Effects of Continuous-Wave (670-nm) Red Light on Wound Healing

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BACKGROUND Recent work suggests that injuries can heal faster if treated by lasers emitting 670-nm red light. LED lights emitting 670-nm light are now available. This suggests that inexpensive and easy-to-use 670-nm LED lights might help accelerate cutaneous wound healing.

OBJECTIVE The objective was to evaluate the effect of 670-nm LED light on wound healing in SKH-1 hairless mice.

METHODS To study 670-nm light effects on incisional injury, animals were left unexposed or exposed to equal doses of high-, medium-, or low-flux light. Burn injuries were treated with high-flux light or left unexposed. Healing was assessed by measurement of the burn area and the gap remaining to closure of incisional injury.

RESULTS Mice exposed to 670-nm red light showed significantly faster healing than control mice. High, medium, and low fluxes of light were all effective after incisional injury. In burn injury, there was improvement in wound healing initially, but the time to repair was unchanged.

CONCLUSIONS A 670-nm LED red light source accelerates healing in skin of SKH-1 hairless mice after incisional injuries, but is not as effective for burn injuries. These data that suggest red light exposure may be helpful in postoperative wound repair.

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Lacerations and burns are a common presenting complaint in primary care, intermediate care, and emergency medical facilities. Previous studies with continuous-wave 670-nm near infrared laser light have shown promise in enhancing injury repair in some laboratory animals.[1] This light source, however, is cumbersome and expensive for routine use. Recently, NASA has used an LED (light-emitting diode) lamp as the source of 670-nm light to study its effects on injury repair.[2] This light source is thought to be superior to laser light because it is more cost-efficient, can irradiate more surface area, requires less energy, and produces less heat byproduct than a laser.[2] The efficacy of this light source in attenuating some types of injury has been demonstrated in recent work showing that high-flux 670-nm LED light can inhibit the toxic effects of methanol on the retina.[3] Other reports have shown exposure to 670-, 726-, or 880-nm LED red light can decrease healing time in chronic ischemic ulcers in rats.[2,4] Reports also indicate that 670-nm light exposure is helpful in chemotherapy-induced mucositis in humans.[2] Collectively, these studies suggest that 670-nm LED may be helpful in repair of acute cutaneous wounds. If 670-nm light can decrease healing time, it may be a benefit for patient comfort and decrease the risk of infection in skin injury. We therefore performed a series of studies using a 670-nm LED lamp to

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determine its efficacy and flux requirements in improving healing of laceration and burn injuries.

Methods

Mice

Inbred hairless mice of the SKH1-*hr* albino strain were purchased from Charles River Laboratory (Wilmington, MA). These mice are widely used to study cutaneous wound healing. Their skin contains fine cysts which are the remnants of hair follicles. Mice were housed three to five per cage. This strain was used because they have an intact immune system and are hairless and so effects of light will not be altered by the presence of hair.

Injuries

This injury protocol was approved by University of Rochester Strong Memorial Hospital's governing body for animal research, UCAR (University Committee on Animal Resources). The burn injury model produces a full-thickness insensate burn. Before any thermal injury, the mice were deeply anesthetized by injecting 60 mg/kg ketamine and 5 mg/kg xylazine IP. A surgical plane of anesthesia was obtained and assessed by lack of response to toe pinch. To produce burns, a 65-gauge cylindrical copper rod was heated in boiling water to 100°C and applied to the flank of a deeply anesthetized mouse for 10 seconds.[5,6] The injury results in reproducible and well-tolerated wounds. Such burns also have a predictable depth and border, permitting precise measurement of wound area during repair and leaving sufficient tissue at the wound border to permit analysis of tissue histology. Sham-treated control mice were also anesthetized, and an unheated rod was applied to the skin for 10 seconds. After the burn injury, to prevent shock, the mice were resuscitated using 0.1 mL/kg IP Ringer's lactate. Animals were then individually housed in standard plastic cages with standard bedding material.

Incisional injuries were created using a No. 11 blade to make three parallel cuts, 6 mm in length, in the upper midscapular region. While under anesthesia, the mouse was laid on its side, the skin was separated from the underlying tissue, and a full-thickness incisional injury was produced. Incisions were made 4 mm apart.

Pain relief for the animals was provided using 2 mg buprenorphine/kg of body weight. This was readministered every 12 hours as needed if animals exhibited behaviors characteristic of pain. Acetaminophen was also provided in drinking water at a dose of 2 mg/mL. Wounds were left uncovered. Animals were euthanized at 24, 48, and 72 hours after incisional injury and 1, 7, 14, 21, and 28 days after burn injury, using CO_2 inhalation followed by cervical dislocation. After euthanization, incised areas were excised, and burn areas were biopsied and then fixed in formalin for further analysis.

Light Source

A cage apparatus equipped with red light–emitting diodes (Quantum Devices, Barneveld, WI) was used in this experiment. Analysis of the light's emission spectra and power was provided by Kodak (Rochester, NY). The diode array consists of two panels that emit light between 660 and 680 nm with peak emission at 670 nm (Figure 1). The irradiation distance in the apparatus was 2 to 3 cm from the backs of the mice. Water and food were removed while animals were irradiated. The cage and light apparatus has a cooling airflow fan built into it to maintain a constant temperature during light exposure. Control mice were sham irradiated for the same length of time.

LED Irradiation

Mice received either no light (controls) or were exposed to one of three different fluxes of light. Mice received either a 90-second LED exposure at an intensity of 40 mW/cm^2, a 450-second exposure at an intensity of 8 mW/cm^2, or a 37.5-minute exposure at an intensity of 1.6 mW/cm^2, so that the total treatment dose in each group was the same. Light was administered daily Monday through Friday to mice in these groups; light was not administered on day of sacrifice. For all exposure groups the total dose per exposure was 3.6 J/cm^2 (Quantum SpectraLife, LED

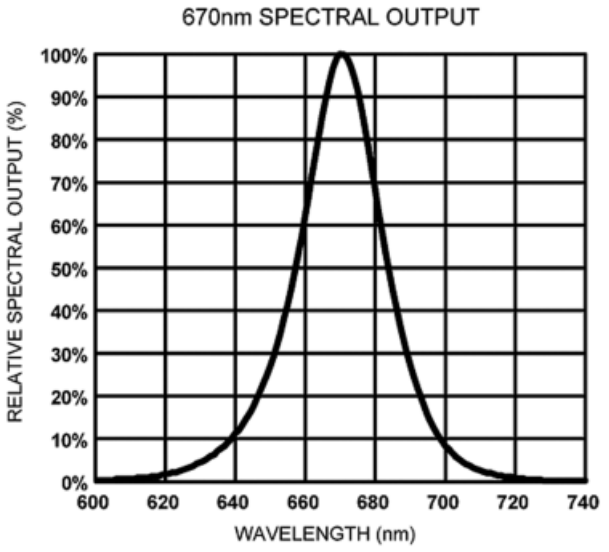

Figure 1. Spectral graph of light output from LED source. This graph represents the wavelength that the LED source emits. The source emits light between 660 and 680 nm with peak emission at 670 nm. The full bandwidth at half-maximum is approximately 25 to 35 nm.

array). This dose is in the range that has been shown to be efficacious in many animal models and in a human mucositis model.[2]

Morphologic Studies and Measurements

After euthanization, skin specimens were fixed in 10% neutral buffered formalin, routinely processed, and paraffin embedded, and 5-μm-thick sections were prepared. Sections were either stained with hematoxylin and eosin or processed for immunohistochemistry using bromodeoxyuridine (BrdU) incorporation as a proliferation marker. Animals were injected with 0.4 cm^3 of a 20 mM solution of BrdU IP 3 hours before sacrifice. BrdU was detected using a BrdU staining kit purchased from Zymed Laboratories Inc. (San Francisco, CA), according to manufacturer's instructions, and used to check for proliferation at the periphery of incisions and burns. Sections were counterstained briefly with hematoxylin.

The H&E slides were examined at 10 ×. Wound repair was evaluated by measuring the length of the unepithelialized wound bed, i.e., the distance between ingrowing epithelial sheets. Measurements were all taken using digital images obtained at 10 × magnification using the computer program Spot (Diagnostic Instruments Inc., Sterling Heights, MI). Reviewers were blinded to the identity of the slides.

In the burn model, healing was evaluated by measuring the gross burn diameter. Digital photographs were taken at a fixed distance at time of euthanization. The burn area was then assessed by measuring the largest and smallest diameter of the gross burn and then averaging them to obtain the mean diameter. Burn area was then calculated using the mean diameter.

Results

Incisional Model

The incisional model was designed to mimic human incisional injuries, such as lacerations and surgical wounds. On histologic examination, significant effects of 670-nm red light on wound healing were observed. On Day 1 (24 hours after injury), measurement of the gap remaining in the incisional wounds showed a significant improvement in mice exposed to low-, medium-, and high-flux light compared to controls (Figure 2A).

On Day 2, measurements of distance between the epithelial tips under the incisional injury crust revealed that mice exposed to high-, medium-, or low-flux light continued to have significantly better epithelial repair than did controls (Figure 2B). In fact, epithelial integrity was restored in a larger number of incisions by Day 2 in all the treatment groups compared to controls (Table 1). On Day 3, reepithelialization was complete in all groups. Epidermal proliferation measured using BrdU showed proliferation was evident in all three groups, but no significant difference was noted between LED-treated and control animals on either day (data not shown). Histologically, there was no detectable difference in inflammation between groups.

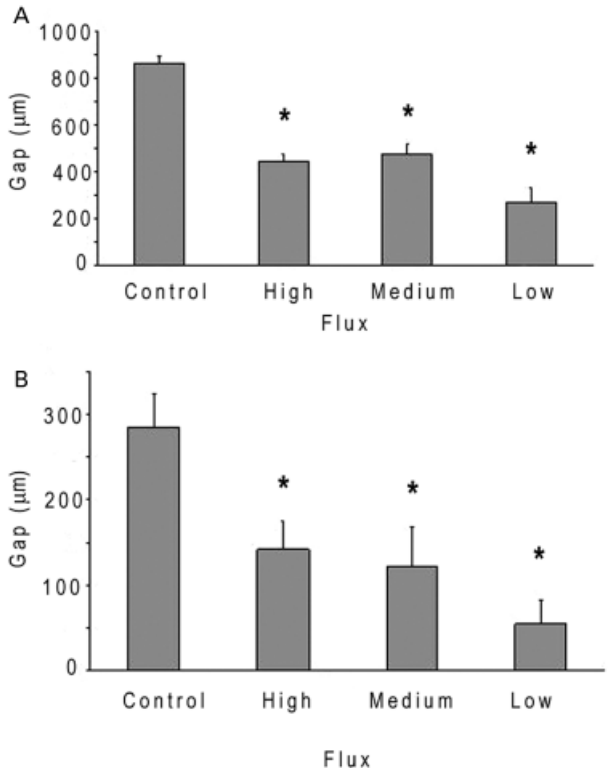

Figure 2. Effect of red light exposure on incisional gap. Red light (670 nm) markedly accelerates wound healing in incisional injuries in mice exposed to high-, medium-, or low-flux light compared to controls. (A) Twenty-four hours after injury and one light exposure and (B) 48 hours later after two light exposures. Mice were treated and euthanized, and healing was assessed by measuring the incisional gap as described under Methods. Data represent mean for control ($n=5$) and high ($n=3$), medium ($n=3$), and low ($n=3$) flux at each time point. There was a significant difference between red light–exposed animals and the controls for Day 1 ($p<.001$) and Day 2 ($p<.001$), Wilcoxon rank sum test. Bars, $\pm$ SE.

Burn Model

In mice treated with seven doses of light, euthanized 1 week after injury, gross burn diameter showed a significant improvement compared to controls (Figure 3A). By the end of the second week, the light-exposed animals still had a slightly smaller burn area, but the difference between groups was no longer statistically significant (Figure 3B). By the third week, the burns were almost completely healed and there was no significant difference between the two groups (Figure 4). At Week 4, all wounds were fully healed (Figure 4).

Conclusions

In this study we demonstrated that 670-nm red light emitted from an LED lamp can accelerate the healing process in both mouse burn and mouse incisional injury models. In the incisional model, the improved healing led to earlier complete reepithelialization. This was apparently due to increased epithelial migration, since BrdU incorporation was unchanged. Some contribution from myofibroblast activity, as noted by Medrado and colleagues,[7] cannot be excluded. In the burn model, the repair process was initially faster in LED-treated animals, but by the second week, repair in both groups was nearly the same. Potentially, this poorer response at the later time point is due to the thick overlying burn eschar blocking access of the light to the growing epithelium.

Our studies confirm that wound healing can be improved by red light and also that it can be helpful when delivered by an LED device that can deliver light evenly over larger wound areas than a laser. All flux groups healed significantly faster than the controls. Of interest is the finding that healing in the lowest flux group appeared to be better than either the high- or medium-flux groups. This difference may be due to the slightly younger age (2 weeks younger: 6 weeks vs. 8 weeks) of the mice in the

TABLE 1. Red Light Exposure Increases Number of Incisional Wounds Closed on Day 2*

		Flux		
	Control	*High*	*Medium*	*Low*
Percent healed	23	54	67	80

*After two doses of red light (670 nm), incisional injuries in treated animals showed a dramatically greater response to those of the control group. A larger percentage of wounds were completely healed in the high-, medium-, and low-flux group compared to controls. One could infer from these results that being treated with red light (670 nm) can reduce healing time by an entire day.

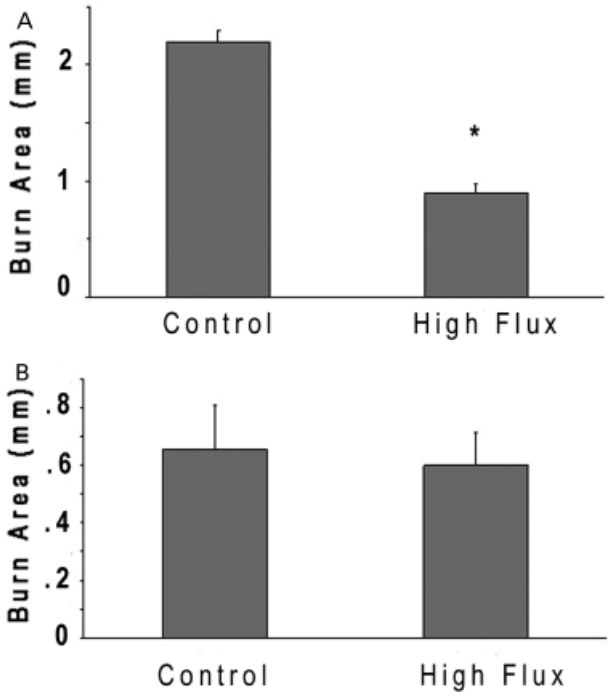

Figure 3. Effect of red light on burn repair. Burns at 1 week after seven doses of 670-nm red light show marked improvement over controls. The area of the burn was calculated as described under Methods. Data represent the mean area of burn for control ($n=3$) and high-flux ($n=3$) groups. (A) There is a significant difference between the high flux group and the control group 1 week after injury ($p<.002$) Wilcoxon rank sum test. Bars, $\pm$ SE. (B) After 2 weeks, there is no longer a statistically significant difference between the two groups. Data represent mean area of burn for control ($n=2$) and mean for high-flux ($n=3$) groups. Bars, $\pm$ SE.

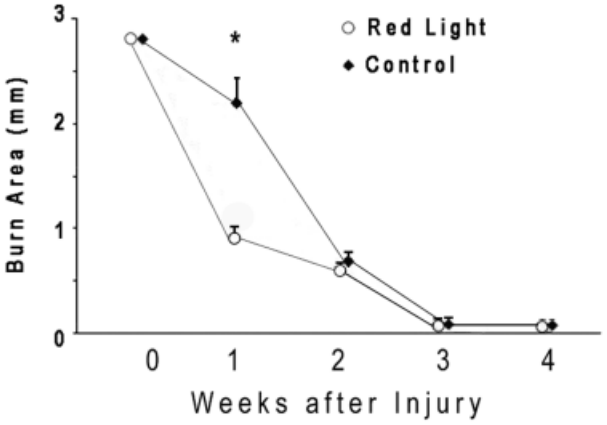

Figure 4. Time course of burn repair in red light–treated mice. Burns initially healed faster due to 670–nm red light exposure. There is a clear difference at Week 1 ($p<.002$). By Week 2, this difference is almost gone, and the difference is no longer statistically significant. At Week 3 most burns are healed, and by Week 4 they are all completely healed. Data points represent mean burn area at each week for each group.

low-flux group compared to those exposed to high- and medium-flux light. Healing in the younger control animals, however, was the same as that of the slightly older mice. Alternatively, this result may be due to the fact that a lower flux of light allows for a smaller amount of power to be delivered over a longer time period, more continuously stimulating the repair process. More investigation into understanding how a lower flux of light may be given to achieve a maximum effect and where the falloff point in efficacy occurs would be worthwhile.

Past experiments have shown much promise for the use of red and other visible light wavelengths in wound healing. Several past studies have found laser light sources of multiple wavelengths to be helpful. Accelerated healing as well as increased collagen synthesis was noted by use of HeNe and Argon lasers (514.5 nm) on skin wounds in rats.[8] One study showed that a single application of 4 J/cm^2 670 nm light from a GaA1As laser reduced edema and inflammation, increased collagen deposition, and induced proliferation of myofibroblasts in cutaneous wounds in rats.[8] In a study performed by Mester and colleagues,[9] mechanical wounds and burns were created on the dorsa of mice, and laser-treated wounds healed faster than those wounds not treated. Another report shows evidence that a HeNe laser (904 nm) was able to improve wound tensile strength in rabbits.[10]

The mechanism of action of 670 nm light is not fully understood, but the mechanism appears to directly affect those cells involved in wound repair. It appears likely that the light stimulates photoreceptor molecules in the mitochondrial respiratory chain.[11] This theory suggests that overall electron transfer in the respiratory chain is accelerated, and thus more adenosine triphosphate is available for use in the wound healing process.[11] Other contributory mechanisms may include Cu-Zn superoxide dismutase reactivation by red light, causing a decrease in reactive oxygen species, thereby facilitating wound healing by preventing tissue destruction.[12] Photolysis of nitric oxide generated in

injured tissue has also been suggested as a mechanism. This hypothesis postulates free-radical nitric oxide can inhibit cellular respiration by binding to cytochrome *c* oxidase, and its photolysis is therefore protective.[12–24] Additional work is needed to clearly identify the mechanism at work here. Such information will be very useful to optimize use of 670-nm light for wound repair.

At present, studies in humans with 670-nm LED light have addressed only mucositis. Results of our experiments suggest that further studies in humans should be pursued. A search for the exact mechanism by which improved wound repair occurs is also an important next step. If 670-nm light improves wound repair in human subjects with acceptable or no adverse effects, it could be useful to reduce morbidity and health care costs associated with wound care in medical facilities.

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