



The Effect of Photobiomodulation with Red and Near-Infrared Wavelengths on Keratinocyte Cells

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Abstract—Photobiomodulation (PBM) is defined as the use of non-ionizing photonic energy to trigger photochemical changes, particularly in mitochondrial-sensitive cellular structures. Photobiomodulation is a form of treatment used in medicine in a practical and noninvasive way and it has a significant role in inflammation, ache, and pain reduction, wound healing, and tissue regeneration. It triggers proliferation and the activity of the cell, primarily by utilizing light from the near infrared-red to visible wavelength of the light (630-1000 nm). This *in vitro* study has analyzed comparatively the most appropriate energy doses with the wavelengths in the red and near-infrared spectrum to induce photobiomodulation on the keratinocyte cells. 1, 3, and 5 J/m² energy densities of 655 nm and 808 nm diode lasers were used, which might affect wound healing mechanism and cell proliferation. The potential stimulating effect of photobiomodulation to promote wound healing and cell proliferation on human keratinocyte cells was analyzed via microscopic imaging of cell morphology, MTT analysis for cell proliferation and scratch assay for wound closure after light applications. The highest increase in cell viability was obtained with a rate of 112.6% after the triple treatment of 655-nm wavelength at 1 J/cm². The best wound closure was achieved with a rate of 45% after the triple treatment of 655 nm wavelength at 3 J/cm². This study revealed that PBM with 655-nm of wavelength was an effective tool to induce cell proliferation and speed up the wound healing process with specific energy doses.

Keywords—Photobiomodulation, wound healing, keratinocytes, 808-nm, 655-nm

I. INTRODUCTION

Many studies have been conducted on the potential interaction using any light source and tissue as a target. In medicine, different kinds of light sources, especially Lasers, have been used in many areas such as Low Level Light Therapy (LLLT) or photobiomodulation, phototherapy, and photodynamic therapy [1]. Laser light is used to allow the dissemination of intense energy levels [2]. LLLT, or photobiomodulation, is a therapeutic perspective that results from light exposure of tissue or cells to induce cell proliferation, differentiation, pain reduction, and accelerated wound healing process with near-infrared and/or visible light by lasers or other light sources [3].

Even though LLLT is used today to treat a wide range of diseases from tissue repair to chronic diseases, it is still controversial as a treatment, as the fundamental biochemical

mechanisms are not efficiently understood and many parameters should be optimized, such as power density, wavelength, pulse structure, fluency, the timing of the light applied. The most important point for all light applications is to keep the dose parameters in optimum condition [4].

Photobiomodulation is defined as the use of non-ionizing photonic energy to trigger photochemical changes, particularly within mitochondrial-sensitive cellular structures [5]. In the field of biomedical optics, it is a rapidly developing field with triggering factors that play a significant role in cell proliferation, wound healing, tissue regeneration, inflammation, pain, and pain reduction *in vitro* studies [6]. Besides, photobiomodulation is the practical treatment method using the light that ranges from the red to the near-infrared region of the spectrum (630-1000 nm). PBM can be triggered by various light sources that emit visible light and NIR light, along with modulating multiple cellular functions such as; accelerating wound healing, improving ischemic damage of the heart, improving mitochondrial energy metabolism and production, providing weakened degeneration of the injured optic nerves, reducing pain and inflammations, preventing decreased tissue viability and dissemination of cell cultures by spreading them [7-9]. In this *in vitro* study, 1, 3, and 5 J/m² energy densities of 655 nm and 808 nm diode lasers were used to induce photobiomodulation mechanism for wound healing and cell proliferation purposes on the keratinocyte cells.

II. MATERIAL AND METHODS

A. Cell Line and Culture Conditions

In this study, human keratinocyte cells (HS2) were used, which are the epithelial cells located in the upper part of the epidermis [10]. It has several important roles in the wound healing process. They are involved in the initiation, maintenance and completion mechanisms of wound healing as epithelization [11]. Wounds on the skin are partially repaired by keratinocytes migrating through the nesting movement during wound healing process to fill the gap in the wound area [12].

Keratinocyte cells were cultured in 75 cm² tissue culture flasks with 10% FBS, DMEM supplemented with 1% Penicillin/Streptomycin and % L-Glutamine to prevent bacterial contamination. When the cells reached a minimum of 80% confluency, they were detached with 0.005% trypsin and 0.02% EDTA. 2.5x10³ cells were seeded in 24-well plates for scratch assay and 5x10³ cells were seeded in 96-well plates for MTT

assay. At the end of this procedures, keratinocyte cells were made ready for the LLLT applications.

B. Experimental Setup

The laser devices used as the source of photobiomodulation during the experiments were in İzmir Katip Çelebi University, Biomedical Engineering Department, Biomedical Optics and Laser Applications Laboratory. A diode laser (PS4 III. LED; Changchun New Industries Optoelectronics Tech. Ltd., China) was used as a red light source to irradiate at 655-nm of wavelength. It irradiates at a maximum power of 1 Watt, and its output power can be adjusted in the range of 0-1 Watt. A diode laser operating at the 808-nm wavelength was used as a near-infrared light source. This laser device (Teknofil, Istanbul, Turkey) irradiates at a maximum power of 3 Watts. Its output power and application time can be adjusted on the device.

Before PBM applications, 655 nm and 808 nm diode lasers were sterilized with 70% alcohol. Then, they were placed in the biological safety cabinet and kept under UV to prevent contamination. Finally, the environment is ready for photobiomodulation studies and LLLT applications with the setup and adjustment of the optical devices according to the experimental procedures. In accordance to the experimental procedures, 50 mW power for 655 nm diode laser and 125 mW power for 808 nm diode laser were used to obtain energy doses of 1, 3, 5 J/cm². The output power was checked with a power meter (Thorlabs, USA) before each experiment to ensure that each well containing cells received homogenous light exposure.

C. Scratch Assay for Wound Healing

Scratch assay, also known as a wound-healing assay, is a laboratory technique that mimics cell migration and cell-cell interaction during *in vivo* wound healing [13-16]. HS2 cells incubated in 24-well plates for 24 hours were used for the scratch assay. After 24 hours of cell cultivation, the medium in the wells was removed and a flat scratch in the middle of the wells was created on a single layer of cells attached to the bottom of the plate using a sterile small pipette tip to simulate a wound. The formation of the wound *in vitro* was confirmed under the inverted microscope (Olympus, CKX41). Then 500 µl of DMEM was added to each well and the photobiomodulation protocol was performed on keratinocyte cells with 655 nm and 808 nm of wavelengths. Experimental groups were listed in Table 1.

Table 1. Experimental groups with two diode lasers for scratch assay

Groups	Cell Type	Lazer Type	Pulse Parameters	Energy Density	Power	Irradiation Time
G1	Keratinocyte Cells	655 nm diode laser	Continuous Wave	1 J/cm ²	50 mW	475 s
G2	Keratinocyte Cells	655 nm diode laser	Continuous Wave	3 J/cm ²	50 Mw	1426 s
G3	Keratinocyte Cells	655 nm diode laser	Continuous Wave	5 J/cm ²	50 mW	2376 s
G4	Keratinocyte Cells	808 nm diode laser	Continuous Wave	1 J/cm ²	125 mW	39 s
G5	Keratinocyte Cells	808 nm diode laser	Continuous Wave	3 J/cm ²	125 Mw	118 s
G6	Keratinocyte Cells	808 nm diode laser	Continuous Wave	5 J/cm ²	125 mW	196 s

Irradiation for each group was repeated in triplicate at 24-hour intervals and three samples were irradiated for each group. At the same time, the wound repopulation for each group was evaluated with the inverted microscope at 12-hour intervals. The wound closure rates for HS2 cells after each light applications were examined and compared with each other and the control group. The morphologies of human keratinocyte cells were examined with the inverted microscope both before and after

applications, and cell images were taken at 4X and 10X magnifications in every 12 hours until the wounds were closed. Thus, the effect of the light color and energy intensities on the cells was determined.

D. Cell Viability Analysis - MTT Assay

MTT analysis is the most commonly used colorimetric analysis worldwide to determine the growth rate and cell viability [17]. Cell metabolism and viability were evaluated using the MTT assay for HS2 cell lines. Cells incubated in 96-well plates for 24 hours used for MTT assay. The photobiomodulation protocol was performed on keratinocyte cells with 655 nm and 808 nm of wavelengths. Experimental groups were listed in Table 2.

Table 2. Experimental groups with two diode lasers for MTT assay.

Groups	Cell Type	Lazer Type	Pulse Parameters	Energy Density	Power	Irradiation Time
G1	Keratinocyte Cells	655 nm diode laser	Continuous Wave	1 J/cm ²	50 mW	141 s
G2	Keratinocyte Cells	655 nm diode laser	Continuous Wave	3 J/cm ²	50 Mw	424 s
G3	Keratinocyte Cells	655 nm diode laser	Continuous Wave	5 J/cm ²	50 mW	707 s
G4	Keratinocyte Cells	808 nm diode laser	Continuous Wave	1 J/cm ²	125 mW	39 s
G5	Keratinocyte Cells	808 nm diode laser	Continuous Wave	3 J/cm ²	125 mW	118 s
G6	Keratinocyte Cells	808 nm diode laser	Continuous Wave	5 J/cm ²	125 mW	196 s

Irradiation for each group was repeated in triplicate at 24-hour intervals and three samples were irradiated in each group. The medium was removed from the wells containing the cells, after the third laser application. The 10% MTT solution was prepared at dark. A freshly prepared sterile 5 mg/mL MTT solution (10%) and 0% serum-free culture medium were added to all control and test wells containing the cells to reach a final volume of 100 µl. After the newly prepared sterile MTT solution was added, the cells were incubated for 4 hours. Following incubation, all media was removed from the wells and DMSO was added to the wells. They were closed with aluminum foils to prevent the light. After 10-minute waiting period, the absorbance values in each wells were measured at 595 nm with a spectrophotometer (Multimode Microplate Reader Biotek Synergy HTX, Biotec). The measured absorbance value was used as an expression to show the cell viability in each well after light treatments.

E. Statistical Analysis

The microscopic images of the scratch assay were analyzed with the help of ImageJ for wound area calculation. All acquired data with MTT assays and wound area calculation were analyzed with the help of Student t-test. The statistically significant difference ($p \leq 0.05$) was evaluated between the control groups and light therapy groups, and the results were compared.

III. RESULTS

A. Scratch Assay after Triple Treatment

Triple light treatment was applied to the keratinocyte cells with three different energy densities of 655 nm and 808 nm diode lasers for 24-hour intervals. Besides, scratch assay images of keratinocyte cells were taken in every 12 hours starting at 0th and ending at 60th hours for 655 nm and 808 nm diode laser irradiation groups of 1, 3, 5 J/cm² energy densities and the control group. The images in Fig. 1 and Fig. 2 show the closure in the scratched wound models of the application groups. These images were analyzed with the help of ImageJ for area calculation.

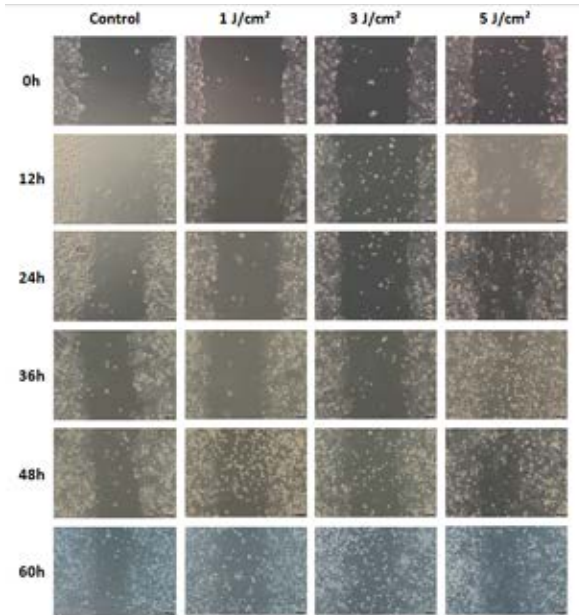


Fig. 1. Microscopic images of HS2 cells in Scratch Assay taken in every 12 hours starting at 0th and ending at 60th hours after triple light treatment with 655 nm diode laser at 1, 3, 5 J/cm² energy densities and the control group.

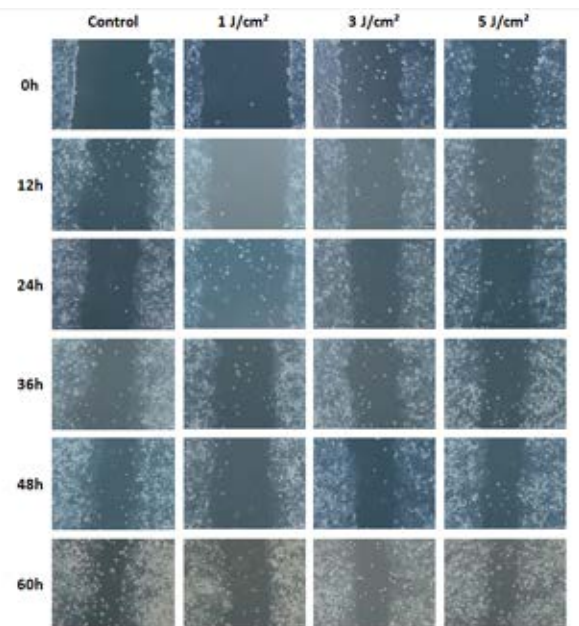


Fig. 2. Microscopic images of HS2 cells in Scratch Assay taken in every 12 hours starting at 0th and ending at 60th hours after triple light treatment with 808 nm diode laser at 1, 3, 5 J/cm² energy densities and the control group.

Triple light treatment of 655 nm diode laser on HS2 cells resulted in maximum wound closure (minimum wound area) at an energy density of 3 J/cm² as shown in Fig. 2. Also, Fig. 2 shows the wound area percentages at 1, 3, and 5 J/cm² energy densities with the rates of 91.4%, 54.9%, and 67.6% according to the control group at the end of 60 hours evaluation, respectively.

Triple light treatment of 808 nm diode laser on HS2 cells resulted in maximum wound closure (minimum wound area) at an energy density of 5 J/cm² as shown in Fig. 3. Also, Fig. 3 shows the wound area percentages at 1, 3, and 5 J/cm² energy densities with the rates of 123.6%, 93.1%, and 75.0% according to the control group at the end of the 60 hours evaluation, respectively.

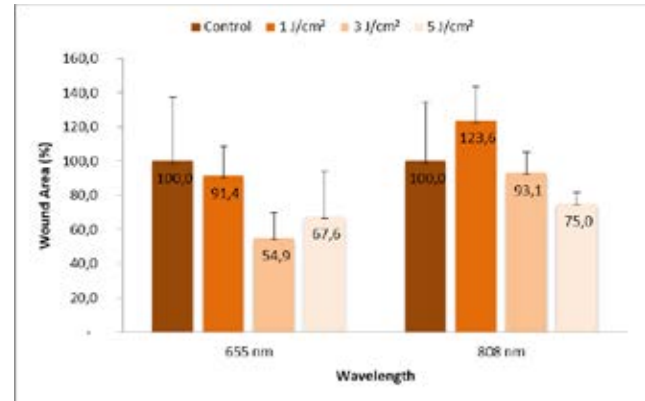


Fig. 3. Changes in wound area as a percentage in control, 1, 3, and 5 J/cm² energy density groups after triple treatment at 655 nm and 808 nm of wavelengths on HS2 cells.

B. Cell Viability Assay after Triple Treatment

Cell viability changes on HS2 cells after light irradiation with 655 nm and 808 nm diode lasers at 1, 3, and 5 J/cm² energy densities were assessed by MTT Assay after triple light treatment.

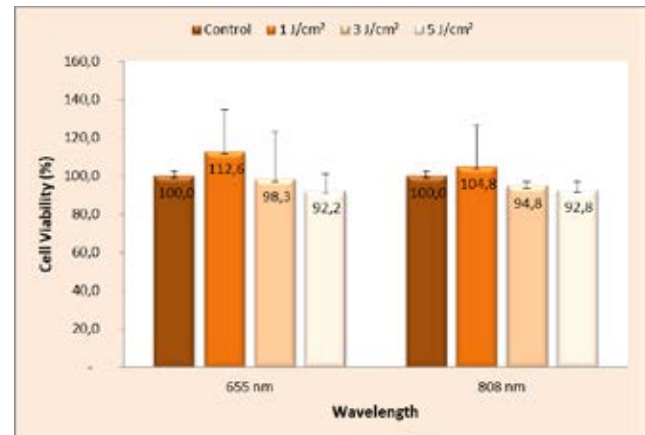


Fig.4. Cell viability in control, 1, 3, and 5 J/cm² energy density groups after triple treatment at 655 nm and 808 nm of wavelengths on HS2 cells.

The cell viability increased at 1, 3, and 5 J/cm² energy densities after triple treatment of 655-nm of wavelength with the rates of 112.6%, 98.3%, and 92.2%, respectively. So it was understood that triple light treatment with 655 nm of wavelength on HS2 cells resulted in maximum increases in cell viability at an energy density of 1 J/cm² as shown in Fig. 4. Likewise, triple laser treatment with 808 nm of wavelength on HS2 cells resulted in maximum cell proliferation at an energy density of 1 J/cm² as shown in Fig. 4. The cell viability increased at 1, 3, and 5 J/cm²



energy densities after triple treatment of 808-nm of wavelength with the rates of 104.8%, 94.8%, and 92.8%, respectively.

IV. DISCUSSION

Photobiomodulation is a practical treatment using light that ranges from visible to near-infrared region of the electromagnetic spectrum (630-1000 nm). In this study, the effects of two different wavelengths (655 nm and 808 nm) on human keratinocyte cells were investigated and their effects on wound healing and cell proliferation were compared. Also, the potential stimulating effect of low-level laser therapy to increase wound healing and proliferation in the cultured human keratinocyte cell was analyzed by changing the laser parameters such as wavelength, energy dose, and application time. The highest cell viability increase was obtained from triple treatment of a 655 nm diode laser with an energy density of 1 J/cm² as 112.6% increase. Likewise, the best wound healing was achieved from the triple treatment of 655 nm diode laser with a 54.9% wound area at an energy density of 3 J/cm², which corresponds to a wound closure around 45%.

To assess the impacts of photobiomodulation, *in vitro* study of Basso et al. irradiated human keratinocyte cells with a 780 nm diode laser using 0.5, 1.5, 3, 5 and 7 J/cm² of energy. The cell viability assay showed that the results were more effective for 0.5, 1.5, and 3 J/cm² [18]. In this study, it was observed that 808 nm of wavelength was the most effective at 5 J/cm² energy densities with a 75 % wound area in Scratch Assay. However, when MTT results were examined, 1 J/cm² energy density was more effective with a 104.8% increase in cell viability. This results were consistent with the study of Basso et al. However, 655-nm of wavelength was more effective to induce proliferation and cell migration in our study when the outcomes resulted from the applications with 655-nm and 808-nm of wavelengths were compared. 808-nm of wavelength has the capacity to induce photothermal effect which may cause cellular stress on keratinocytes. This may be the reason to obtain more positive results after the applications with 655-nm of wavelength.

In conclusion, we understood that the adjusted laser parameters can promote biostimulation, proliferation, and migration of cell for wound healing with the findings we obtained from this study. We anticipate that these encouraging *in vitro* results will guide future studies, including their effects in *in vivo* tissue healing process. The growth rate of cultured cells, including stem cells can be increased, which will be very useful in tissue engineering and regenerative medicine with the proper use of photobiomodulation.

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