

Evaluation of the Effects of Polarized Light (λ 400–200 nm) on the Healing of Third-Degree Burns in Induced Diabetic and Nondiabetic Rats*

Priscila Chagas Oliveira, Ph.D.,¹ Antônio Luiz Barbosa Pinheiro, Ph.D.,^{1,2,3} Isabele C. V. de Castro, D.D.S.,¹ João Alves Reis Junior, D.D.S.,¹ Manuela Pimentel Noia, M.Sci.,¹ Clarissa Gurgel, D.D.S.,⁴ Maria Cristina Teixeira Cangussú, Ph.D.,⁵ and Luciana Maria Pedreira Ramalho, Ph.D.^{1,2,6}

Abstract

Objective: The study evaluated, by light microscopy, the repair process on third-degree burns on diabetic and nondiabetic rats, illuminated or not, with a polarized light (PL) source. **Background data:** Burns are severe injuries that result in the loss of fluid and destruction of tissue, infection, and shock that may result in death. Diabetes mellitus is a public health problem that, being uncontrolled, causes severe disturbance to the body metabolism, including on wound healing. PL sources have been shown to be effective in improving healing in many situations. **Materials and methods:** Ninety male Wistar rats were divided into two groups ($n=45$): non-diabetic and diabetic. In one of the groups, diabetes mellitus was induced by streptozotocin. A third-degree burn, measuring $1.5 \times 1.5 \text{ cm}^2$, was created in the dorsum of each animal. Phototherapy (λ 400–2000 nm, 10.2 or 20.4 J/cm^2) started immediately after burning and was repeated daily until animal death (7, 14, and 21 days). Specimens were taken, processed, and stained with H&E and Sirius red and immunomarked with cytokeratin (CK) AE1/AE3. Descriptive analysis was performed by light microscopy. **Results:** Animals subjected to phototherapy showed an acceleration of the repair, the dose of 10.2 J/cm^2 being the one that caused best results, including higher deposition of collagen, quicker inflammatory reaction, and improved revascularization. **Conclusions:** Our results suggest that the use of PL (10.2 J/cm^2) improves the healing of third-degree burns on both diabetic and nondiabetic animals.

Introduction

BURNS ARE SEVERE INJURIES that may result in the loss of tissue fluids, tissue destruction, infection, and shock that may ultimately result in death. Severe social and psychological impairment are also seen in most burn victims.^{1–4} Wound healing is an integral part of recovery of critically ill patients. They often are at risk for impaired healing because of their compromised body systems and multiple risk factors including infection, diabetes, immunosuppression, obesity, and malnutrition.³

The loss of glycemic control causes impaired wound healing as a result of several factors, primarily related to defects in the inflammatory response because of impaired

granulocytic function and chemotaxis.⁵ Other abnormalities associated with wound healing of diabetic individuals include impaired neovascularization, a decreased synthesis of collagen, increased levels of proteinases, and defective macrophage function. However, the exact mechanisms for impaired healing of wounds in diabetics are still poorly understood.^{6–8}

Alternative light sources have been used to improve wound healing, and previous reports suggested that polarization is the characteristic of laser light responsible for the biomodulation; because of that, other polarized light (PL) sources may also biomodulate biological systems.⁹

The Bioptron[®] lamp (Biptron, Wollenrau, Switzerland) shows 95% polarization, is polychromatic and incoherent,

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¹Center of Biophotonics, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil.

²National Institute of Optics and Photonics, Physics Institute, São Paulo University, São Carlos, São Paulo, Brazil.

³Biomedical Engineering, Universidade Camilo Castelo Branco, São José dos Campos, São Paulo, Brazil.

⁴Laboratory of Surgical Pathology, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil.

⁵Oral Epidemiology and Community Public Health, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil.

⁶Oral Medicine, School of Dentistry, Federal University of Bahia, Salvador, Bahia, Brazil.

and works at low intensities. Previous reports have shown positive effects of this light source on quickening wound repair and healing.^{10,11}

The incoherent light emitting PL is able to induce biostimulative effects in living cells similar to low-level lasers. As the Bioptron® lamp combines visible light at 440–700 nm and infrared (IR) light at 1700–2000 nm, it is a low-power light source such as low-level laser, but it is polychromatic and incoherent.¹²

Several mechanisms are responsible for the stimulating effects of both parts of the electromagnetic spectrum present in these polychromatic light sources. These lead to the same final photoresponse, but start the cascade of metabolic events at different cellular levels. One of the main effects of the absorption of visible light is the stimulation of the mitochondria, which results in increased cell energy and activation of the nucleic acid synthesis, essential for wound repair. On the other hand, the use of IR light also has similar results, being the process initiated by a response at the membrane level. Biomodulation may be then influenced by a common characteristic of polarization of both types of light.¹¹

A linearly PL has a particular effect on the bilipid layer of the cellular membrane as the polarized ends of lipids tend to rotate toward the electrical source, changing its structure. Transference of energy from the lipids to proteins and consequent reorganization of the cellular membrane occurs because of a closer contact. These aspects interfere with all membrane-regulated processes.¹³ Tada et al.¹⁴ in study that compared different types of polarization of light using light-emitting diode (LED), showed that the linearly PL and light circularly polarized promoted a significant stimulation of the fibroblast cells with reduced wound area and increased the expression of type 1 procollagen mRNA.

A recent study¹⁵ assessed the effect of a linearly polarized polychromatic light on the treatment of second-degree burns

on rodents. Clinical assessment showed less hyperemia and edema and smaller burn size on illuminated subjects in comparison to their controls. Histological analysis evidenced better re-epithelialization and angiogenesis on illuminated subjects. Ouvir

The present study aimed to assess the effects of a PL source on the healing process of third-degree burns on diabetic and nondiabetic rodents treated with 10.2 or 20.4 J/cm².

Materials and Methods

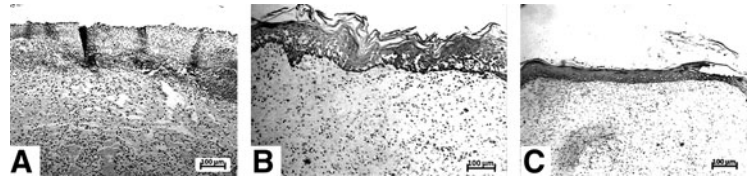
Following approval by the Animal Experimentation Ethics Committee of the School of Dentistry of the Federal University of Bahia (Protocol number 013/06) 90 young adult male Wistar rats weighing 200–230 g were obtained from the Animal House of the School of Veterinary Medicine of the Federal University of Bahia, and were kept at the Animal Experimentation Laboratory of the School of Dentistry of the Federal University of Bahia during the experimental time. The animals were kept in individual plastic cages lined with wood chips and maintained at 22°C in a 12 h day/night light cycle. The animals were fed a standard laboratory pelleted diet and had water available *ad libitum*.

We used 90 animals that were divided into 2 main groups (nondiabetic and diabetic) that were further subdivided into 18 subgroups with 5 animals each. In 1 of the main groups, the animals were kept unfed for 12 h and then injected with streptozotocin (Sigma Aldrich, St. Louis, MO) diluted in citrate buffer (0.1M, pH 4.5, 60 mg/kg) for induction of diabetes mellitus.¹⁶ Forty-eight hours after injection, the blood sugar level was measured and only animals with blood sugar levels of ≥ 350 mg/100ml entered the study.¹⁶ Under intraperitoneal general anesthesia (0.12 ml/100 g of ketamine and 0.06 ml/100 g of xylazine) the animals of both groups had their dorsum shaven and cleaned. While anesthetized, a

TABLE 1. CRITERIA USED FOR LIGHT MICROSCOPY ANALYSIS

Criterion		Score		
Inflammation	Acute	Discrete: Presence of <25% neutrophils of all cells present on the field	Moderate: Presence of <25–50% neutrophils of all cells present on the field	Intense: Presence of >50% neutrophils of all cells present on the field
	Chronic	Discrete: Presence of <25% chronic inflammatory cells on the field	Moderate: Presence of <25–50% chronic inflammatory cells present on the field	Intense: Presence of >50% chronic inflammatory cells present on the field
	Mixed	Discrete: Presence of <25% chronic inflammatory cells and neutrophils on the field	Moderate: Presence of <25–50% chronic inflammatory cells and neutrophils present on the field	Intense: Presence of >50% chronic inflammatory cells and neutrophils present on the field
Re-epithelialization	Absence		Discrete: Covering of 1–25% of the wound Moderate: Covering of 25–50% of the wound Intense: Covering of 50–100% of the wound	
Amount of fibroblasts		Discrete: Presence of <25% of fibroblasts among other cells	Moderate: Presence of <25–50% of fibroblasts among other cells	Intense: Presence of >50% of fibroblasts among other cells
Amount of collagen fibers		Discrete: Sirius red staining is less intense than that seen on the healthy adjacent tissue	Moderate: Sirius red staining is similar to that seen on the healthy adjacent tissue	Intense: Sirius red staining is more intense than that seen on the healthy adjacent tissue
Neoangiogenesis		Discrete: Amount smaller than that seen on healthy adjacent tissue	Moderate: Amount similar to Intense: More than that seen on healthy adjacent tissue	Intense: More than that seen on healthy adjacent tissue

FIG. 1. Photomicrograph of a specimen from the nondiabetic group. (A) control; (B) 10.2J/cm²; and (C) 20.4J/cm² on the 14th day post-burn. Re-epithelialization was not seen in the control group. Complete coverage by epithelium could be seen in specimens illuminated with 10.2 and 20.4J/cm² (CK AE1/AE3, 100 μm).



specially designed instrument measuring 1.5×1.5 cm² was heated until red and incandescent and then applied to the skin for 20 sec to induce formation of a third-degree burn. The successful creation of the burn was both microscopically and clinically confirmed.

For phototherapy (Biopton®; 400–2000 nm, 0.04 W/cm², round irradiation area = 23.7 cm², 0.95 W, 255 or 510 s, 10.2 or 20.4 J/cm², Biopton AG, Monchaltorf, Switzerland), the animals of each group were divided subgroups (*n* = 15) as follows: G1: untreated nondiabetic control; G2: nondiabetic treated with PL dose 10.2 J/cm²; G3: nondiabetic treated with PL dose 20.4 J/cm²; G4: untreated diabetic control; G5: diabetic treated with PL dose 10.2 J/cm²; G6: diabetic treated with PL dose 20.4 J/cm².

The phototherapy started immediately post-burning and was repeated daily until the day before animal death. The energy was applied transcutaneously respecting the focal distance of 10 cm as recommended by the manufacturer. The dose was 10.2 or 20.4 J/cm² (255 or 510 s). The measurement of local temperature was not performed as, according to the manufacturer, temperature changes caused by the use of the device do not occur when it is used following its specifications.

At each time point chosen (7, 14, and 21 days post-burning) and following macroscopic examination, each animal was killed by an overdose of general anesthetic. Specimens were taken and kept in 10% formalin during 24 h, were then routinely cut and processed to wax, cut (5 μm) and stained with H&E and Sirius red and immunomarked with CK AE1/AE3 (Dako Cytomation® + streptavidin-biotin-peroxidase, 1:200, Dako do Brasil, São Paulo, São Paulo, Brazil) antibody and underwent histological analysis performed by an experienced pathologist in a double-blind manner. Three slides were made from each specimen and the whole area of the burn was analyzed. The criteria used on this analysis used are shown in Table 1 and had been used previously.^{16,17}

Results

Seventh day

Nondiabetic. Control specimens showed areas of coagulation necrosis and edema. The inflammatory reaction was

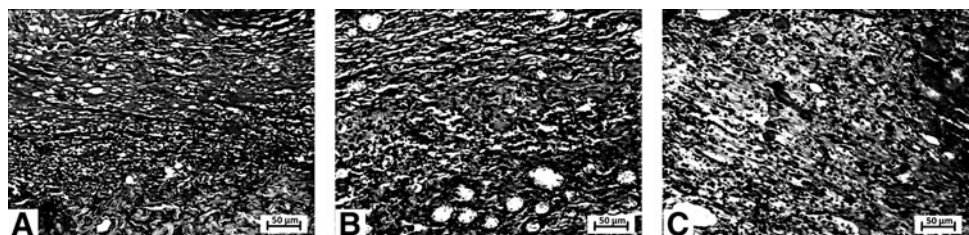
chronic and discrete. Re-epithelialization was found discrete in 80% of the control group. On 80% of the subjects illuminated with 10.2 J/cm² and on all subjects illuminated with 20.4 J/cm² no re-epithelialization was detected at this stage. Fibroblastic proliferation was moderate on 60% of control specimens. Discrete fibroblastic proliferation was seen on 80% of the cases illuminated with 10.2 J/cm² and on 60% of the ones treated with 20.4 J/cm². Collagen deposition was graded as discrete on 80% of both controls and subjects illuminated with 10.2 J/cm², and on 60% of subjects illuminated with 20.4 J/cm². Angiogenesis was found discrete on 60% of both control and illuminated subjects (10.2 J/cm²). Forty percent of subjects in these groups showed a moderate angiogenesis. This parameter was graded as discrete in 100% of subjects illuminated with 20.4 J/cm².

Diabetic. All specimens showed the presence of necrotic skin fragments extending down to the muscle. Most control (80%) and illuminated subjects (60%) (20.4 J/cm²) showed chronic inflammation at this time. Subjects illuminated with 10.2 J/cm² showed variable inflammatory infiltrate: acute, (20%), chronic (40%), or mixed (40%). Angiogenesis and fibroblast number were significantly lower in subjects illuminated with 20.4 J/cm² when compared to both control and subjects illuminated with 10.2 J/cm². Controls and specimens from subjects illuminated with 10.2 J/cm² were mostly similar in regard to re-epithelialization, which was mostly discrete. Subjects illuminated with 20.4 J/cm² did not show evidence of re-epithelialization in most of the cases.

Fourteenth day

Nondiabetic. The inflammatory reaction at this time varied greatly. Control subjects showed chronic inflammation in 60% of the cases and mixed in the other 40%. Subjects illuminated with 10.2 J/cm², in 80% of the cases, showed a discrete chronic inflammatory reaction. On the group illuminated with 20.4 J/cm², inflammation was acute in 20% of the cases and chronic in 80% of the cases. Re-epithelialization was not seen on control group. Subjects illuminated with 10.2 J/cm² showed a variation: intense in 40% of the cases, moderate in 20%, discrete in 20%, and absent in 20%. In the

FIG. 2. Photomicrograph of a specimen from the diabetic group. (A) control; (B) 10.2 J/cm²; and (C) 20.4 J/cm² on the 14th day post-burn. Few blood vessels could be seen in the control. Both illuminated groups showed moderate to intense angiogenesis with congested blood vessels (H&E, 50 μm).



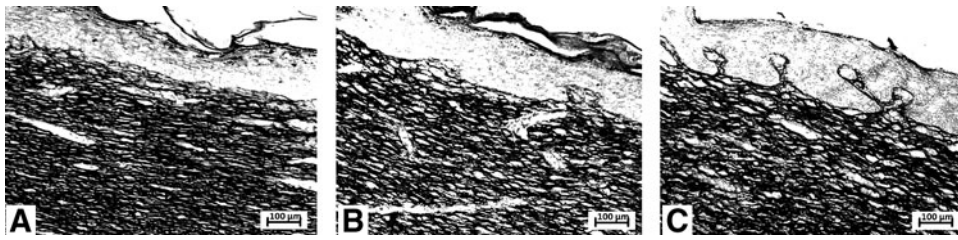


FIG. 3. Photomicrograph of a specimen from the nondiabetic group. (A) control; (B) 10.2 J/cm²; and (C) 20.4 J/cm² on the 21st day post-burn. The specimens from both groups show intense deposition of mature collagen fibers parallel to the wound surface (Sirius red, 100 µm).

group illuminated with 20.4 J/cm², there was an absence of re-epithelialization in 40% of the specimens, moderate re-epithelialization in 20% and intense re-epithelialization in 40% (Fig. 1). Fibroblast proliferation was moderate in 100% of controls and in 60% of both groups that were illuminated. Collagen deposition was intense in 80% of the control specimens; moderate in 60% and intense in 40% of the subjects illuminated with 10.2 J/cm²; in specimens of the group illuminated with 20.4 J/cm² it was discrete in 60% and intense in 40%. Angiogenesis was moderate in all controls, discrete in 80% of subjects treated with 10.2 J/cm², and varied from discrete (40%), to moderate (20%) or intense (40%) in subjects illuminated with 20.4 J/cm².

Diabetic. Fourteen days after burning, 60% of control specimens showed evidence of a mixed moderate inflammatory infiltrate. Illumination (10.2 J/cm²) caused wide variation in the inflammatory reaction: acute (20%), chronic (40%), or mixed (40%). Subjects illuminated with 20.4 J/cm² showed a moderate or intense chronic inflammatory reaction in 80% of the cases. These specimens also showed significantly less crusting than the controls. Both illuminated groups showed a significant increase in angiogenesis when compared to their controls (Fig. 2). Specimens illuminated with 20.4 J/cm² showed a significant increase in the number of fibroblasts when compared to both control and 10.2 J/cm² groups. Re-epithelialization was more evident on control specimens than on subjects illuminated with 10.2 J/cm². Collagen deposition was significantly higher (increased density) on both control and 10.2 J/cm² illuminated subjects when compared to the ones illuminated with 20.4 J/cm².

Twenty-first day

Nondiabetic. Inflammation was mostly chronic and discrete in 80% of controls and in 100% of the illuminated with 10.2 J/cm²; on subjects treated with 20.4 J/cm² the inflammation was moderate in 100% of the cases. Re-epithelialization was intense in 100% of the controls and those illuminated

with 10.2 J/cm², and in 60% of those illuminated with 20.4 J/cm². Fibroblastic proliferation was moderate in all the controls and in subjects illuminated with 20.4 J/cm², and in 80% of those illuminated with 10.2 J/cm². Collagen deposition was intense in 100% of the controls and 10.2 J/cm² illuminated subjects and in 80% of subjects illuminated with 20.4 J/cm² (Fig. 3). Angiogenesis was discrete in all control specimens, and moderate in 60% of the subjects treated with 10.2 J/cm² and in 80% of subjects illuminated with 20.4 J/cm².

Diabetic. At the end of the observation time, abscess and edema were observed on two specimens illuminated with 20.4 J/cm². Control specimens showed intense mixed inflammatory reaction in 60% of the animals. Illuminated subjects showed a predominantly lymphoplasmocitary inflammatory infiltrate, this being observed in 60% of the subjects illuminated with 10.2 J/cm² and 80% of those illuminated with 20.4 J/cm². Sixty percent of control specimens showed a discrete number of fibroblasts at this stage. In 60% of the subjects illuminated with 20.4 J/cm² there was a moderate number of these cells, and on 60% of the specimens illuminated with 10.2 J/cm², the number of fibroblasts was marked and these cells were placed parallel to the surface. Specimens illuminated with 20.4 J/cm² showed significantly higher angiogenesis and collagen deposition when compared to their controls (Fig. 4). Most of subjects (60%) illuminated with 10.2 J/cm² showed complete epithelial pavingmenting; only one subject illuminated with 20.4 J/cm² showed complete pavingmenting. A summary of the results is presented in Table 2.

Discussion

Despite important advances in the understanding of the healing of soft tissue lesions, burns remain an important cause of high morbidity and mortality, and these are further compromised when patients also have metabolic disturbances capable of interfering with the healing process. Increased morbidity causes impairment in the performance of daily activities, including working.¹⁷⁻²⁰

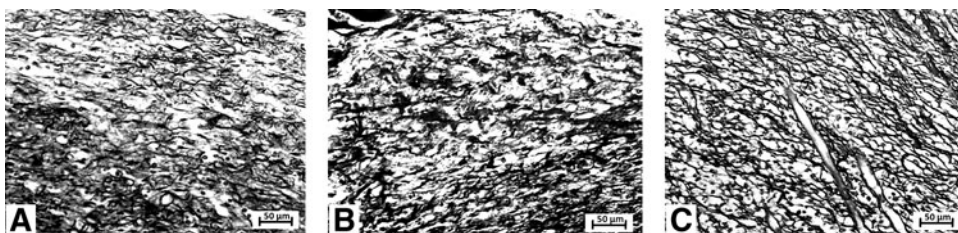


FIG. 4. Photomicrograph of a specimen from the diabetic group (A) control; (B) 10.2 J/cm²; and (C) 20.4 J/cm² on the 21st day post-burn. Discrete deposition of not-well-organized mature collagen matrix fibers could be seen in the control and specimens illuminated with

10.2 J/cm²; the 20.4 J/cm² illuminated specimens show moderate deposition of collagen and are better organized (Sirius red, 50 µm).

TABLE 2. SUMMARY OF THE RESULTS: NONDIABETIC ANIMALS

Group	7 days			14 days			21 days		
	Control	10.2 J/cm ²	20.4 J/cm ²	Control	10.2 J/cm ²	20.4 J/cm ²	Control	10.2 J/cm ²	20.4 J/cm ²
Inflammation	80%Discrete 20%Moderate	100% Discrete	60%Discrete	40%Discrete 20%Moderate	80%Discrete 20%Moderate	20%Moderate 20%Discrete 40%Moderate 20%Intense	80%Discrete 20%Moderate	100%Discrete	100%Moderate
Chronic									
Mixed									
Re-epithelialization	20% Absent 80%Discrete	80% Absent 20%Discrete	100% Absent	100% Absent	20% Absent 20%Discrete 20%Moderate 40%Intense	40% Absent 20%Moderate 40%Intense	100%Intense	100%Intense	20% Absent 20%Moderate 60%Intense
Amount of fibroblasts	40%Discrete 60%Moderate	80%Discrete 20%Moderate	60%Discrete 40%Moderate	100%Moderate	40%Discrete 60%Moderate	40%Discrete 60%Moderate	100%Moderate	80%Moderate 20%Intense	100%Moderate
Amount of collagen fibers	80%Discrete 20%Moderate	80%Discrete 20%Moderate	60%Discrete 40%Moderate	20%Moderate 80%Intense	60%Moderate 40%Intense	60%Discrete 40%Intense	100%Intense	100%Intense	20%Moderate 80%Intense
Neovascularization	60%Discrete 40%Moderate	60%Discrete 40%Moderate	100%Discrete	100%Moderate	80%Discrete 20%Moderate	40%Discrete 20%Moderate 40%Intense	100%Discrete	20% Discrete 60%Moderate 20%Intense	80%Moderate 20%Intense

DIABETIC ANIMALS									
Group	7 days			14 days			21 days		
	Control	10.2 J/cm ²	20.4 J/cm ²	Control	10.2 J/cm ²	20.4 J/cm ²	Control	10.2 J/cm ²	20.4 J/cm ²
Inflammation	20%Discrete 60%Moderate	20%Discrete 40% Discrete	20%Discrete 60%Discrete	20%Intense 20%Discrete	20%Discrete 20%Moderate	40%Moderate 40% Intense	20%Discrete 20%Moderate	20%Discrete 20%Moderate 20%Intense	20%Moderate 20%Discrete 60%Moderate
Chronic									
Mixed									
Re-epithelialization	40% Absent 60%Discrete	20% Absent 60%Discrete 20%Moderate	60% Absent 40%Discrete	20% Absent 80%Discrete	80% Absent 20%Discrete	60% Absent 40%Discrete	60%Discrete 20%Moderate 40%Intense	20% Absent 20%Discrete 60%Intense	80%Discrete 20%Intense
Amount of fibroblasts	20%Discrete 60%Moderate 20%Intense	60%Discrete 40%Moderate	80%Discrete 20%Moderate	80%Discrete 20%Moderate	20%Discrete 80%Moderate	20%Moderate 80%Intense	60%Discrete 40%Intense	20% Absent 20%Discrete 20%Discrete 20%Moderate 60%Intense	80%Discrete 20%Intense 60%Moderate 40% Intense
Amount of collagen fibers	40%Discrete 60%Moderate	60%Discrete 40%Moderate	80%Discrete 20%Moderate	80%Moderate 20% Intense	20%Discrete 80%Moderate	80%Discrete 20%Moderate	60%Discrete 20%Moderate 20%Intense	60%Discrete 40%Moderate 40%Moderate 60%Moderate 40%Intense	20% Discrete 80%Moderate
Neovascularization	60%Moderate 40%Intense	60%Moderate 40%Moderate 40% Intense	20%Discrete 40%Moderate	80%Discrete 20%Intense	20%Discrete 60%Moderate 20%ntense	60%Moderate 40%Intense	100%Moderate 20%Intense	60%Moderate 40%Intense	20%Moderate 80%Intense

Several reports have shown the efficacy of PL on quickening wound healing.²¹⁻²³ However, only a few of these associated this light source with systemic diseases such as uncontrolled diabetes. Because of the lack of a consensus on both action and protocol in the use of light therapies on burns, we opted to use a very severe model of wounding, a third-degree burn on subjects with uncontrolled diabetes. We used a similar treatment protocol to one used previously.^{21,23,24} The choice of using different doses was because of the lack of an agreement on the ideal protocol for treating such lesions with light sources.

One major advantage of using this PL source is its multi-wavelength emission and the association of both visible (λ 480–700 nm) and IR (λ 700–2000 nm) wavelengths. These wavelengths are known to be either highly absorbed or to have increased penetration on the tissue. These wavelengths are known to increase cell activity and proliferation as well as the release of both growth and angiogenic factors, essential for the healing process.¹¹

One may question why a quantitative analysis was not performed in the present study. We opted for using a qualitative assessment of the tissue response to the PL. However, the results of this study will most likely be further analyzed in the near future by using a quantitative analysis. In the present study, we found that the use of different levels of energy caused different responses from the tissues. Subjects treated with 10.2 J/cm² showed better results on most of the criteria used. We found increased angiogenesis on the 7th day in both diabetic and nondiabetic animals as well as increased re-epithelialization on the 21st day. Irradiated diabetic animals showed a similar pattern of healing to that of the nondiabetic ones. These findings are aligned with those from previous reports from our team^{12,21,23} that showed that the use of PL (10.2 J/cm²) resulted in a quicker and better repair. The use of 20.4 J/cm² caused more specific and localized effects, such as on fibroblastic proliferation. These differences may be attributed to the therapeutic window that assumes that high amounts of energy may cause inhibitory effects.²⁵

The use of the PL caused a marked positive effect on the animals with the metabolic unbalance (diabetes). In this case, the energy given seemed to be more effective in the presence of some energetic deficit.^{11,26} This findings is aligned with previous reports from Al-Watban & Andres²⁶ who used LED light on burns and found no significant improvement on the healing of the burns in non-diabetics.

An *in vitro* report from South Africa²⁷ in which fibroblasts cultured in both regular and hyperglycemic medium were exposed to HeNe laser light (λ 638 nm, 3 mW/cm²) and doses of 5 or 16 J/cm². The authors found that the use of 5 J/cm² on “diabetic cells” resulted in responses similar to those observed on controls. A large cellular migration was observed on irradiated areas; a phenomenon important in tissue healing. No cytotoxic effect or genetic alterations were reported with this dose. On the other hand, the use of 16 J/cm² caused inhibitory effects, probably by excessive energy being delivered to the cells. In the present study, the use of the PL caused a significant increase in angiogenesis in most illuminated groups, independent of the systemic status of the animal. It is important to note that the vascular response is very important in diabetic wound healing, as vascular diseases are a common complication in patients with uncontrolled

diabetes and that that causes delay in the repair process.²⁸ Oliveira et al.²⁹ showed, in the recent study, the effectiveness of PL with the low dose (10.2 J/cm²) in improving the healing of third-degree burns in diabetic animals at both early (mainly on the angiogenesis) and late (mainly on re-epithelialization of repair) stages.²⁹

Meireles et al.¹⁷ have also confirmed the positive effect of the use of phototherapy on the healing of burns. However, the positive effect was found with the use of a laser device, which is very costly when compared to PL devices. It is very important to provide effective therapeutics at lower costs for both professionals and patients. Lower costs would stimulate professionals to use the therapeutics on a larger number of patients and would therefore make its benefits more widespread.

We may conclude that the use of a PL source with 10.2 J/cm² resulted in a positive stimulating effect on the healing process of third-degree burns, especially in diabetic animals, reflected in both the re-epithelialization and angiogenesis of the burns.

Author Disclosure Statement

No conflicting financial interests exist.

References

- Sheridan, R.L., Hinson, M.I., Liang, M.H., Nackel, A.F., Schoenfeld, D.A., Ryan, C.M., Mulligan, J.L., and Tompkins R.G. (2000). Long-term outcome of children surviving massive burns. *JAMA* 283, 69–73.
- Vale, E.C.S.D. (2005). Primeiro atendimento em queimaduras: a abordagem do dermatologista [First care on Burns]. *An. Bras. Dermatol.* 80, 9–19.
- Wassermann, D. (2002). Évaluation et premiers soins d'une brûlure thermique. *Rev. Prat.* 52, 2228–2233.
- Sharma, B.R. (2007). Infection in patients with severe burns: causes and prevention thereof. *Infect. Dis. Clin. North Am.* 21, 745–759.
- Alves, C., Andion, J., Brandão, M., and Menezes, R. (2007). Pathogenic aspects of the periodontal disease associated to diabetes mellitus. *Arq. Bras. Endocrinol. Metabol.* 51, 1050–1057.
- Kumar, M.B.B.S., Abbas, A.K., and Fausto, N. (2005). *Robins & Cotran Patologia Humana [Human Pathology]* (7th ed.). Rio de Janeiro: Elsevier, Chapt 124-0 Sistema Endócrino [The endocrine system], pp. 1243–1262.
- Gupta, A., and Raghbir, R. (2005). Energy metabolism in the granulation tissue of diabetic rats during cutaneous wound healing. *Mol. Cell. Biochem.* 270, 71–77.
- Ferreira, S.R.G., and Vannucci, M.G. (2004). Noções de diabetes mellitus para o não especialista. [Basic concepts of diabetes mellitus for non-specialist], in: *Periodontia Médica: Uma abordagem integrada [Medical Periodontology: An Integrated Approach]*. C. M. Brunette (ed.). São Paulo: Senac, pp 149–170.
- Iordanou, P., Baltopoulos, G., Giannakopoulou, M., Bellou, P., and Ktenas, E. (2002). Effect of polarized light in the healing process of pressure ulcers. *Int. J. Nurs. Pract.* 8: 49–55.
- BIOPTRON AG 2007: <http://www.bioptron.eu/?m=407> accessed November 18, 2008.
- Tunèr, J., and Hode, L. (2002). *Laser therapy: clinical practice and scientific background*. Grangeberg: Prima Books AB, Chapt 1—Some basic laser physics, pp 1–44.
- Pinheiro, A.L., Pozza, D.H., Oliveira, M.G., Weissmann, R., and Ramalho, L.M. (2005). Polarized light (λ 400–2000 nm)

- and non-ablative laser ($\lambda 685\text{ nm}$): a description of the wound healing process using immunohistochemical analysis. *Photomed. Laser Surg.* 23,485–492.
13. Ribeiro, M.S., Da Silva, D.F.T., De Araújo, C.E.N., Oliveira, S.F., Pelegrini, C.M.R., Zorn, T.M., and Zezell, D.M. (2004). Effects of low-intensity polarized visible laser radiation on skin burns: A light microscopy study. *J. Clin. Laser Med. Surg.* 22, 59–66.
 14. Tada, K., Ikeda, K., and Tomita, K. (2009). Effect of polarized light emitting diode irradiation on wound healing. *J. Trauma* 67, 1073–1079.
 15. Karadag, C.A., Birtane, M., Aygıt, A.C., Ozunca, K., and Doganay, L. (2007). The efficacy of linear polarized polychromatic light on burn wound healing: an experimental study on rats. *J. Burn Care Res.* 28, 291–298.
 16. de Almeida, S.M., Ferreira, R.I., and Boscolo, F.N. (2002). Influência da irradiação sobre o conteúdo de colágeno, durante a cicatrização, em ratos diabéticos [Influence of irradiation on the collagen content during healing on diabetic rats]. *Pesqui. Odontol. Bras.* 16, 293–298.
 17. Meireles, G.C.S., Santos, J.N., Oliveira, P.C., Moura, A.P., and Pinheiro, A.L.B. (2008). A comparative study of the effects of laser photobiomodulation on the healing of third-degree burns: a histological study in rats. *Photomed. Laser Surg.* 26, 159–166.
 18. Santos, N.R.S., Santos, J.N., Reis, J., Jr., Chagas, P.O., Sousa, A.P.C., Carvalho, C.M., Soares, L.G.P., Marques, A.M.C., and Pinheiro, A.L.B. (2009). Influence of the use of laser phototherapy ($\lambda 660$ or $\lambda 790\text{ nm}$) on the survival of cutaneous flaps on diabetic rats. *Photomed. Laser Surg.* doi: 10.1089/pho.2009.2500.
 19. Martins, C.B.G., and Andrade, S.M. (2007). Queimaduras em crianças e adolescentes: análise da morbidade hospitalar e mortalidade [Burns on children and teenagers: Analysis of hospital morbidity and mortality]. *Acta Paul Enferm.* 20, 464–469.
 20. Mendes Junior, E.C.S., Viterbo, F., and Rosa, C.S. (2007). Análise histológica e morfométrica da área cruenta tratada com membrana de silicone: estudo em ratos. [Histological and morphometric analysis of wounds treated of silicon membrane: Study on rats]. *Rev. Assoc. Med. Bras.* 53, 395–400.
 21. Campanha, B.P., Weismann, R., Oliveira, M.G., et al. (2002). Luz polarizada (400–2000nm): descrição do processo de reparo em feridas através da avaliação imunohistoquímica. [Polarized light (400–2000nm): Description of the repair process by immunohistochemistry]. *Rev. Assoc. Paul. Cir. Dent.* 56, 175–179.
 22. Stasinopoulos, D., Stasinopoulos, I., and Johnson, M.I. (2005). Treatment of carpal tunnel syndrome with polarized polychromatic noncoherent light (bioptron light): a preliminary, prospective, open clinical trial. *Photomed. Laser Surg.* 23, 225–228.
 23. Pinheiro, A.L., Meireles, G.C.S., Carvalho, C.M., de Barros Vieira, A.L., Santos, J.N., and Ramalho, L.M. (2006). Biomodulative effects of polarized light on the healing of cutaneous wounds on nourished and undernourished Wistar rats. *Photomed. Laser Surg.* 24, 616–624.
 24. Monstrey, S., Hoeksema, H., Saelens, H., Depuydt, K., Hamdi, M., Van Landuyt, K., and Blondeel, P. (2002). A conservative approach for deep dermal burn wounds using polarised-light therapy. *Br. J. Plast. Surg.* 55, 420–426.
 25. Sommer, A.P., Pinheiro, A.L., Mester, A.R., Franke, R.P., and Whelan, H.T. (2001). Biostimulatory windows in low-intensity laser activation: lasers, scanners, and NASA's light-emitting diode array system. *J. Clin. Laser Med. Surg.* 19, 29–33.
 26. Al-Watban, F.A.H., and Andres, B.L. (2003) Polychromatic LED therapy in burn healing of non-diabetic and diabetic rats. *J. Clin. Laser Med. Surg.* 21, 249–258.
 27. Houreld, N.N., and Abrahamse, H. (2007). *In vitro* exposure of wounded diabetic fibroblast cells to a helium-neon laser at 5 and 16 J/cm² Photomed. Laser Surg. 25, 8–84.
 28. Ekmektzoglou, K.A., and Zografos, G.C. (2006). A concomitant review of the effects of diabetes mellitus and hypothyroidism in wound healing. *World J. Gastroenterol.* 7, 2721–2729.
 29. Oliveira, P.C., Pinheiro, A.L.B., Reis Junior, J.A., de Castro, I.C., Gurgel, C., Noia, M.P., Meireles, G.C., Cangussu, M.C., and Ramalho, L.M. (2010). Polarized light (1400–2000 nm) on third-degree burns in diabetic rats: immunohistochemical study. *Photomed. Laser Surg.* 28, 613–619.

Address correspondence to:
 Antônio Luiz Barbosa Pinheiro
 Center of Biophotonics
 School of Dentistry
 Federal University of Bahia
 Av. Araújo Pinho, 62
 Canela, Salvador-BA
 CEP 40110-150
 Brazil

E-mail: albp@ufba.br

