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Blue light does not impair wound healing *in vitro*

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ABSTRACT

Irradiation with red or near infrared light promotes tissue repair, while treatment with blue light is known to be antimicrobial. Consequently, it is thought that infected wounds could benefit more from combined blue and red/infrared light therapy; but there is a concern that blue light may slow healing. We investigated the effect of blue 470 nm light on wound healing, in terms of wound closure, total protein and collagen synthesis, growth factor and cytokines expression, in an *in vitro* scratch wound model. Human dermal fibroblasts were cultured for 48 h until confluent. Then a linear scratch wound was created and irradiated with 3, 5, 10 or 55 J/cm^2. Control plates were not irradiated. Following 24 h of incubation, cells were fixed and stained for migration and fluorescence analyses and the supernatant collected for quantification of total protein, hydroxyproline, bFGF, IL-6 and IL-10. The results showed that wound closure was similar for groups treated with 3, 5 and 10 J/cm^2, with a slight improvement with the 5 J/cm^2 dose, and slower closure with 55 J/cm^2 $p < 0.001$). Total protein concentration increased after irradiation with 3, 5 and 10 J/cm^2, reaching statistical significance at 5 J/cm^2 compared to control ($p < 0.0001$). However, hydroxyproline levels did not differ between groups. Similarly, bFGF and IL-10 concentrations did not differ between groups, but IL-6 concentration decreased progressively as fluence increased ($p < 0.0001$). Fluorescence analysis showed viable cells regardless of irradiation fluence. We conclude that irradiation with blue light at low fluence does not impair *in vitro* wound healing. The significant decrease in IL-6 suggests that 470 nm light is anti-inflammatory.

1. Introduction

It is now well documented that treatment with red or infrared light with less than 200 mW/cm^2 irradiance and with wavelength in the range of 600 nm to 1000 nm promotes the repair process of skin in experimental animal and human wounds [1–4], ligament [5,6], tendon [7,8], bone [5,9], and cartilage [10,11]. The evidence indicates that red and near infrared light advance tissue repair by enhancing fibroblast migration and proliferation, collagen synthesis [8,12], and by modulating the timing and release of growth factors and cytokines, including basic fibroblast growth factor (bFGF), interleukin 6 (IL-6) and IL-10 [13]. Similarly, accumulating evidence indicates that light between 400 to 480 nm wavelengths, commonly referred to as blue light, is antimicrobial and has been shown to suppress *Staphylococcus aureus*—including methicillin-resistant *S. aureus* [14–21], *Propionibacterium acnes* [22,23], *Pseudomonas aeruginosa* [14], *Salmonella enterica* serovars Typhimurium and Heidelberg [24] and other bacteria [23,25–27].

Also, blue light has been shown to improve tissue perfusion by release of nitric oxide (NO) from nitrosyl complexes with hemoglobin in a skin flap model in rats [28]. Since NO formation leads to vasodilatation and subsequent increase in microcirculatory blood flow, the use of blue light might be of great importance for wound healing of diabetic and venous ulcers [29]. Moreover, blue light has been shown to enhance angiogenesis [30] and to be anti-inflammatory [22,31].

These findings suggest that the combination of red/near infrared light and blue light could promote healing and suppress wound infection simultaneously; since red or near infrared light will, as expected, engender faster tissue healing concurrently as infection is kept away with blue light treatment. However, although blue light is well-known to suppress bacterial growth, its potential effect on wound healing remains unknown. The fact that it is antimicrobial suggests that it may suppress tissue repair.

Thus, the purpose of this study was to determine the effect of blue 470 nm light on wound healing, by measuring (1) the rate of wound closure, (2) collagen synthesis, (3) total protein synthesis, (4) growth factor release and (5) cytokines expression, in an *in vitro* scratch model of wound repair. Wounded or scratched, fibroblast monolayers respond to the disruption of cell–cell contacts by secreting more growth factors at the wound margins and promote healing by a combination of proliferation and migration [32–34]. Thus, the scratch assay presents a simple

Abbreviations: bFGF, basic fibroblast growth factor; IL-6, interleukin 6; IL-10, interleukin 10; NO, nitric oxide; PBS, phosphate-buffered saline; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; EDTA, ethylenediaminetetraacetic acid; LED, light-emitting diode; DPBS, Dulbecco's phosphate-buffered saline; EthD-1, ethidium homodimer-1; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate; HCl, hydrochloric acid.

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experimental model of healing in which the cell sheet serves as a surrogate tissue, enabling precise observation of the effect of an experimental intervention [13,32]. The model has been used successfully to reproduce a wound environment *in vitro* and has proven to be a valuable inexpensive tool for gaining initial insight into the effect of experimental treatments on wound healing [35–39]. In this model, the surrogate wound is produced in a monolayer of experimentally cultured fibroblasts, the major cell type in the dermis [38], which promote contractile reapproximation of the wound edges and are vital in synthesizing collagen and other components of the extracellular matrix [40–43].

2. Material and Methods

2.1. Cell Culture

Human dermal fibroblasts isolated from adult skin (Cat. No. C-013-5C) were obtained from Life Technologies Corporation (Carlsbad, CA). Cells were grown in 75 cm^2 cell culture flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, and 100 μg/mL streptomycin, in a controlled humidified cell culture incubator (37 °C, 5%CO_2/95% air). The medium was changed every two days. When cells became confluent, the medium was removed, the cell layer was washed with phosphate-buffered saline (PBS) and trypsinized with 0.25% trypsin in buffered ethylenediaminetetraacetic acid (EDTA). Cells were counted in automated cell counter [Cellometer® Auto T4 (Nexcelom Bioscience, Lawrence, MA)] [44]. Prior to the tests, experimental cultures were prepared by seeding cell suspensions at concentrations of 3×10^4 cells/well (1 mL/well) in 24-well microplates and incubated for 48 h in a controlled humidified cell culture incubator to obtain confluent cell growth.

2.2. Scratch Assay

After 48 h of incubation, microplates were observed under an inverted microscope [Olympus® IX51 (Olympus America Inc., Melville, NY)] to confirm the presence of cell growth monolayer. Consistently linear wounds were made using a sterile 5.0 mL pipette tip across the center of each well, creating a cell-free area [35,38,39]. Any cellular debris created from the scratching was removed by gently washing each well twice with DMEM and then 1.0 mL of fresh DMEM (containing 2.5% FBS) was added to each well [45]. The presence of the *in vitro* wound was confirmed under an inverted microscope before plates were irradiated with blue light.

2.3. Light Source and Cell Irradiation

A light-emitting diode (LED) device, the Dynatron Solaris® 708 (Dynatronics Corp., Salt Lake City, UT) fitted with a 470 nm light probe, was used to irradiate the wounded fibroblasts. The applicator, which has a cluster of 32 LEDs, emits blue light with a spectral width of 455–485 nm, and a rating of 150 mW average power and 30 mW/cm^2 irradiance. The light applicator was clamped at a distance of 1–2 mm perpendicularly above each open plate. The device automatically timed the duration of treatment needed per dose [15,16,18–21,24,44]. As detailed in a previous report, no measurable increase in temperature is generated by the device within the range of fluences used [16]. The fluences used to irradiate the cells were 3, 5, 10 and 55 J/cm^2 based on our previous study [44].

Irradiated groups and non-irradiated controls were subjected to the same environmental conditions in terms of humidity, temperature and time within or outside the incubator, and light–dark cycle. Plated fibroblasts were incubated for 24 h and assayed as summarized in Fig. 1.

2.4. Cell Migration/Wound Closure Analysis

To assess cell migration (wound closure/repopulation), images were acquired immediately after wounding (0 hour-time point), and also after irradiation and 24 h of incubation (24 hour-time point), using an inverted microscope (Olympus® IX51 [Olympus America Inc., Melville, NY]) equipped with a digital camera (Olympus® DP70). Cell migration

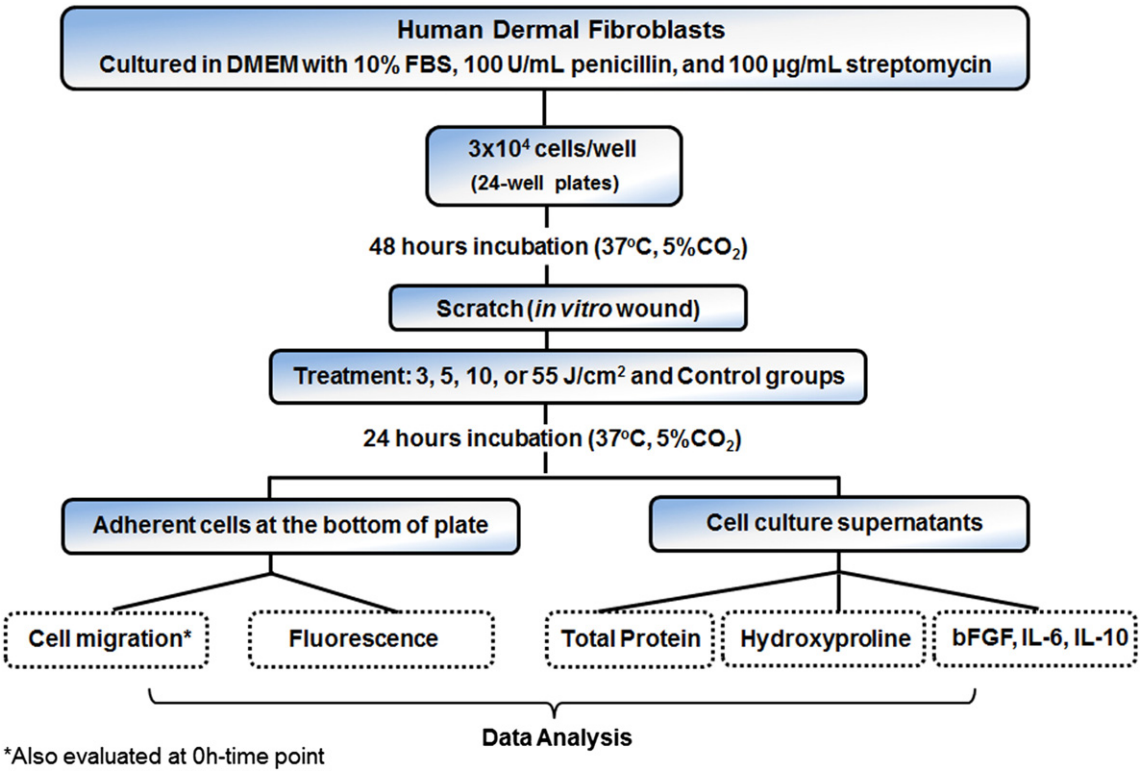

Fig. 1. Experimental design.