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Effect of LED Phototherapy of Three Distinct Wavelengths on Fibroblasts on Wound Healing: A Histological Study in a Rodent Model

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Abstract

Aim: The aim of the present investigation was to evaluate histologically fibroblastic proliferation on dorsal cutaneous wounds in a rodent model treated or not with light-emitting diodes (LEDs) of three wavelengths. ***Background:*** Fibroblasts secrete substances essential for wound healing. There are few reports of LED phototherapy on fibroblast proliferation, mainly in vivo. ***Animals and Methods:*** Following approval by the Animal Experimentation Committee of the School of Dentistry of the Federal University of Bahia, we obtained 16 young adult male Wistar rats weighing between 200 and 250 g. Under general anesthesia, one excisional wound was created on the dorsum of each animal; they were then randomly distributed into four groups of four animals each: G0, untreated control; G1, red LED (700 ± 20 nm, 15 mW, 10 J/cm^2); G2, green LED (530 ± 20 nm, 8 mW, 10 J/cm^2); and G3, blue LED (460 ± 20 nm, 22 mW, 10 J/cm^2). The irradiation started immediately after surgery and was repeated every other day for 7 days. Animals were killed 8 days after surgery. The specimens were removed, routinely processed to wax, cut, and stained with hematoxylin/eosin (HE). Fibroblasts were scored by measuring the percentage of these cells occupying the area corresponding to wound healing on stained sections. ***Results:*** The quantitative results showed that red LED (700 ± 20 nm) and green LED (530 ± 20 nm) showed a significant increase in fibroblast numbers ($p < 0.01$ and $p = 0.02$) when compared with the control group. ***Conclusion:*** The use of green and red LED light is effective in increasing fibroblastic proliferation on rodents.

Introduction

Wound repair is a complex process involving several cellular and biochemical responses that may be affected by several local and/or systemic factors. One main reaction of the healing tissue is fibroblastic activity, proliferation, and secretion.[1] The granulation tissue present at early stages of the healing process will mature and progress to scar tissue. Fibroblasts will secrete several substances, including glycosaminoglycans and collagens, that are essential for the formation of the extracellular matrix and for its density.[1–3] Despite being essentially the same for different wounds, the pattern of healing may change for both intrinsic and/or extrinsic factors. These factors include age, gender, hormonal factors, nutritional deficiencies, the use of drugs, and some diseases.[2,4–6]

Several therapeutic approaches have been used to improve healing; these include the use of stem cells,[7–9] growth factors,[10] drugs,[5] hyperbaric oxygen,[11] and phototherapies.[11–16] In recent years, several reports on the benefits of using LEDs operating at several wavelengths, both in vitro and in vivo, for both normal and pathologic conditions have been published,[17–27] as well as for facial rejuvenation.[28,29] LED technology has been shown to be an effective alternative for the treatment of both cutaneous and mucosal wounds.[11]

Despite encouraging reports found in the literature, it is essential that controlled studies be carried out to establish adequate and effective clinical protocols.[19,24,30] The present study aimed to assess histologically the effects of LED phototherapy (LedPT) of different wavelengths on the fibroblasts of cutaneous wounds on a rodent model.

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Table 1. Summary of the Parameters Used in the Study

Parameters	Groups		
	G1 LED	G2 LED	G3 LED
Wavelength (nm)	700 ± 20	530 ± 20	460 ± 20
SAEF (J/cm^2)		10	
Output (mW)	15	8	22
Illuminated area (cm^2)		1	
Spot (cm^2)	2.01	2.01	2.01
Intensity (mW/cm^2)	7.46	3.98	10.94
Time (per session)	11 min 08 sec	20 min 50 sec	7 min 36 sec
SAEF (treatment) (J/cm^2)		40	

SAEF, spatial average energy fluency.

Table 2. Criteria Used for Fibroblast Measurement

	Discrete	Moderate	Intense
Fibroblastic proliferation	Presence of <25% of fibroblasts	Presence of <50% of fibroblasts	Presence of >50% of fibroblasts

Animals and Methods

Following approval by the Animal Experimentation Ethics Committee of the School of Dentistry of the Federal University of Bahia, 16 adult male Wistar rats weighing 20–250 g, obtained from the Centro de Criação de Animais da Faculdade de Medicina Veterinária da Universidade Federal da Bahia, were kept at the Animal Experimentation Laboratory of the School of Dentistry of the Federal University of Bahia. The animals were held in individual plastic cages bedded with wood chips and maintained at 22°C in a day–night light cycle. The animals were fed with standard pelted laboratory diet and had water ad libidum. After regular quarantine, under intraperitoneal general anesthesia (GA) (60 mg/kg of ketamine (Coopazine®) and 10 mg/kg of xylazin (Virbaxil™), the dorsum of each animal was shaved and cleaned. A 1- by 1-cm cutaneous wound was created with a scalpel on the dorsum of each animal. The wound was left without suturing or dressings. The animals were randomly distributed into four groups with four animals in each: G0, control; G1, LED 700 ± 20 nm (15 mW, diameter ~16 mm); G2, LED 530 ± 20 nm (8 mW, diameter ~16 mm); and G3, LED 460 ± 20 nm (22 mW, diameter ~16 mm).

LED phototherapy used prototype LED devices, started immediately after surgery, and was repeated at every other day for 7 days. The spatial average energy fluency (SAEF) per session was $10\,J/cm^2$ for all groups (Table 1). LED light was applied over the wounded area. The time of application varied according to the equipment used and was automatically set. Because of the lack of previous studies using the chosen model, the choice of wavelengths was made based on the effects of the treatment parameters chosen, since different wavelengths will have different absorption and penetration.

If an animal presented any evidence of pain, a nonsteroidal analgesic would be used. But this was not the case in any group. Following macroscopic examination, each animal was killed by an overdose of general anesthetic at day 8 after surgery. Specimens were taken and kept in 10% formalin for 24 h. They were then routinely cut and processed to wax, stained with HE, and underwent histological analysis carried out by an experienced pathologist in a double-blind manner. One slide was made from each specimen and the whole fragment was analyzed. The criteria used for this analysis are shown in Table 2. Each criterion was scored as discrete, moderate, or intense according to the percentage of the phenomena observed. The percentage of occurrence of each score was analyzed using Minitab15®software (Globaltech, BeloHorizonte, Brazil) using the Kruskal–Wallis test. The significance level was 5%.

Results

The histological analysis of the specimens showed that on control specimens showed a discrete number of fibroblasts in 50% of the cases. These cells were dispersed on a discrete amount of granulation tissue (Fig. 2). On 700-nm LED-irradiated subjects, there was a marked amount of young fibroblasts located parallel to the surface (Fig. 3). When illuminated with 530 nm, a marked amount of fibroblasts was observed on 75% of the cases, and they were parallel to the wound surface (Fig. 4). On 460-nm illuminated subjects, a marked amount of fibroblasts was seen on 50%, and this was discretely organized on the dermis of the wound (Fig. 5). Statistical analysis showed that the amount of fibroblast on the subjects illuminated with a 700- or 530-nm LED was significantly higher than for the controls ($p < 0.01$ and $p = 0.02$, respectively). The group LED 460 ± 20 nm showed no significant increase in fibroblast proliferation when compared with the control (Fig. 1).

Discussion

The results of the present study showed that LedPT (700 ± 20 nm/530 ± 20 nm) was able to stimulate fibroblastic proliferation. Many in vitro and in vivo studies have shown that the use of LEDs (630–1000 nm) has positive biomodulatory effects on the expression of genes coding for improved wound healing in diabetic mice,[31] on rat-derived osteoblasts, rat-derived skeletal muscle cells and human

Table 3. Summary of the Findings Observed in the Present Investigation

	Control (a)	LED λ700 nm (b)	LED λ530 nm (c)	LED λ470 nm (d)
Fibroblastic proliferation[*ab,*ac]	50%	0%	0%	25%
	25%	0%	25%	25%
	25%	100%	75%	50%

*$p < 0.005$.
[a—d]Group with significant intergroup differences. Percents are, respectively, discrete, moderate, and intense.

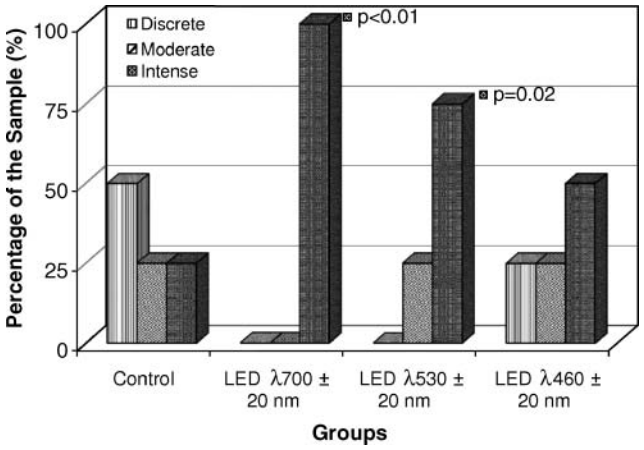

FIG. 1. Comparison between control and LEDs regarding the amount of fibroblasts.

epithelial cells,[11] on angiogenesis,[30] on the oxidative metabolism of the mitochondria in vitro,[19] and on both the prevention and treatment of both oral mucosites[22,32] and radiation-induced dermatitis.[33]

In accordance with the positive results for red LedPT in this study, previous reports on the effect of red LedPT (625 to 635 nm, 1 J, 3 mW/cm^2) on fibroblasts showed in vitro maintenance of viability and an increase in collagen synthesis on illuminated samples.[34] A previous report[11] showed increased mouse-derived fibroblastic proliferation in vitro following illumination with a combination of LEDs (670, 728, and 880 nm, 4/8 J/cm^2, 50 mW/cm^2). The authors also found that the stimulation occurred in the growth phase but not n the stationary phase, and these findings may indicate that red LED light does not cause excessive cell proliferation or malignant transformation. It has been demonstrated that red LED irradiation (628 nm, 0.88 J/cm^2, daily irradiation, 3 days) was able to stimulate human fibroblast proliferation in vitro by regulating the expression of several genes involved in cell proliferation and the suppression of apoptosis.[35]

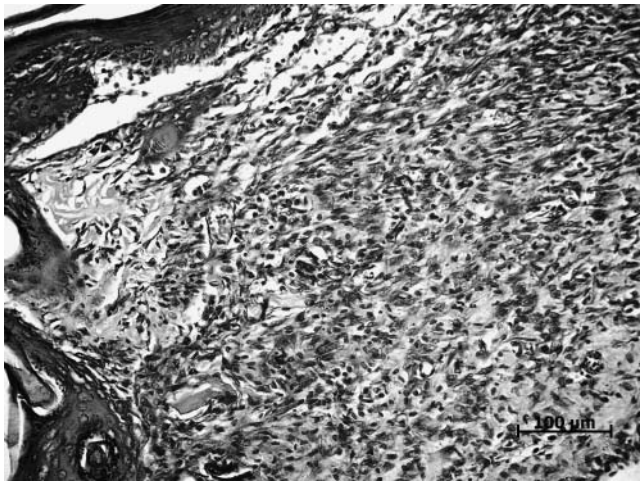

FIG. 2. Photomicrograph of control specimen showing young fibroblasts, collagen fibers, and congested blood vessels. Epithelium was keratinized and atrophic. HE, ×20.

FIG. 3. Photomicrograph of specimen irradiated with red LED showing the wound covered by orthokeratinized and atrophic epithelium. The dermis shows large numbers of young fusiform fibroblasts parallel to the wound surface. Congested blood vessels may also be seen. HE, ×20.

There are a few studies in the literature using LedPT with wavelengths other than red and infrared (IR). In vitro study has demonstrated stimulation of the fibroblastic proliferation using green LED on both conventional medium (570 nm, 0.1 J/cm^2, 10 mW, 3 min, 24-h interval, 3 days)[23] and on hyperglycemic medium.[24] Our findings in vivo confirm these findings, since green LED (530 ± 20 nm) illumination was able to increase fibroblast proliferation.

Previous studies using 400- to 500-nm wavelengths demonstrated that these may positively affect tissues due to high absorption by flavins, mainly dehydrogenases, while wavelengths higher than 500 nm are better absorbed by the cytochrome c oxidase.[36–38] Lewis and colleagues[39] reported that blue-light irradiation (380–500 nm) may either enhance or suppress mitochondrial succinate dehydrogenase activity

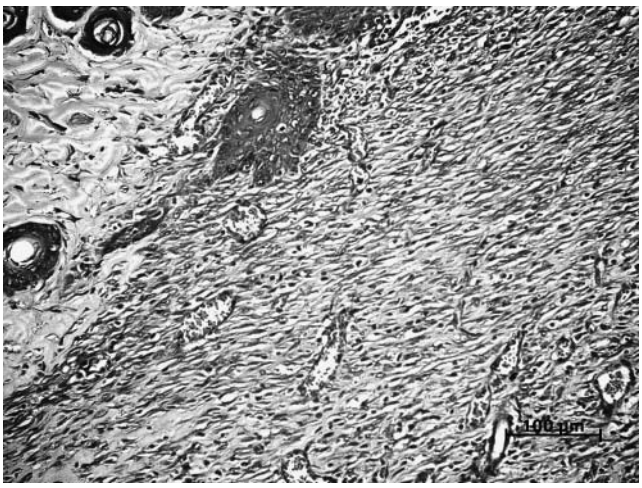

FIG. 4. Photomicrograph of specimen irradiated with green LED showing marked number of fibroblasts parallel to the wound surface at the dermis, tortuous and congested blood vessels, and a small number of mono- and polymorphnuclear leukocytes. HE, ×20.

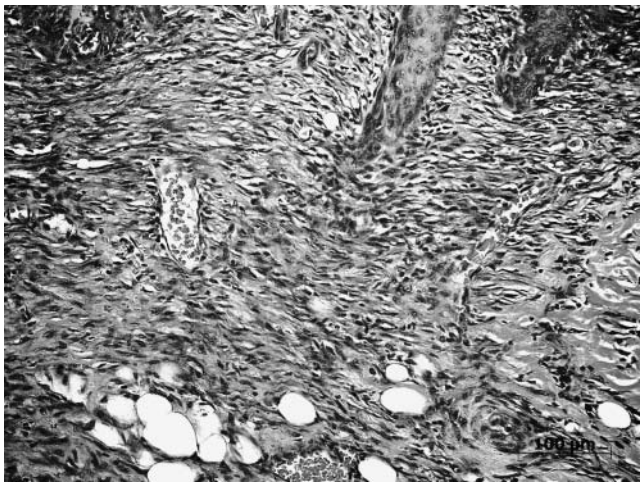

FIG. 5. Photomicrograph of specimen irradiated with blue LED showing the wound covered by keratinized epithelium. The dermis shows numerous fibroblasts parallel to the surface, 18 collagen, and congested bloodvessels; a few adipocytes vessels may also be seen. HE, ×20.

depending on the cell type irradiated; they also showed an increased production of reactive oxygen species (ROS) in different levels when compared with distinct cultured cell type responses. ROS is known to induce oxidative stress, which is prejudicial to cellular activity. On other hand, there is evidence that blue LED light irradiation (470 nm, 1.8 mW/cm^2) is effective in promoting neurite outgrowth of PC12 cells in vitro, whereas neurite outgrowth was slightly suppressed under 455, 525, 600, 630, 880, and 945 nm of LED irradiation. Light irradiation of 470 nm may cause specific response to transduction signals in PC12 cells, although the underlying mechanism is not clear.[26] However, the current study showed that fibroblast proliferation was not increased by blue LedPT (460 nm) when using the specified parameters.

Controversy exists over which properties of light are responsible for the effect on tissue. Coherence was the first accounted for property. However, over the past years, properties such as wavelength, which are specific to a particular photoreceptor, have been accounted for as important factors for the final response to treatment. Another important observation was that coherence is mostly lost during light propagation within the tissue.[40]

Another property also considered to play a role in tissue response is monochromaticity. LED light also possesses monochromaticity, and this may cause the same observed responses on tissues as the response seen when laser light is used, even considering the spectral band present on LEDs.[11,28,34] It has been suggested that high monochromaticity is not necessary to achieve positive results, but it is essential that the spectral band be on the absorption spectrum of the photoreceptor.[40]

Another important aspect to consider is that our findings were based on superficial wounds, and these may not occur deeper in the tissue. Although coherence may be lost at the first tissue layers,[41] coherent and noncoherent light differ in their penetration and distribution within the tissue. However, there is no evidence that this aspect affects tissue responses.[42,43]

The mechanism of the action of LedPT on biological systems remains unclear. However, there is evidence that LED light used at the same wavelengths as lasers has a similar biochemical effect. It seems that the basic mechanism of interaction between LED light of 630–1000 nm and the tissues involves the mitochondria and that the cytochrome c oxidase present at the cell membrane is responsible for absorption of the light, thus increasing cellular adenosine triphosphate (ATP) production.[44–47] A previous study has suggested that macrophages are the first target of noncoherent 660-nm light.[48] Other studies have also suggested the involvement of genomic expression, which may regulate positively or negatively the response, as well as the arrangement of the cascade of the signaling pathway of the cells.[28,31] The choice of the parameters for LedPT may be vital for determination of the pattern of genomic activity.[28]

Sommer and colleagues[49] showed the relationship among the energy density, irradiation time, and power density and that the latter has to be higher than the minimal threshold necessary for laser LPT. The authors also assume that the threshold for the power density is wavelength dependent. Further studies are necessary to determine the therapeutic windows for LedPT.

It is concluded that LedPT at 700 ± 20 or 530 ± 20 nm presented a positive biomodulatory effect on fibroblastic proliferation.

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Author Disclosure Statement

No competing financial interests exist.

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