630 nm-OLED Accelerates Wound Healing in Mice Via Regulation of Cytokine Release and Genes Expression of Growth Factors

SangJoong Mo¹⁴, Phil-Sang Chung²³, and Jin Chul Ahn¹²⁴*

¹Medical Laser Research Center, Dankook University, Cheonan 31116, Korea
²Beckman Laser Institute Korea, College of Medicine, Dankook University, Cheonan 31116, Korea
³Department of Otolaryngology-Head and Neck Surgery, College of Medicine, Dankook University, Cheonan 31116, Korea
⁴Center for Bio-Medical Engineering Core Facility, Dankook University, Cheonan 31116, Korea

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Photobiomodulation (PBM) using organic light emitting diodes (OLEDs) surface light sources have recently been claimed to be the next generation of PBM light sources. However, the differences between light emitting diodes (LEDs) and OLED mechanisms in vitro and in vivo have not been well studied. In vivo mouse models were used to investigate the effects of OLED irradiation on cellular function and cutaneous wound healing compared to LED irradiation. Mice in the LED- and OLED-irradiated groups were subjected to irradiation with 6 J/cm² LED and OLED (630 nm), respectively, for 14 days after wounding, and some mice were sacrificed for the experiments on days 3, 7, 10, and 14. To evaluate wound healing, we performed hematoxylin-eosin and Masson’s trichrome staining and quantified collagen density by computerized image analysis. The results showed that the size of the wound, collagen density, neo-epidermis thickness, number of new blood vessels, and number of fibroblasts and neutrophils was significantly influenced by LED and OLED irradiation. The tissue levels of interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α were investigated by immunohistochemical staining. LED and OLED irradiation resulted in a significant increase in the tissue IL-1β and IL-6 levels at the early stage of wound healing (P < 0.01), and a decrease in the tissue TNF-α level at all stages of wound healing (P < 0.05), compared to the no-treatment group. The expression levels of the genes encoding vascular endothelial growth factor and transforming growth factor-beta 1 were significantly increased in LED and OLED-irradiated wound tissue at the early stage of wound healing (P < 0.01) compared to the no-treatment group. Thus, OLED as well as LED irradiation accelerated wound healing by modulating the synthesis of anti-inflammatory cytokines and the expression levels of genes encoding growth factors, promoting collagen regeneration and reducing scarring. In conclusion, this suggests the possibility of OLED as a new light source to overcome the limitations of existing PBMs.

Keywords: Light-emitting diode, Organic light-emitting, Photobiomodulation, Wound healing

OCIS codes: (170.0170) Medical optics and biotechnology; (000.1430) Biology and medicine; (170.1420) Biology; (170.1610) Clinical applications

I. INTRODUCTION

A wound is a damaged or broken area (tear, cut, or hole) on the body, where the damage occurs relatively quickly. Wounds are classified as acute or chronic; one type of wound is a sharp injury, which damages the dermis [1-3]. The skin protects the skeletal and muscular system, so damage to the skin can be dangerous [4]. Therefore, timely treatment is needed to restore and regenerate damaged skin tissue without side effects. Wound healing and tissue

*Corresponding author: jcahn@dankook.ac.kr, ORCID 0000-0002-0501-7210
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regeneration are complex processes involving coagulation, inflammation, the formation of granulation tissue, synthesis of collagen, and activation of tissue remodeling. These processes are regulated by a complex signaling network involving growth factors, cytokines, and chemokines, and the interactions of various cell types, including lymphocytes, mononuclear cells, epithelial cells, and fibroblasts [5-7]. Following their recruitment to sites of injury, neutrophils promote inflammation by releasing cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) [8]. However, excess TNF-α suppresses wound healing, resulting in slow-healing wounds [9]. Moreover, the free radicals released by neutrophils are toxic to cells and tissues [10]. Therefore, strategies to promote wound healing should suppress the release of proinflammatory cytokines to reduce their adverse effects.

Photobiomodulation (PBM) is non-invasive, selective, and safe. The light-emitting diode (LED) light source has a narrow wavelength bandwidth, which emits light of a specific wavelength, so it does not emit harmful ultraviolet rays or unnecessary infrared rays. The U.S. FDA permits the use of treatments with LED light sources in the visible and near-infrared regions for humans. In particular, LEDs are frequently used for PBM in dermatology and psychiatry, and for the treatment of cancer and Alzheimer’s disease [11]. In terms of skin healing, LED light sources can treat a large wound area using a power far lower than that applied to small areas by a high-power laser [12, 13]. In PBM using LEDs, photons are absorbed by chromophores or photoreceptors in mitochondria, which enhances the production of ATP, resulting in the synthesis of reactive oxygen species (ROS) and nitric oxide. This modulates gene expression by activating redox-sensitive transcription factors such as nuclear factor-kappa B (NF-kB) [14, 15], leading to the induction of protein synthesis, proliferation, and migration; modulation of the synthesis of cytokines, growth factors, and inflammatory mediators; and an increase in tissue oxygenation [16-18]. LED irradiation (630 nm) of a wound ameliorates inflammation by inhibiting prostaglandin E2 synthesis in human gum fibroblasts [19]. LED light of visible and near-infrared wavelengths increases collagen production in the skin and inhibits the synthesis of matrix metalloproteinases. As such, LEDs may be useful for tissue repair or wound healing. However, LED devices are bulky and heavy [20, 21], generate localized heat, and may not produce uniform light [22]. In contrast, organic light-emitting diodes (OLEDs) are thin, lightweight, and flexible. In addition, because the OLED surface can be shaped into the desired form by a deposition process, they are able to irradiate more uniformly than LEDs. Therefore, OLEDs enable frequent and/or repeated PBM treatments and facilitate wound healing. A novel PBM patch based on an OLED light source that attaches to the body reportedly promotes wound healing [23]. Thus, OLEDs show promise as next-generation wearable light sources for PBM. However, biological and biochemical evidence of the effect of OLED based PBM on wound healing is lacking. Thus, studies of the mechanism of the wound-healing effect of OLEDs are needed. We investigated the effect of LEDs and OLEDs with a wavelength of 630 nm on wound healing in mice.

II. METHODS

2.1. Study Design

Male ICR mice (5 weeks of age; 20-25 g body weight) were purchased from Nara Biotech Co. (Seoul, Republic of Korea). The mice were housed in a specific pathogen-free facility with appropriate temperature and humidity and had ad libitum access to food and water. The mice were randomized into the following groups (n = 15 each): no-treatment group (control), wounded but not irradiated; LED-irradiated group; wounded and irradiated by a 630 nm LED; and OLED-irradiated group, wounded and irradiated by a 630 nm OLED. All animal procedures were performed according to the guideline of Institutional Animal Care and Use Committee of Dankook University (Approval # DKU-18-012).

2.2. Wound Induction

The mice were anesthetized by intramuscular injection of Zoletil (30 mg/kg; Virbac Laboratories, Carros, France) and Rompun (10 mg/kg; Bayer Korea, Seoul, Republic of Korea). The temperature of the anesthetized mice was adjusted to ~37°C using a warming light. The mice were allowed to selfbreathe room air. The mice were shaved thoroughly using a razor blade (HA 944; Hwasung, Yangsan, Republic of Korea), and their skin was disinfected with 70% (v/v) alcohol; a full-thickness excision wound was then created 2 cm from the lumbar vertebrae using a 6 mm biopsy punch (Kai Medical, Solingen, Germany).

2.3. Wound Irradiation

Immediately after wounding, the mice were irradiated using a 630 nm LED and OLED (WON TECH Co., Ltd, Daejeon, Republic of Korea) for 14 days (20 minutes per day). Figure 1(a) shows the experimental conditions for the 630 nm red-light LED and OLED groups. The LED and the OLED were designed to fit over a standard multiwell plate (12.5 × 8.5 cm) or polyethylene terephthalate plate for animals (8.0 × 3.6 cm) and had an emission wavelength that peaked at 630 nm. The wavelength spectrum of the LED and OLED was measured with a Varian Cary Eclipse Fluorescence Spectrophotometer (Palo Alto, CA, USA) (Fig. 1(b)). Electroluminescence spectrum analysis of the LED and OLED devices both showed a maximum emission peak at 633 nm. The spectral bandwidth is from 590 nm to 670 nm for LED and from 579 nm to 750 nm for OLED, respectively. The irradiance at the skin surface was measured using a power meter (Orion; Ophir Optronics,
Jerusalem, Israel). To obtain an energy dose of 6 J/cm², the exposure time of the LED array and the OLED module was set at 20 minutes at a power density of 5 mW/cm² (1 milliwatt × second = 0.001 joules).

2.4. Wound Healing Assay

To evaluate the rate of wound healing, images of the wound were captured using a digital camera at days 0, 3, 7, and 14 after wounding. The photographs were analyzed using ImageJ software (National Institutes of Health [NIH], Bethesda, MD, USA). Photographs were obtained with the mouse adjacent to a metric ruler, which was used for distance calibration and standardization, and the wound area was calculated. The percentage wound closure was calculated as follows: (area of the wound measured d days after scar ÷ area of the wound measured immediately after scar) × 100%.

2.5. Histological Staining

For histological analysis, wound tissue was harvested at days 3, 7, 10, and 14 after wounding. Specimens were fixed in 10% (v/v) buffered formaldehyde, dehydrated in a graded ethanol series, and embedded in paraffin. The specimens were sliced into 5 μm sections using an RM2125 rotary microtome (Leica, Wetzlar, Germany) and stained with H&E. The rate of re-epithelialization and length of the regenerated epithelium were analyzed using H&E-stained sections under a DX53 microscope equipped with a DP74 digital camera and the bundled software (Olympus, Tokyo, Japan). MT staining of collagen was performed to assess tissue fibrosis, and collagen synthesis was quantified using ImageJ (NIH) according to the modified method of Ying et al. [24].

2.6. Immunohistochemistry

An IHC analysis of cytokines in wound tissue was performed at days 3, 7, 10, and 14 after wounding. Cryosections were cut to 10 µm thickness, mounted on Colorfrost Plus microscope slides, and maintained at -70°C. For IHC analysis, cryosections were fixed in acetone, exposed to 3% (v/v) hydrogen peroxide to eliminate endogenous peroxidase activity, and blocked with bovine

FIG. 1. Experimental design and comparison of 630 nm-LED and OLED emitting spectrum. (a) Schematic representation of the experimental design used for evaluating cutaneous wound healing by light-emitting diode (LED) and organic light-emitting diode (OLED) irradiation in male ICR mice. Five-week-old male ICR mice were divided into three groups and irradiated or not by an LED or OLED (630 nm; 6 J/cm²). The mice were euthanized and hematoxylin and eosin (H&E), Masson’s trichrome (MT), and immunohistochemical (IHC) staining was performed at days 3, 7, 10, and 14 after wounding. (b) Electroluminescence spectra of 630 nm-LED and OLED.
2.7. Semi-quantitative Reverse Transcriptase-polymerase Chain Reaction

Wound tissue obtained at days 7 and 10 after wounding was stored in RNA later solution (Ambion, Austin, TX, USA) and homogenized in TRIzol reagent to isolate total RNA (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s protocol. Contaminating DNA was removed by treatment with DNase I (Invitrogen) for 30 minutes at 37°C. First-strand cDNA was synthesized from 1 μL of total RNA using oligo(dT) 12-18 primers and reverse transcriptase (SuperScript III; Invitrogen) in a 20 μL reaction for 50 minutes at 50°C. The RNA template was subsequently removed by digestion with 2 IU RNase H (Invitrogen) for 20 minutes at 37°C using cDNA (1 μL) as the template. For standard polymerase chain reaction (PCR), 200 μM dNTPs (Roche, Mannheim, Germany), 1 IU Taq DNA polymerase (Denville Scientific, Metuchen, NJ, USA), and 0.2 μM primers (Table 1) were used. The number of semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) cycles was selected such that the amplification products were within the linear range, as assessed by densitometry. The PCR products were electrophoresed in 2% (w/v) agarose gels and stained with ethidium bromide. Band intensity was quantified by densitometry using Quantity-One software (Bio-Rad, Hercules, CA, USA). The experiments were performed at least in triplicate, and gene expression was normalized to that of GAPDH.

### TABLE 1. Primers used for RT-PCR

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>VEGF-F</td>
<td>5'-AAAGAGCTTCAAGTGGTCTGAG-3'</td>
</tr>
<tr>
<td>VEGF-R</td>
<td>5'-GGTTGGAACGGGCATCTTTATC-3'</td>
</tr>
<tr>
<td>TGF-β1-F</td>
<td>5'-CCTGTTCAAAACTAAGAC-3'</td>
</tr>
<tr>
<td>TGF-β1-R</td>
<td>5'-GTTTTTCTCTATAGATGGCG-3'</td>
</tr>
<tr>
<td>IL-10-F</td>
<td>5'-TGCCCAAGAAATCAAGGAGC-3'</td>
</tr>
<tr>
<td>IL-10-R</td>
<td>5'-CACCACTCTCAATACACACT-3'</td>
</tr>
<tr>
<td>GAPDH-F</td>
<td>5'-TCACCACCATGGAGAAGGC-3'</td>
</tr>
<tr>
<td>GAPDH-R</td>
<td>5'-GCTAGGAGTGTGGTCA-3'</td>
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2.8. Statistical Analysis

Statistical analysis was performed using Prism for Windows software (version 7.0; GraphPad Software Inc., San Diego, CA, USA). Data are expressed as means ± standard deviation (SD). Statistical significance was determined by two-way analysis of variance (ANOVA) with the Bonferroni’s post hoc test. A value of $P < 0.05$ was taken to indicate statistical significance.

III. RESULTS

3.1. Effect of LED and OLED Irradiation on Wound-healing

The closure of skin wounds in ICR mice was monitored daily. Wound healing differed significantly between the no-treatment group and the LED and OLED-irradiated groups at days 3, 7, 10, and 14 after wounding (Fig. 2(a)). Acceleration of wound healing was significantly observed from day 3 in the LED- and OLED-irradiated groups compared to the no-treatment group ($P < 0.05$ and $P < 0.01$, Fig. 2(b)). Although the all wound size in the LED- and OLED-irradiated groups continued to steadily decrease, significance was observed only in the OLED-irradiated group at days 7 and 10 after wounding compared to the no-treatment group ($P < 0.05$). However, there was no significant difference in wound closure rate among all groups after day 14.

3.2. Neo-epithelial Thickness and Newtissue Formation

We next performed a histological analysis of wound-tissue sections by H&E staining and assessed neo-epidermis thickness and new-tissue formation (Fig. 3(a)). The damaged epidermis was not completely healed at day 14 in any group, but most of the epidermis was regenerated to form a distinct middle layer of regular thickness (Fig. 3(a)). Although the thickness of the neo-epidermis could not be measured at day 3 after wounding due to removal of the keratinocyte layers, it was significantly decreased ($P < 0.05$) in the OLED-irradiated group compared to the no-treatment group at day 7 after wounding. There was also a significant difference in the thickness of the neo-epidermis between the LED and LED-irradiated groups ($P < 0.05$) (Fig. 3(b)) but not between the no-treatment and LED-irradiated groups. At day 10 after wounding, the LED- and OLED-irradiated groups continued to steadily decrease, thickness among the groups after day 14, which was almost similar to the positive control (Fig. 3(b)).
We also investigated the formation of new tissue during wound healing. Rapid accumulation of neutrophils and fibroblasts were respectively observed during the early and middle phases of wound healing (days 3 and 7 after wounding) in the LED- and OLED-irradiated groups, but not in the no-treatment group (blue circles and black ovals, Fig. 3(a)). Histological analysis of H&E-stained wound tissue showed that the number of blood vessels in the dermis was increased in the LED- and OLED-irradiated groups compared to the no-treatment group at day 10 after wounding. Formation of new blood vessels was enhanced in the LED-irradiated group compared to the OLED-irradiated

FIG. 2. Comparison of the effect of LED and OLED irradiation on wound closure. The wounds were photographed to observe the wound areas at days 0, 3, 7, 10, and 14 after wound; mice were sacrificed at day 14 after wound. (a) The representative images of wounds in each group at the indicated time points are shown. (b) Wound-closure rate. Wound areas were determined using ImageJ (NIH) and are percentages of those immediately after wounding. Error bars, means ± standard deviation (SD) (n = 3 mice/group). Two-way analysis of variance (ANOVA) with Bonferroni’s post hoc test. *P < 0.05, **P < 0.01 compared to no treatment.

FIG. 3. Histological evaluation of wounded skin. (a) Representative H&E-stained images obtained at days 3, 7, 10, and 14 after wounding (magnification, 100×). Blue circles and black ovals, neutrophils and fibroblasts, respectively; black dotted oval, new blood vessels; black arrows, non-epithelialized areas; blue arrows, hair follicles. Scale bars, 100 µm (bottom). E, epidermis; D, dermis; K, keratinocyte. Positive control is normal tissue that does not cause wound. (b) Epidermal thickness determined based on H&E-stained sections from the center of the wound at days 7, 10, and 14. Error bars, mean ± SD (n = 3 mice/group). Two-way ANOVA with Bonferroni’s post hoc test. *P < 0.05, **P < 0.01, ***P < 0.001 compared to no treatment.
group (black dotted oval, Fig. 3(a)). In addition, re-epithelialization of the edge of the wound occurred more rapidly in the OLED-irradiated group than in the other two groups (black arrow). Finally, the restoration of skin structures (e.g., hair follicles) occurred more rapidly in the LED and OLED-irradiated groups than in the no-treatment group, similar to normal tissue (positive control) (blue arrow, Fig. 3(a)).

3.3. Collagen Density

To further investigate the effect of LED and OLED irradiation on the healing of skin wounds, we examined collagen deposition. Because the presence of mature collagen fibrils is a marker of healing, an immature collagen fibril is deemed a marker of poor healing. Therefore, we performed MT staining at days 3, 7, 10, and 14 after wounding to investigate collagen deposition. At day 3 after wounding, immature collagen was present in the dermal tissue of the LED- and OLED-irradiated groups, and most of the dermal tissue of irradiated two groups was filled with mature collagen at day 10 after wounding compared to the no-treatment group (Fig. 4(a)). Also, collagen density was increased in the LED- and OLED-irradiated groups compared to the no-treatment group at day 7 after wounding, but the highest significance was shown only the LED-irradiated group ($P < 0.001$) (Fig. 4(b)). At day 10 after wounding, on the other hand, collagen deposition was significantly increased in the OLED-irradiated group compared to the no-treatment group ($P < 0.001$), and in the OLED-irradiated compared to the LED-irradiated group ($P < 0.001$). At day 14 after wounding, there was a significant increase in collagen density only in the OLED-irradiated group, as compared with the no treatment group ($P < 0.05$).

3.4. Levels of Proinflammatory Cytokines in Wound Tissue

We next assayed the levels of proinflammatory cytokines in wound tissue by IHC analysis. The IL-1β and IL-6 levels in the LED and OLED-irradiated groups were higher at day 3 after wounding, and lower at day 10 after wounding, than those in the no-treatment group. In contrast, the TNF-α level in the LED and OLED-irradiated groups was lower than that in the no-treatment group at day 3 after wounding, and was further decreased at day 10 (Fig. 5(a)). As result of quantification of cytokine in wound, the IL-1β and IL-6 levels in the LED and OLED-irradiated groups were significantly higher compared to those in the no-treatment group at day 3 after wounding ($P < 0.01$) but declined thereafter. The IL-1β level was significantly lower in the LED-irradiated (day 10, $P < 0.05$; day 14, $P < 0.01$) and OLED-irradiated ($P < 0.01$) groups than in the no-treatment group (Fig. 5(b)). However, the TNF-α level was lower in the LED and OLED-irradiated groups compared to the no-treatment group at day 3 after wounding (LED group, $P < 0.01$; OLED group, $P < 0.05$), and further decreased thereafter ($P < 0.001$) (Fig. 5(b)).

3.5. Expression Levels of Growth Factors and Cytokines

We assayed the expression levels of vascular endothelial growth factor (VEGF), transforming growth factor-beta 1 (TGF-β1), and IL-10. At day 3 after wounding, the expression levels of VEGF and TGF-β1 were higher in the LED- and OLED-irradiated groups compared to the no-treatment group. The expression level of VEGF, but not that of TGF-β1, was significantly greater in the LED and OLED-irradiated groups than in the no-treatment group at day 10 after wounding. In contrast, the expression level of IL-10 in the LED and OLED-irradiated groups at day 3

![FIG. 4. Collagen deposition. (a) Representative micrographs of MT staining of collagen at days 3, 7, 10, and 14 after wounding (magnification, ×100). Blue, collagen. Scale bars, 100 µm. (b) Quantification of collagen staining by ImageJ (NIH). Error bars, mean ± SD (n = 3 mice/group). Two-way ANOVA with Bonferroni's post hoc test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to no treatment.](image-url)
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and 10 after wounding was not significantly different compared to that in the no-treatment group (Fig. 6(a)). The quantitative result of the transcript level of the corresponding genes, the magnitude of the upregulation of VEGF and TGF-β1 was greater in the LED and OLED-irradiated groups than in the no-treatment group on initiation of wound healing (LED group: VEGF, \( P < 0.001 \), and TGF-β1, \( P < 0.01 \); OLED group: VEGF and TGF-β1, both \( P < 0.001 \)). However, the expression level of IL-10 did not differ significantly among the three groups at any stage of wound healing. The expression level of VEGF was markedly upregulated in the LED and OLED-irradiated groups compared to the no-treatment group until the late stage of wound healing (\( P < 0.05 \) and \( P < 0.01 \), respectively), while the level of TGF-β1 was significantly higher only in the OLED-irradiated group (\( P < 0.05 \); Fig. 6(b)).
The use of PBM has progressively increased over the past decade, and has shown promise for accelerating wound healing in animal models and clinical studies. For instance, PBM ameliorated the slow wound healing associated with diabetes mellitus [25-28] and was used clinically to alleviate the inflammation and pain experienced by patients with head-and-neck cancer undergoing radiotherapy and chemoradiotherapy [29]. However, low-level laser therapy known as PBM has a clinical limitation that it is very difficult to provide a standardized treatment because the different responses of the cells or tissues to the irradiation wavelength and energy. In order to solve this problem, it is urgent to standardize parameters such as irradiation wavelength, energy intensity and irradiation time according to condition of clinic treatment. In addition, the clinical efficacy of PBM is well-established, but the underlying molecular mechanisms are unclear, so further research is needed.

Recently, Jeon et al. have demonstrated that OLEDs with 630 and 650 nm wavelengths have a more effective wound healing effect than OLEDs with 670 and 690 nm wavelengths at low energy densities. These results were consistent with the results of previous studies by Wu et al. We also compared the wound healing effect of LEDs with 630 and 660 nm wavelengths, and the wound healing effect was slightly better for LEDs with 630 nm wavelength (data not shown). Cell proliferation assays were performed using human skin fibroblast cells to determine the optimal light intensity and time. When fibroblast cells were irradiated with 6 J/cm² LED light for 20 minutes, it showed a significant increase of about 52% as compared with control (see Appendix, Fig. 7). As a result of increasing the light irradiation time and energy, cell proliferation no longer increased significantly. We studied the effect of LED and OLED with 630 nm wavelength on wound healing. We also examined the release of inflammatory cytokines and the formation of new tissue. Our results showed that OLED and LED irradiation increases the levels of IL-1β and IL-6 at the initial stage of wound healing and decreases the level of TNF-α at all stages of wound healing. In addition, OLED and LED irradiation increased the expression levels of VEGF and TGF-β1, which are involved in the initial stage of wound healing, and enhanced the formation of new tissue and re-epithelialization of the wound, which is consistent with Wu et al. result. The effect of OLED irradiation on the synthesis of proinflammatory cytokines, the expression of growth factors, and collagen deposition was greater than that of LED irradiation.

During the initial stage of wound healing, proinflammatory cytokines play an important role in inducing infiltration by inflammatory cells, which is necessary to remove debris and kill bacteria [7]. In the present study, the IL-1β level in wound tissue was increased in the LED- and OLED-irradiated groups compared to the no-treatment group. Martins and colleagues reported that PBM increased the IL-1β level during the inflammatory stage of healing of oral wounds in mice (day 1 after wounding) [30], which is consistent with our results [30]. Thus, PBM promotes early IL-1β synthesis during the healing of oral wounds.

Martins et al. asserted that the increase in the IL-1β tissue level induced by PBM at the early stage of wound healing is associated with angiogenesis, which is essential for wound healing [7, 30]. This is consistent with our result that new blood vessels formed more rapidly in the LED and OLED-irradiated groups than in the no-treatment group. Indeed, angiogenesis was enhanced in the LED-irradiated group at day 10 (Fig. 3(a)). The growth factors VEGF and TGF-β1 are important modulators of angiogenesis [31-33]. In this study, the mRNA levels of VEGF and TGF-β1 were increased in the LED- and OLED-irradiated groups compared to the no-treatment group at the early stage of wound healing. Histopathologically, PBM induced re-epithelialization and collagen formation, which are important events during the proliferative phase required for restoration of cellular homeostasis. These results are in agreement with those of Wagner et al., who demonstrated that the increase in the IL-1β level induced by PBM at the early stage of wound healing exerts a proangiogenic effect, as new blood vessels are essential for progression to the proliferative stage of healing [30].

The acute-phase proinflammatory cytokine TNF-α increases vascular permeability and recruits inflammatory cells to the site of injury. While a low level of TNF-α promotes wound healing by indirectly stimulating inflammation and increasing macrophage production of growth factors [34-37], a high level of TNF-α inhibits the synthesis of collagen and fibronectin, delaying wound healing. In this study, the level of TNF-α, but not that of IL-1β, was decreased in the LED and OLED-irradiated groups (Fig. 5(b)). IL-1β and TNF-α exert a positive feedback effect on each other’s expression, which promotes wound healing. Thus, significantly decreased expression of TNF-α during wound healing may have induced the high expression of IL-1β. The complex regulatory system between IL-1β and TNF-α resulted in rapid and organized re-epithelialization and increased collagen deposition (Figs. 3 and 4), and increased the mRNA levels of the growth factors VEGF and TGF-β1 (Fig. 6). Martins et al. asserted that PBM can increase the tissue level of IL-1β at the early stage of oral-wound healing and decrease the tissue level of TNF-α during all stages, and suggested that regulation of cytokine release induces angiogenesis to accelerate wound healing. Their arguments were consistent with our results that LED, and OLED irradiation increased the tissue level of IL-1β and reduced that of TNF-α, and promoted wound healing by enhancing re-epithelialization, collagen deposition, angiogenesis, and the expression of growth factors. Interestingly, this study confirmed that OLEDs were by far superior to LEDs for PBM therapy in terms of re-epithelialization, collagen deposition, and expression of growth factors. In particular, the surface
emission characteristics of OLEDs are superior to point emitting LEDs in that light can be irradiated to cells or tissues with an even energy intensity. This could contribute to the positive effects seen in this study. In addition, OLEDs have a physical characteristic with wide spectral bandwidth, and besides the maximum emission peak of 630 nm, other wavelengths within the broadband (e.g., 650 nm, 660 nm, 670 nm) may be beneficial factors to speed up healing [38, 39]. Thus, OLEDs show promise for next-generation wearable PBM systems for the treatment of wounds.

V. CONCLUSION

We investigated the effects of LED and OLED irradiation on wound healing. LED and OLED irradiation had slightly different effects, but both promoted wound healing markedly compared to no treatment. Wound size, histopathologic changes, levels of proinflammatory cytokines, and levels of gene expression were significantly influenced by LED and OLED irradiation. Also, OLED irradiation accelerated epithelial regeneration and increased collagen density compared to LED irradiation. In addition, OLED irradiation significantly increased the expression levels of VEGF and TGF-β1 compared to LED irradiation. We report here the mechanism by which OLED irradiation modulates inflammation and wound healing, and show the promise of OLEDs for PBM therapy.

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APPENDIX

Cell Proliferation Assay

Cell proliferation was measured using the MTT assay, according to the manufacturer’s protocol. Human skin fibroblasts were purchased from the American Type Culture Collection (ATCC, Virginia) and maintained in Dulbecco’s Modified Eagle’s Medium (DMEM; Life Technologies, New York) with 10% (v/v) fetal bovine serum (FBS; Equitech-Bio Inc., Texas) and 1% (w/v) penicillin and streptomycin (Gibco, New York) supplemented with 7.5 mM L-glutamine, 5 ng/ml of human recombinant fibroblast growth factor beta, 5 μg/ml of recombinant human insulin, 1 mg/ml of hydrocortisone and 50 mg/mL of ascorbic acid; cells were grown under 5% (v/v) CO₂ at 37°C. Cells were seeded at a density of 1 × 10⁵ cells/well in 96-well flat-bottom microtiter plates and incubated at 37°C in the presence of 5% CO₂ for 24 hr. After incubation, cells were refreshed with same DEME culture medium, exposed to different irradiation energy doses (3, 6, 9 J/cm²) and times (5, 10, 20, 30 min) with 630 nm-LED in the dark room and further incubated at 37°C in 5% CO₂ atmosphere for another 24 hr. Cells were then incubated with MTT (0.25 mg/ml) for 2-4 h at 37°C. The formazan crystals in the cells were solubilized with a solution containing 50% dimethylformamide and 20% SDS (pH 4.7). Finally, the level of MTT/formazan was determined by measuring absorbance at a wavelength of 540 nm using a microplate reader (SPECTRA; Tecan Group, Ltd., Männedorf, Switzerland). Cell proliferation was calculated as: Proliferation (%) = (mean OD of treated wells ÷ mean OD of control wells) × 100.

REFERENCES


