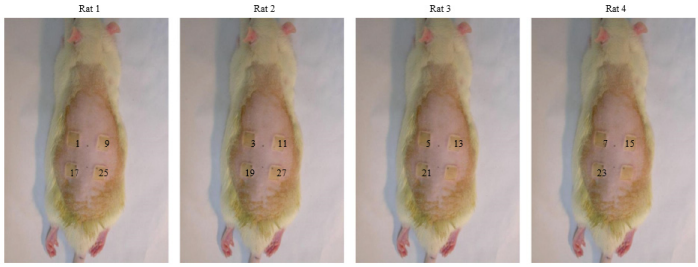

Figure 2: Each group had 4 rats with 4 wounds in each. Each of the 16 wounds were numbered according to the day (postburn day 1,3,5,7,9,11,13,15,17,19,21,23 and 25) wound area was measured and excisional biopsy was taken

Induced Diabetic Rats

After 24 hours of starvation, Alloxan monohydrate in a dose of 150mg/kg was injected into the peritoneum. On day 14 and day 28 after the injection, blood samples were taken for the fasting glucose level measurements. Animals with serum glucose less than 250mg/dl were excluded from the study.

Burn Infliction

Burn wounds were created 4 weeks after the induction of diabetic rats. Rats were anesthetized with 50mg/kg phenobarbital injected intra-peritoneally. The back of the animals was shaved

with electrical razor followed by the application of hair removal lotion. The area was then washed with normal saline and sanitized with 70% isopropyl alcohol. Four 1 cm^2 burns were created by direct contact with a heated metal rod (100°C) on the back of each rat for 20 seconds.

Light Treatment

Light emitting diode at wavelength 630nm was used as the light source. The power was 14 mW/cm^2 and the energy density was 4 J/cm^2. The diabetic rats were randomly divided into LED treated group and LED non-treated group. In the former, LED wound treatment for 286 seconds was performed on the biopsy days. The distance of the LED probe to the wound was kept at 4cm apart.

Wound Contraction and Re-Epithelialization Measurement

Wound contraction was represented as a percent change in the wound surface area compared to the original wound's size. Digital pictures of the wounds were taken on biopsy days. Using computerized image analysis software SimplePCI, wound areas were measured and the time to complete wound healing was recorded. In Group 2 and Group 3, biopsies for histological studies were taken on biopsy days. The burn wounds were excised together with 0.5cm normal skin along the border of the burn wounds. Each 1x1 cm^2 burn tissue was divided into 5 equal pieces, which were then made into 5μm specimens and stained with Hematoxylin and Eosin. The area of re-epithelialization was assessed under the light microscope.

Cell Count

Pictures of the specimens were taken at 40X magnification, followed by macrophage, Poly Morphonuclear Neutrophil (PMN) and fibroblast cell count.

Statistical Analysis

Unpaired Student's t-test was used for statistical analysis for wound area measurements and histological studies, significance was defined as p value <0.05.

Results

In the diabetic rats (n=24), the mean serum glucose concentration on day 14 was 447 ± 129 mg/dl and 476 ± 88 mg/dl on day 28, as compared to 61 ± 9 mg/dl before induction ($p<0.05$).

Gross Assessment

Figure 3 showed the wound contraction rate in the non-diabetic rats and diabetic rats. On day 19, 100% wound healing was achieved in Group 1, 80% in Group 3 and 40% in Group 2 ($p<0.01$).

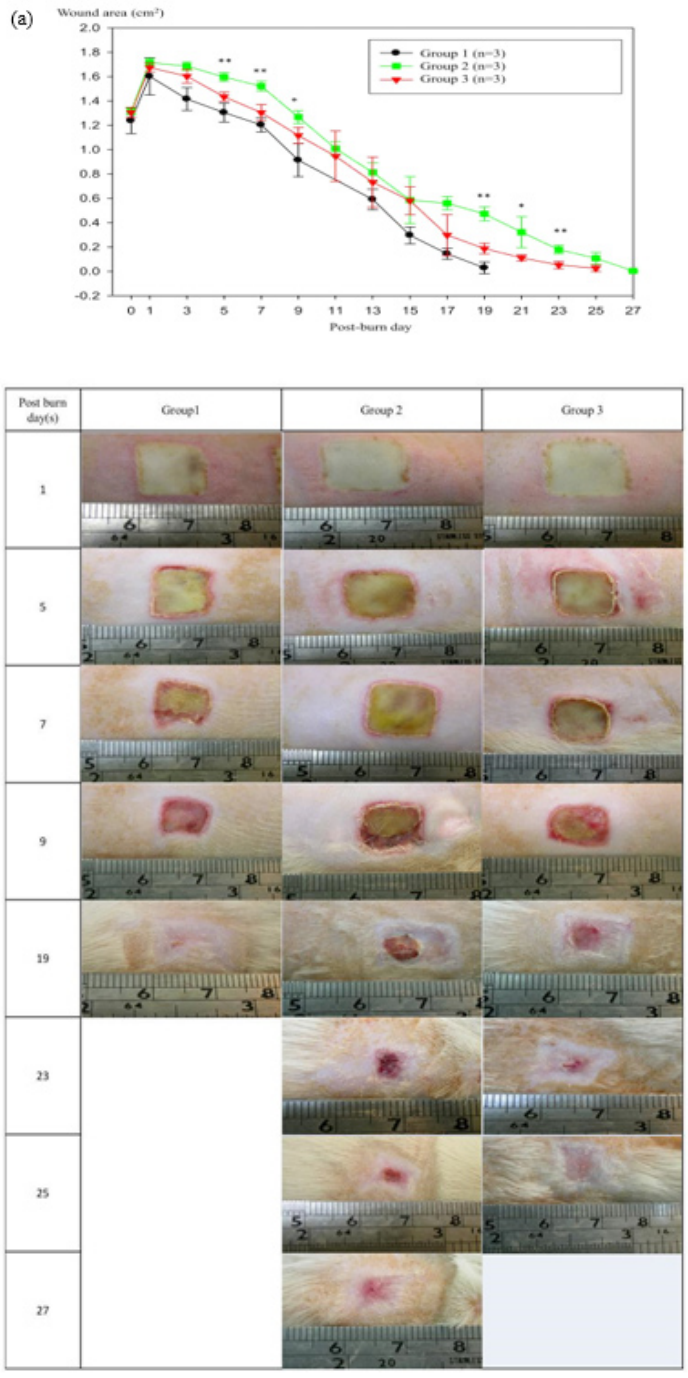

Figure 3: Compare the wound contraction rate among non-diabetic rats, diabetic rats with or without LED treatments. On day 19, 100% wound closure was noted in Group 1, 80% in Group 3 and 40% in Group 2 ($p<0.01$).

Histological Studies

On day 7, the mean area of re-epithelialization was 23.3 ± 5.9 mm^2 in Group 3, compared to 10.3 ± 2.9 mm^2 in Group 2 (p

<0.05). As shown in Figure 4, diabetic burns treated with LED irradiations had a faster rate of re-epithelialization than that without ($p < 0.05$). There were no significant differences in the cell counts of PMN, macrophage and fibroblast between Group 2 and Group 3 ($p > 0.05$).

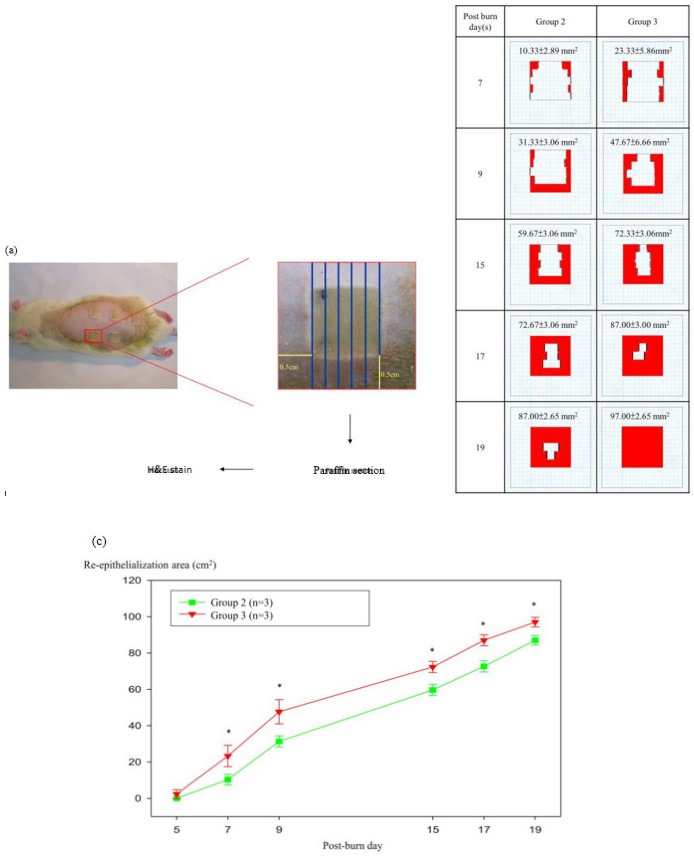

Figure 4: Compare the rate of re-epithelialization in diabetic rats treated with LED and that without. On day 7, the mean area of re-epithelialization was 23.3 ± 5.9 mm² in Group 3, compared to 10.3 ± 2.9 mm² in Group 2 ($p < 0.05$)

Discussion

Our study confirmed that LED_{630} with power 14 mW/cm² and the energy density 4 J/cm² improved wound contraction and re-epithelialization in an alloxan-induced diabetic burn rat model. However, no significant cell proliferation was noted using this irradiation parameter. The study also confirms the negative effect of hyperglycemia on burn healing which has also been reported by Al-Watban FA, et al. [24,25] who stated that burn healing in the diabetic wound was delayed by 46.2%, compared to 60% in our study. The results were also compatible to a study showing that wound healing times were decreased in diabetic patients with lower HbA1c [26].

The cellular mechanisms of phototherapy on burn healing has been investigated in previous studies [27,28]. It activates mitochondria, increasing Adenosine Triphosphate (ATP) production and thus promoting phagocytosis, cell proliferation, cell migration, and collagen production during the process of wound healing. The increased fibroblast proliferation [6,7,29,30] and collagen production [1,6-12] serves as a ground substance to facilitate epithelial cell migration. In other words, phototherapy provides addition energy to improve the function of PMN, macrophage and fibroblast. However, the optimal irradiation parameters remain undetermined. Using diabetic rats, Yu, et al [31] found that treatments with 630-nm argon dye laser at fluence of 5 J/cm² enhanced the percentage of wound healing area. Stadler, et al, [19] reported that irradiations with 830-nm 5 J/cm² diode lasers improved wound tensile strength in diabetic rats, while Reddy, et al. [12] showed that 632.8-nm helium-neon laser at fluence of 1 J/cm² accelerated collagen production and promoted connective-tissue stability in incisional wounds of diabetic rats. In Maiya GA, et al. study [32], excision wounds in diabetic rats treated with He-Ne laser (632.8 nm wavelength) at a dose of 4.8 J/cm2 healed faster than the control group. In short, despite the absence of standard treatment protocol, various irradiation parameters of phototherapy used in different studies have shown to have beneficial effect on wound healing.

Based on our previous cell culture study results showing significant cell proliferations upon light treatments [33], similar irradiation parameters were applied to this animal model. However, significant cell proliferations were not achieved in the irradiated wounds. The incompatible results could be explained by insufficient energy delivery in the animal model compared to that delivered in the cell culture. This is due to the complicated irradiation environment in the rat model affecting the true energy delivered to the cells. In addition, the irradiation wavelength used in our study was shorter than that in others (660nm [34], 670nm [35], 694.3nm [21] and 904nm [10]) showing increased cell proliferation, implying wavelength longer than 630nm is needed in the delivery of sufficient energy for significant cell proliferation. In fact, it has been mentioned that energy density of 9.86J/cm² was more effective in promoting cellular responses related to neoangiogenesis, decreasing inflammation and collagen fibers reorganization [36].

This study showed that wound contraction and re-epithelialization was the main feature of wound healing in a burned rat model. However, the increase in wound contracture strength and collagen production was not investigated in the study. We assumed that the epithelial cells were activated by LED_{630} and migrated at a faster rate from the skin appendages left in the remaining dermis as well as from the leading wound edges.

The optimal irradiation parameters are difficult to be determined as they involve a large probability of combinations. Dif-

ferent irradiation parameters have been used in cell proliferation studies [24,25,37,38]. However, some studies stated that energy greater than 10 J/cm^2 or lower than 2 J/cm^2 are ineffective. A study of long wavelength laser therapy (830nm) in postsurgical wounds has shown no apparent benefit on healing [39]. Similarly, studies have shown that a fluence of 5 J/cm^2 stimulates diabetic wound healing *in vitro*, while 16 J/cm^2 is inhibitive [38]. This could be explained by the possibility that longer wavelength delivery causes higher energy density which may have negative impact on cell proliferation due to thermal effect. Although fluence of 5 J/cm^2 has shown to stimulate cell proliferation, our study proved that LED_{630} at energy density 4J/cm^2 was inadequate to stimulate significant cell proliferation in a diabetic burn wound model. Energy density that have shown to have increased cell proliferation in our previous cell culture study did not achieve similar effects in animal or clinical model. The reasons may be due to the fact that necrotic wound tissue, topical antibiotic creams, wound depth and microorganisms affect the ultimate energy delivered to the target cells. This indicates that wound pretreatment is necessary to attain maximum photo-stimulation effect. Infection should be eradicated as the micro-bacteria may absorb light energy leading to wastage or ineffective treatment. Furthermore, background light should be cut off to avoid light interference.

The main limitation in the study was variation in the initial wound size created immediately after burn as wound enlarged after burn, leading to study error. In addition, the model mimicked wound healing in the state of hyperglycemia, but did not reflect healing in a complicated chronic diabetic situation which required a follow-up of 24 weeks in an induced diabetic rat [40]. Poor perfusion and neuropathy that are commonly found in chronic diabetic ulcers have not been demonstrated in this model.

Conclusions

In conclusion, this study confirms hyperglycemia impairs wound healing and that LED_{630} improved wound contraction and re-epithelialization. Wavelength > 630nm is suggested for the induction of significant cell proliferation in animal model.

Acknowledgments: We thank Professor Kuo-Liong Chien and the statistical analyst Heng-Hsiu Liu for statistical consultation.

Source of Funding: There was no funding or financial support for the study.

Conflict of Interest Disclosure Statement: All authors have no relevant conflicts of interest to disclose.

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