Effect of Visible and Infrared Polarized Light on the Healing Process of Full-Thickness Skin Wounds: An Experimental Study

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Abstract

Objective and Background Data: Polarized light has already been experimentally and clinically used in an effort to promote wound healing, but the findings have been equivocal. The aim of this study was to evaluate the effect of visible and infrared polarized light of a specific range of wavelength (580–3400 nm) on the secondary healing of full-thickness skin wounds in rats. Materials and Methods: Forty male Wistar rats were used, divided in two groups of 20 animals each. A standardized open full-thickness skin wound was created on the back of each animal. In the first group the rats were exposed to polarized light (40 mW/cm² and 2.4 J/cm²) for 7 min on a daily basis (total daily dose 16.8 J/cm²), while the second group acted as controls. Clinical and histological evaluation of wound healing were performed on days 5, 10, 15, and 20 post-wound. The size of the wounds was measured with the use of planimetry, whereas epithelialization, inflammatory response, neovascularization, and collagen formation were histologically assessed. Results: According to our findings, the group exposed to light therapy showed statistically significantly faster epithelialization seen on days 10 and 15 post-wound compared to controls, as well as better quality of the healing process (although not statistically significantly) at all time points. Conclusion: In conclusion, this specific fraction of polarized light seems to have beneficial effects on wound healing, leading to faster epithelialization and qualitatively better wound healing.

Introduction

Nonhealing acute or chronic wounds represent a source of pain and disability for thousands of patients, and have a great impact on their quality of life. In addition, they represent a significant economic burden to society due to loss of income and increased health care costs. The development of an effective, fast-acting, and low-cost treatment for these kinds of wounds would have enormous benefits for both patient quality of life and the economic burden.1,2

To date, a wide range of local and systemic treatment modalities have been used, both experimentally and clinically, in an effort to improve and accelerate wound healing. Among these, certain physical applications have been tried, such as pulsed electromagnetic fields, low-power laser therapy, and ultrasound, which have in common the delivery of energy to the target tissues.3–5

It is generally accepted that low-power laser light promotes wound healing, and this is attributed to its biomodulating effect, which depends on several parameters, such as wavelength, dose, coherence, and power density. There are also some studies that support the beneficial effects of non-coherent light on wound healing.6–7,15–17 The data indicating the potential beneficial effects of polychromatic polarized light on wound healing are limited and controversial, and few experimental and clinical studies have been conducted using this spectrum of polarized light.7–9,14–17

The aim of this study was to investigate the effect of polarized polychromatic light of a specific range of wavelength (580–3400 nm), which represents the yellow, orange, red, and near-middle infrared portions of the spectrum, on secondary skin wound healing in a rat model. This modality uses the fraction of visible light that is capable of deeper tissue penetration, and the fraction of infrared that has only minimal

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Materials and Methods

Forty male Wistar rats, 17 weeks old and weighing 200 ± 30 g, were used and cared for according to Greek and European guidelines regulating animal research. The animals were acclimated for a period of 3 d prior to the experiments, during which time they were examined for any signs of disease. Throughout the study period the animals were kept under stable conditions (temperature 22°C, humidity 30%–70%, and light cycles on a 12/12-h light/dark schedule), and nourished with dried pellets and tap water.

On day 0, a standard full-thickness skin defect was created on the back of each rat. Under intraperitoneal anesthesia (ketamine 3.5 mg/kg body weight and midazolam 7 mg/kg body weight) and aseptic conditions, all animals underwent en bloc excision of the skin and underlying panniculus carnosus of a square-shaped area measuring 2 × 2 cm from their back.

The rats were randomly divided in two groups of 20 rats each. The animals in the first group (experimental group: EG) were exposed to polarized light, and those of the second group (control group: CG) acted as controls. A polarized light source (Bioptron III, Bioptron-AG, Wellezau, Germany) with the following characteristics was used: wavelength = 480–3400 nm, degree of polarization = 95%, power density = 40 mW/cm², and light energy = 2.4 J/cm². However, the wavelength of the polarized light delivered to the animals was modified to 580–3400 nm via the use of a Wratten color gelatin filter (number 25, Eastman Kodak Company, Rochester, NY, USA), and the light cycles on a 12/12-h light/dark schedule, and nourished with dried pellets and tap water.

Beginning on day 0 and then on a daily basis, each animal in the EG was placed in restraints for 7 min in a specially-constructed wooden cage (16 × 8 cm). No metallic parts were used to build the cage to avoid any interference with the polarized light. The light source was vertically centered over the box at a distance of 10 cm from the wound surface. In the EG the animals were exposed to polarized light (total daily dose: 2.4 J/cm² × 7 min = 16.8 J/cm²). In the control group, although the animals were caged for the same time period, the light source was not activated. From the beginning until the end of the experiment the rats were housed individually to avoid cannibalistic behavior. Dressings were not used and antibiotics were not administered.

On days 5, 10, 15, and 20 after wound creation, five rats from each group were sacrificed to evaluate progression of the healing process. Clinical evaluation comprised measure-
wound epithelialization in the experimental group compared to the controls, on days 10 and 15 \( (p < 0.05) \). For the rest of the assessment period, although epithelialization was faster in the experimental group, there were no statistically significant differences between the two groups. The findings are listed in detail in Table 3 and the difference between the two groups is shown in Fig. 1.

**Histopathological findings**

The findings of the histological evaluation, which included assessment of the degree of epithelialization, inflammatory response, neoangiogenesis, and collagen formation, are listed in detail in Table 3 and illustrated in Figs. 2, 3, 4, and 5.

**Epithelialization.** The first complete wound epithelialization was noted on day 15 in the experimental group, and all the wounds were healed by day 20 in this group. In the control group on day 20 the wounds were still not completely healed. Statistical analysis showed significantly enhanced epithelialization in the experimental group at 10 and 15 days post-wound \( (p < 0.05) \). On days 5 and 20, although the epithelialization scores were higher in the experimental group,

<table>
<thead>
<tr>
<th>Day</th>
<th>Experimental group</th>
<th>Epithelialization</th>
<th>Inflammatory response</th>
<th>Neoangiogenesis</th>
<th>Collagen formation</th>
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<td>5</td>
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<td>4.6 (4–5)</td>
<td>1.8 (1–3)</td>
<td></td>
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<tr>
<td></td>
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<td>1.0 (0–1)</td>
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<td>4.2 (3–5)</td>
<td>1.4 (1–2)</td>
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<td>10</td>
<td>2.8 (2–3)</td>
<td>1.6 (1–2)</td>
<td>3.8 (2–4)</td>
<td>2.8 (2–4)</td>
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<td>2.4 (2–3)</td>
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<tr>
<td></td>
<td>Control group</td>
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<td>3.8 (3–4)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control group</td>
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<td>0.4 (0–1)</td>
<td>1.2 (1–2)</td>
<td>3.6 (3–4)</td>
</tr>
</tbody>
</table>

\( ^a \)Statistically significant difference between the two groups by Kruskall-Wallis testing.
the difference was not statistically significant compared to controls. The histopathologic findings were in accordance with those of planimetry.

**Inflammatory response.** Inflammation scores were constantly lower in the experimental group; however, no statistically significantly differences were found at any time point, compared to those of the control group.

**Neoangiogenesis.** In the experimental group, a denser, newly-formed capillary network was noted on days 5 and 10, which became more mature by days 15 and 20, and was confirmed by the use of vascular endothelial growth factor stain and smooth muscle actin/Vimentin stain for endothelial cells and pre- and post-capillary vascular smooth muscle cells, respectively. Vascularization scores, although constantly higher in the experimental group compared to the controls, did not reach a statistically significant level at any time point.

**Collagen formation.** On day 5, the presence of several fibroblasts producing collagen bundles was evident in experimental group, but there were few fibroblasts producing col-

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**FIG. 2.** Photomicrographs of skin wounds at day 5 (vascular endothelial growth factor, 40×). (A) Control group. Immature endothelial cells can be seen in the newly formed capillaries (arrow). (B) Experimental group. A denser capillary network with more mature endothelial cells can be seen (arrows).

**FIG. 3.** Photomicrographs of skin wounds at day 10 (hematoxylin and eosin, 10×). (A) Control group. Slight epithelialization (gray arrow) and dense connective tissue (black arrow) can be seen. (B) Experimental group. Better epithelialization (gray arrow), and fibroblasts organized parallel to the surface of the wound (black arrow) can be seen.

lagen around the margins of the wounds in the controls. On day 10, the amount of collagen formation by fibroblasts was increased in both groups. On day 15, a moderate number of fibroblasts producing abundant bundles of collagen was found in both groups. Finally, on day 20, more mature fibroblasts creating a denser collagen layer beneath the epithelial layer were seen in the experimental group compared to the controls. However, no statistically significant differences were found between the two groups at any time point.

**Discussion**

Wound healing is a complex process, which involves several local and systemic tissue responses regulated by many different cellular and humoral factors.\textsuperscript{19–21} It normally proceeds in four overlapping phases: inflammation, granulation tissue formation, matrix formation, and remodeling.\textsuperscript{22} It is well known that low-power lasers, but also other light sources, can promote wound healing, and this is mainly attributable to the biomodulating effects of light energy. With regard to the mechanisms involved in biophotomodulation, several models have been suggested by the existing literature, such as stimulation of mitochondria, changes in the cell...
membrane due to light absorption, and the photophysical action of light on hydrogen bonds. All of these lead to a similar photobiological action, characterized by increased cell energy and activation of nucleic acid synthesis that are essential for wound healing, but each of these factors acts on the metabolic cascade at a different cellular level.6,23–28

In vitro and in vivo data demonstrate that phototherapy acts during all four healing phases, and has positive effects on each of them. During the inflammatory phase, cellular modulation regulates the local inflammatory response (proliferation and activation of lymphocytes and monocytes, and an increase in phagocytic capacity of neutrophils), and increases the release of growth factors, initiating the proliferative phase. In the second phase, neovascularization is enhanced by photostimulation of endothelial cells. Also, increased proliferation of fibroblasts and keratinocytes, as well as collagen synthesis and deposition contribute towards granulation tissue formation and epithelialization. During the phase of matrix formation and remodeling, phototherapy induces denser collagen deposits with better bundle orientation and maturation.14,29–38

As for the effects polarized light, the results of several studies support the fact that it has a beneficial effect on wound healing. This is primarily attributable to increased release of growth factors and cytokines, and enhanced neovascularization and blood flow to the wounded area and the surrounding skin. Nevertheless, the possible role of polarized light in phototherapy is not fully understood and further experiments are needed.7,8,14,18,24,34,39,40

With regard to the protocol of this study, the effect of visible and infrared light on secondary healing of full-thickness skin wounds was investigated in a rodent model. The light used was polarized and of low power, similar to that of the low-powered laser, in order to prevent thermal damage to regenerating tissues. But it was polychromatic and incoherent, since it combined visible light at 580–760 nm and infrared light at 760–3400 nm. This range of wavelength (580–3400 nm) was used in order to: (1) avoid the use of ultraviolet irradiation, which has some dangerous effects; (2) to benefit from the deeper tissue penetration of the yellow-orange-red fraction of visible light (580–760 nm); and (3) to use only near- to middle-infrared irradiation (760–1500 nm and 1500–3400 nm, respectively), thus avoiding the harmful effects of far-infrared irradiation (5600–10,000 nm).39–41

The use of polychromatic light also has certain benefits over the low-power laser, since it is relatively inexpensive, easier to use, safer, and able to irradiate a larger treatment area. In addition, the range of wavelengths used is wider, and can be easily changed at low cost through the use of filters placed over the light source. In addition to the light

![FIG. 4. Photomicrographs of skin wounds at day 15 (smooth muscle actin, 2X). (A) Control group. Slight epithelialization (gray arrow) and immature fibroblasts within abundant collagen bundles (black arrow) can be seen. (B) Experimental group. A moderate amount of surface epithelialization (gray arrow) and mature fibroblasts (black arrow) can be seen.](image1)

![FIG. 5. Photomicrographs of skin wounds at day 20 (Vimentin, 20X). (A) Control group. A completely epithelialized wound (arrow) is seen here. (B) Experimental group. A completely epithelialized wound with a layer of mature keratinocytes (arrow) is seen here.](image2)
source we used in our study, light-emitting diodes represent another source of polychromatic light, and their use in phototherapy is still in its infancy.44

Our results demonstrate that the visible and infrared light used in this study enhance wound healing as evidenced by statistically significantly accelerated epithelialization at days 10 and 15 in the light-treated animals as compared to the control group. Also, the quality and organization of the epithelium was significantly better for at the same time points, as evidenced by the histologic findings. One possible explanation for the faster and better epithelialization could be increased keratinocyte proliferation and migration due to a local or systemic effect of photomodulation. As for the other histological findings, although no statistically significant difference was found between the two groups, the results in the light-treated group were consistently better than those of the control animals, and a possible explanation could be limited light penetration into the tissues.34,45,46

Conclusion

To summarize, the results of the present study support a positive biomodulatory effect of the visible and infrared light used (580–3400 nm) on the healing process of full-thickness skin wounds through faster re-epithelialization. Nevertheless, further studies are warranted to define the optimal wavelength, dose, and direction of polarized polychromatic light to optimally improve wound healing, and to elucidate the mechanisms behind photomodulation, since the improvements seen in some of the histologic parameters we evaluated were not statistically significant.

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References


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