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The Use of Light Photobiomodulation on the Treatment of Second-Degree Burns: A Histological Study of a Rodent Model

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Abstract

Objective: The aim of this investigation was to compare, by light microscopy, the effects of the use of laser photobiomodulation (LPBM) and polarized light (PL) on second-degree burns on rodents.
Background Data: Burns are severe injuries that result in the loss of tissue fluids, destruction of tissues, infection, and shock. With severe and widespread third-degree burns death may occur. Several light sources have been suggested as being effective for improving wound healing.
Materials and Methods: Forty five rats were used in this study. A second-degree burn was created on the dorsum of each animal, and the animals were divided into four groups: PL (400–2000 nm, 40 mW, 2.4 J/cm^2/min); LPBM-1 (780 nm, 35/40 mW, $\theta \sim 2$ mm, 4×5 J/cm^2); LPBM-2 (660 nm, 35/40 mW, $\theta \sim 2$ mm, 4×5 J/cm^2); and untreated animals acted as controls. The treatment was started immediately post-burn at four points around the burned area (laser: 5 J/cm^2 per site). The illumination with PL was performed according to the manufacturer's instructions. Treatments were repeated at 24-h intervals for 7 d. The animals were sacrifice at 3, 5, and 7 d post-burn. The specimens were routinely cut and stained and analyzed by light microscopy using hematoxylin and eosin and Sirius red.
Results: The analysis of the results demonstrated that the damaged tissue was able to efficiently absorb and process the light at all tested wavelengths. LPBM at 660 nm showed better results at early stages of wound healing. However, the use of 780-nm laser light had beneficial effects throughout the experimental period, with the animals growing newly-formed tissue similar to normal dermis.
Conclusion: Despite our findings that the use of both types of light energy improved the healing of second-degree burns at the early stages, long-term assessment is needed to verify if this improvement will influence the final results of treatment.

Introduction

A BURN IS A LESION resulting from the direct or indirect action of heat and is a significant cause of mortality.[1] Burns are severe injuries that result in the loss of tissue fluids, destruction of tissues, infection, and shock, that may result in death, which is most common with extensive third-degree burns. Burns also cause considerable morbidity and have sequelae, the most serious being functional debility, especially when they affect the hands.[2] Severe social and psychological impairments are also common.[3,4] Several therapeutic methods have been used to ameliorate the outcome of burns, including the use of light sources.

Enwemeka[5] recommended that "phototherapy" be used to describe any noninvasive intervention involving the therapeutic use of light to treat cutaneous and subcutaneous tissues, light-based acupuncture, and transcutaneous irradiation for pain relief. "Photobiomodulation" may also be appropriate to describe these kinds of interventions, but is used more broadly to describe more invasive types of light therapy, such as direct irradiation of stomach ulcers, cardiac infarcts, or fractures.

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There are several reports in the literature on the beneficial effects of laser photobiomodulation (LPBM) on local vascularization, edema, pain, and inflammation, as well as on the extracellular matrix and collagen deposition and organization.[6–15]

The use of LPBM has been shown to result in increased amounts of both acute and chronic inflammatory cells. This hastens the inflammatory response and effectively stimulates the development of granulation tissue, an increased number of fibroblasts, increased deposition of collagen fibers, and improved neovascularization. These aspects are seen at the early stages of the healing process, and are probably due to the effects of laser energy at the cellular level.[6,16]

Polarized light (PL) vibrates in a single direction, perpendicular to its propagation axis. This characteristic allows it to act on the lipid layer of the cell, altering cellular processes regulated by the cellular membrane such as energy production, ion transport, and immune processes.[17]

Alternative light sources have been used to improve wound healing, and previous reports have suggested that polarization is the characteristic of laser energy that is responsible for its biomodulating effects, and this is why other polarized light sources may also have biomodulating effects on biological systems.[17,18]

Several reports in the literature have shown the positive biomodulative effect of linearly PL (400–2000 nm) on wound healing.[17] A report by our group found large amounts myofibroblasts in illuminated tissues, that may result in a wound with a greater potential for contracture, which is undesirable aesthetically, but that could be quite useful in areas where more contraction is needed, such as in large burns.[17]

Polarized light is capable of inducing biostimulative effects in living cells similarly to LPBM. As the Bioptron lamp combines visible light at 480–700 nm with infrared light at 700–2000 nm, it is a low-power light source like a low-level laser, but it is polychromatic and incoherent. One of the main effects of the absorption of visible light is the stimulation of mitochondria, which results in increased cell energy and activation of nucleic acid synthesis that is essential for wound repair.

Most histological studies fail to show different mechanisms of wound healing for burns and cutaneous wounds,[18] and several studies have been performed to assess the effect of LPBM on burns. Most researchers believe that the positive effects of LPBM would hasten healing of burns.[16,19]

Polarized light may reproduce nearly 80% of the effects of LPBM, but non-polarized light does not. Polarization has also been suggested as an important factor in the tissue response to injury.[20,21]

Despite earlier reports that suggested that LPBM may inhibit or have no effect on wound healing, there has been meticulous analysis of the parameters used[22–27] as well as the systemic effects of LPBM.[28,29]

Previous results from our group on the use of both types of light sources have shown similar results,[17,30–32] and this prompted us to assess both laser energy and polarized light for the treatment of second-degree burns.

The aim of the present study was to analyze histologically the process of repair of second-degree burns induced in the dorsum of rats when treated with PL (400–2000 nm) or LPBM (780 nm or 660 nm).

Materials and Methods

Following the approval of the Animal Experimentation Ethics Committee of the School of Dentistry of the Federal University of Bahia, 45 young adult male Wistar rats weighting 200–230 g were obtained from the Centro de Criação de animais da Faculdade de Medicina Veterinária da Universidade Federal da Bahia, and were kept at the Animal Experimentation Laboratory of the School of Dentistry of the Federal University of Bahia. The animals were kept in individual plastic cages bedded with wood chips and maintained at 22°C in a day/night light cycle. The animals were fed a standard pelleted laboratory diet and had water *ad libitum*. After regular quarantine the animals were randomly distributed into four groups: PL (n = 12), LPBM-1 (780 nm; n = 12), LPBM-2 (660 nm; n = 12), and controls (n = 9).

Under intraperitoneal general anesthesia (0.10 mL/100 g of ketamine and 0.25 mL/100 mg of xylazine) each animal had the dorsum shaved and cleaned. A specially designed instrument measuring 1.5 × 1.5 cm was heated in boiling water for 50 sec and applied to the skin for 20 sec to induce the formation of a second-degree burn.[33]

The polarized light protocol was administered with a light source (Bioptron®, Bioptron AG, Monchaltorf, Switzerland) at λ = 400–2000 nm, 40 mW, 2.4 J/cm^2/min, 20 J/cm^2, for 8 min 33 sec, $\theta \sim 2$ cm, with a circular beam. Per the manufacturer's instructions the burn was first illuminated immediately post-burn, and was repeated every 24 h until the animals were sacrificed at 3, 5, and 7 d post-burn. A maximum of seven illumination sessions were performed.

LPBM (Kondortech, São Carlos, SP, Brazil; λ = 780 nm or λ = 660 nm, 20 J/cm^2, 40/35 mW, $\theta \sim 2$ mm, circular beam) was started immediately post-burn and was repeated daily until the day before the animal's sacrifice. Laser light was applied transcutaneously at four equidistant points around the wound margin. The dose per point was 5 J/cm^2 and the dose per session was 20 J/cm^2.

Although the control animals were neither illuminated nor irradiated, they were handled in the same way as the experimental animals to assure the same level of stress.

The choice of the types of treatment was due to the conflicting results seen in the literature with regard to the effects of different treatment parameters on the outcome of LPBM. The use of different wavelengths was due to differences in the absorption and penetration of the light sources, as both superficial and deep tissues were affected by the burns.

Although the same dose was provided for each treatment (20 J/cm^2), the use of identical power densities in all treated groups was impossible due to the lack of adjustments of the equipment.

When the time came for the animal's sacrifice (3, 5, and 7 d post-burn), each animal was killed with an overdose of general anesthetic. Specimens were taken and kept in 10% formalin for 24 h, after which the specimens were routinely cut and embedded in wax. The slides were stained with hematoxylin and eosin and Sirius red and numbered. The numbered specimens were analyzed under light microscopy by an experienced pathologist who was blinded to the experimental group of each slide. The criteria used for this analysis are shown in Table 1. Statistical analysis was carried out by Fisher's exact test. Statistical significance was set at $p < 0.05$.

Table 1. Criteria Used for Light Microscopy Analysis

Criterion	*Score*		
Re-epithelialization	Absent	Present: Covering <50% of the wound Present: Covering >50% of the wound Present: Covering 100% with irregular thickness Present: Covering 100% with regular thickness	
Acute inflammation	Slight: Presence of <25% neutrophils of all cells in the field	Moderate: Presence of <25–50% neutrophils of all cells in the field	Intense: Presence of >50% neutrophils of all cells in the field
Chronic inflammation	Slight: Presence of <25% of chronic inflammatory cells in the field	Moderate: Presence of <25–50% chronic inflammatory cells in the field	Intense: Presence of >50% chronic inflammatory cells in the field
Granulation tissue	Slight: At 400< magnification, a slight presence of fibroblasts, collagen fibers, and inflammatory cells	Moderate: At 400< magnification, a presence of fibroblasts, collagen fibers, and inflammatory cells	Intense: At 400< magnification, an intense presence of fibroblasts, collagen fibers, and inflammatory cells
Amount of fibroblasts	Slight: Presence of <25% of young, undifferentiated fibroblasts among the other cell types	Moderate: Presence of <25–50% of young, undifferentiated fibroblasts among the other cell types	Intense: Presence of >50% of young, undifferentiated fibroblasts among the other cell types
Amount of collagen fibers	Slight: Sirius red staining is less intense than that seen in the healthy adjacent tissue	Moderate: Sirius red staining is similar to than seen in the healthy adjacent tissue	Intense: Sirius red staining is more intense than that seen in the healthy adjacent tissue
Neoangiogenesis	Slight: A smaller amount than that seen on healthy adjacent tissue	Moderate: An amount similar to that seen on healthy adjacent tissue	Intense: An amount greater than that seen on healthy adjacent tissue

Results

Controls

At day 3 post-burn, thermal necrosis extending down to the hypodermis was permeated with remnants of pyknotic nuclei, and no signs of inflammation could be seen at this stage. The presence of fibroblasts, granulation tissue, and collagen deposition was slight in all specimens (Fig. 1). Neoangiogenesis was mostly slight. Re-epithelialization was absent in all the analyzed specimens. At the day 5 post-burn, the epidermal necrosis extended down to the hypodermis and there was crust formation in most of the animals. Acute inflammation was moderate in most of the specimens and chronic inflammation was mostly slight at this time. The presence of fibroblasts varied from slight to intense, and the amount of granulation tissue was moderate in all cases. Collagen deposition was considered slight in most cases. Neoangiogenesis was slight in all the animals (Fig. 2). Re-epithelialization was absent in all the analyzed specimens. At the end of the experimental period, the necrotic tissue still extended down to the hypodermis and was permeated by remnants of pyknotic nuclei. Inflammation remained slight at this time. Collagen deposition was uneven, and when present it was moderate in amount. In all the animals the presence of fibroblasts and neoangiogenesis was moderate (Fig. 3). Re-epithelialization was absent in all the analyzed specimens.

Laser light (660 nm)

At day 3 post-burn, the epidermis was necrotic in all specimens and the necrosis extended down to the hypodermis. There was crust formation in half of the animals. A slight amount of acute inflammation was seen at this stage. Chronic inflammation was present in most cases and varied from slight to moderate. The number of fibroblasts was small in most specimens. Both collagen deposition and neoangiogenesis were slight to moderate. Re-epithelialization was absent in all the analyzed specimens. Five days post-burn, the sections showed epidermal necrosis extending down to the hypodermis. Crust formation was slight in most specimens.

FIG. 1. Photomicrograph of a control specimen 3 d post-burn, showing a slight presence of fragmented collagen fibers (arrows) (Sirius red, approximately 100×).

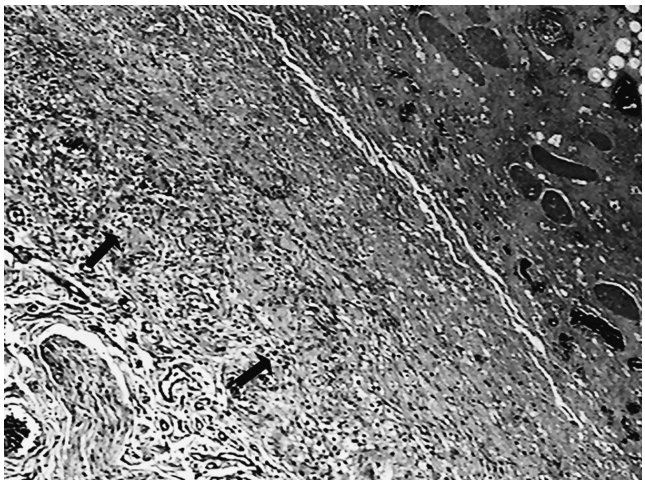

FIG. 2. Photomicrograph of a control specimen 5 d post-burn, showing granulation tissue (arrows) with a few blood vessels (hematoxylin and eosin, approximately 40×).

The acute inflammatory response was also slight in most specimens. In all the animals, chronic inflammation was slight. The number of fibroblasts, amount of collagen deposition, and granulation tissue formation ranged from slight to intense (Fig. 4). Neoangiogenesis also ranged from slight to intense in all specimens (Fig. 5). Re-epithelialization was absent in all the analyzed specimens. At day 7, the sections showed skin fragments exhibiting epidermal necrosis extending down to the hypodermis. Crust formation was moderate in most specimens. In all cases, acute inflammation was intense. Chronic inflammation was slight in most specimens, the number of fibroblasts was moderate, and granulation tissue formation ranged from moderate to intense. In all of the specimens, collagen deposition and neoangiogenesis were moderate at this stage (Figs. 6 and 7). Re-epithelialization was absent in all cases.

Laser light (780 nm)

At the day 3 post-burn, necrotic skin fragments extending down to the hypodermis were seen. Acute inflammation was slight in all specimens. Half of the specimens also showed a slight chronic inflammatory reaction. The number of fibroblasts and the amount of granulation tissue were also slight at this time. Collagen deposition was mostly moderate below the burn and angiogenesis was slight. Re-epithelialization was absent in all cases. Five days post-burn, tissue necrosis was still seen extending down to the hypodermis and crust formation was mostly slight. Acute inflammation was also mostly slight at this time. The chronic inflammatory reaction was slight in all specimens. The number of fibroblasts was small and the amount of granulation tissue, neoangiogenesis, and collagen deposition ranged from moderate to intense (Fig. 8). Re-epithelialization was absent in all cases. At the end of the experimental period (day 7), most specimens had some crust formation. Acute and chronic inflammation were mostly slight. The number of fibroblasts was small and the amount of granulation tissue and collagen deposition were moderate (Fig. 9). Neoangiogenesis varied, ranging from slight to intense (Fig. 10). No signs of re-epithelialization were seen at this time.

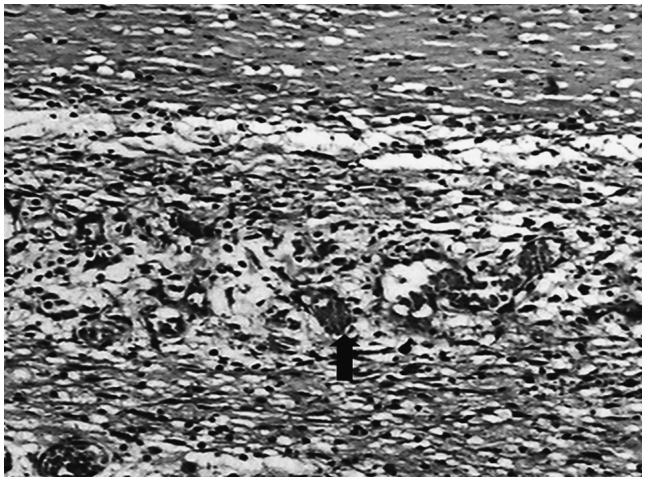

FIG. 3. Photomicrograph of a control specimen 7 d post-burn, showing a slight amount of granulation tissue with a few blood vessels (arrow) (hematoxylin and eosin, approximately 100×).

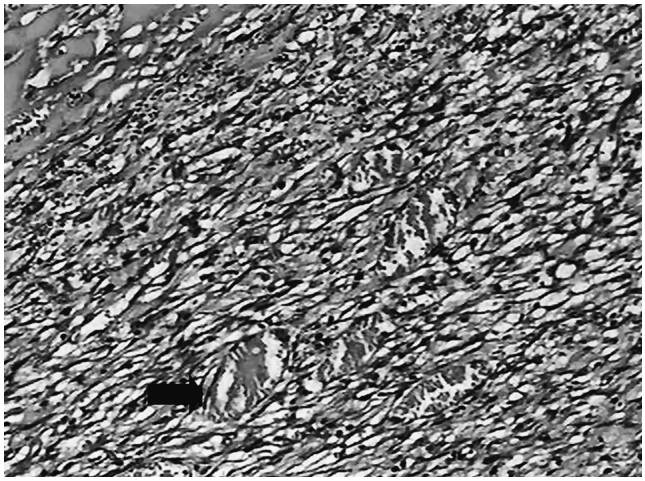

FIG. 6. Photomicrograph of an irradiated specimen (660 nm) 7 d post-burn, showing granulation tissue rich in newly-formed blood vessels (arrow) (hematoxylin and eosin, approximately 100×).

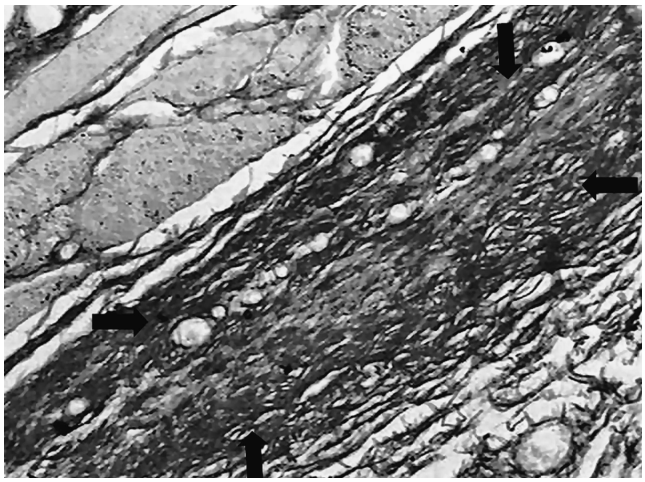

FIG. 4. Photomicrograph of an irradiated specimen (660 nm) 5 d post-burn, showing parallel collagen fibers at an advanced stage of maturation (arrows) (Sirius red, approximately 400×).

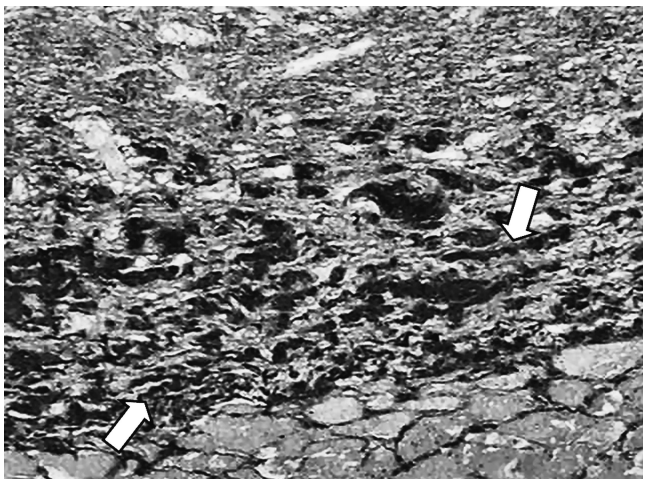

FIG. 7. Photomicrograph of an irradiated specimen (660 nm) 7 d post-burn, showing parallel collagen fibers distributed in a fragmented pattern (arrows). (Sirius red, approximately 100×).

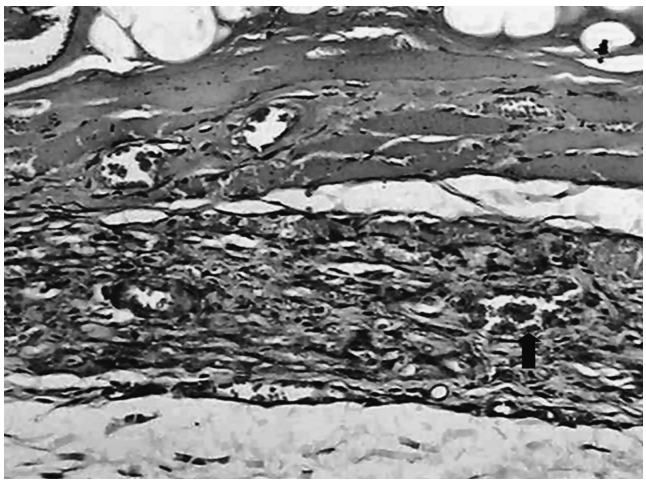

FIG. 5. Photomicrograph of an irradiated specimen (660 nm) 5 d post-burn, showing a narrow zone of granulation tissue with a few blood vessels (arrow) (hematoxylin and eosin, approximately 200×).

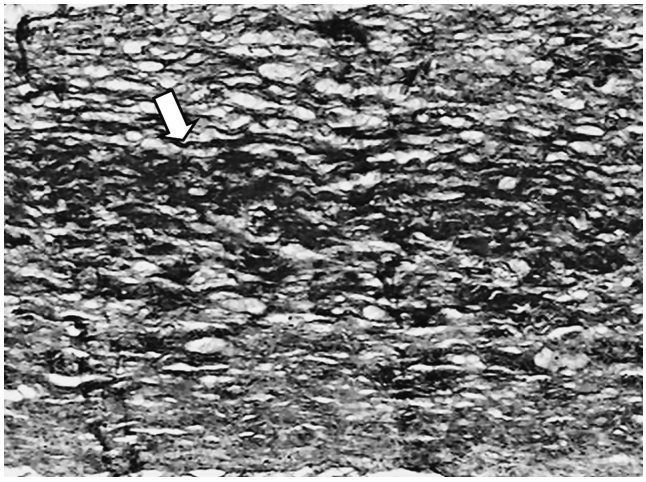

FIG. 8. Photomicrograph of an irradiated specimen (780 nm) 5 d post-burn, showing moderate to intense deposition of mature collagen fibers (arrow). (Sirius red, approximately 200×).

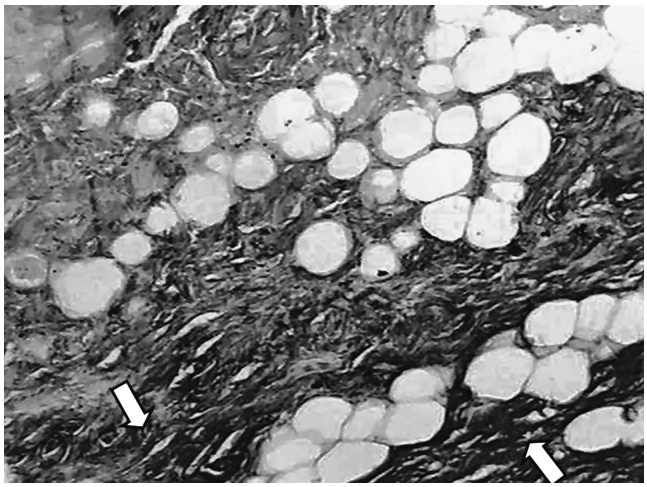

FIG. 9. Photomicrograph of an irradiated specimen (780 nm) 7 d post-burn, showing organized collagen fibers at an advanced stage of maturation (arrows). (Sirius red, approximately 200×).

Polarized light

At day 3 post-burn, there was no sign of cobblestoning and the epithelium was thin. Crusting was seen in one specimen. Acute inflammation was mostly slight and most specimens did not show any evidence of a chronic inflammatory reaction. The number of fibroblasts, and the amount of granulation tissue formation and collagen deposition were slight at this point. Neoangiogenesis ranged from slight to moderate. Re-epithelialization was absent in all specimens. Five days post-burn, the sections showed skin fragments exhibiting epidermal necrosis reaching down to the hypodermis. In most specimens slight crusting was seen. The specimens showed either slight or moderate acute inflammation, and some chronic inflammation was found in half of the specimens. The number of fibroblasts was slight to moderate in all specimens, and the formation of granulation tissue was also mostly slight (Fig. 11). Collagen deposition was more concentrated in the subepithelial area in all specimens, and was either slight (Fig. 12) or moderate. Neoangiogenesis was slight to intense in all specimens. Re-epithelialization was absent in all cases. At the end of the experimental period (day 7), skin fragments exhibiting necrosis extending down to the hypodermis were seen. The formation of granulation tissue was slight at this point (Fig. 13). Some crusting was seen at the edges of the burn in all specimens. Acute inflammation was either slight or intense. Some chronic inflammation was seen in most specimens. The number of fibroblasts and amount of collagen deposition were slight to moderate. Neoangiogenesis ranged from slight to intense. Re-epithelialization was not seen in any specimen. A summary of the results can be seen in Table 2.

The statistical analysis showed a statistically significant difference between the group treated with 780-nm laser light and the other groups at day 7 post-burn, primarily for the criteria of neoangiogenesis and crust formation ($p = 0.029$). The PL group showed better results than the controls at the early stages, primarily for the criterion of acute inflammation ($p = 0.029$).

Discussion

There are several reports in the literature on the beneficial effects of LPBM on local vascularization, edema, pain, and inflammation, as well as on the extracellular matrix and collagen deposition and organization.[34,35] In the present study we used both laser and polarized light sources due to the severity of the burns that deeply affected the skin and made treatment difficult.

As found in the literature, linearly PL can affect the cellular processes regulated by the cellular membrane, including energy production, ion transport, and immune processes.[24] These effects directly influence tissue repair, especially the healing of burns, for which there is a great homeostatic imbalance.[36]

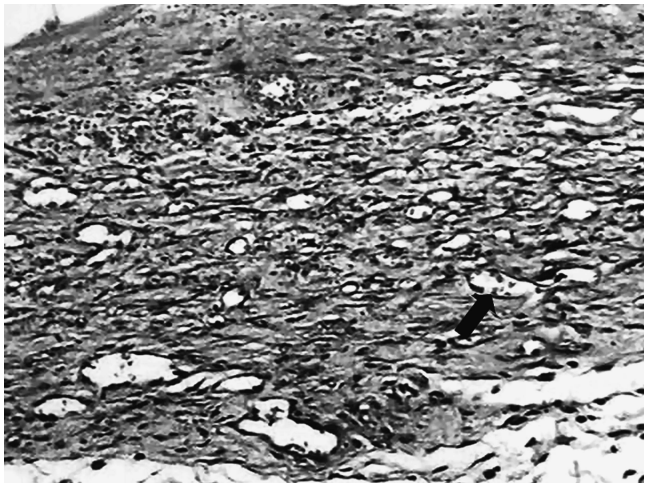

FIG. 10. Photomicrograph of an irradiated specimen (780 nm) 7 d post-burn, showing granulation tissue rich in blood vessels becoming congested (arrow) (hematoxylin and eosin, approximately 100×).

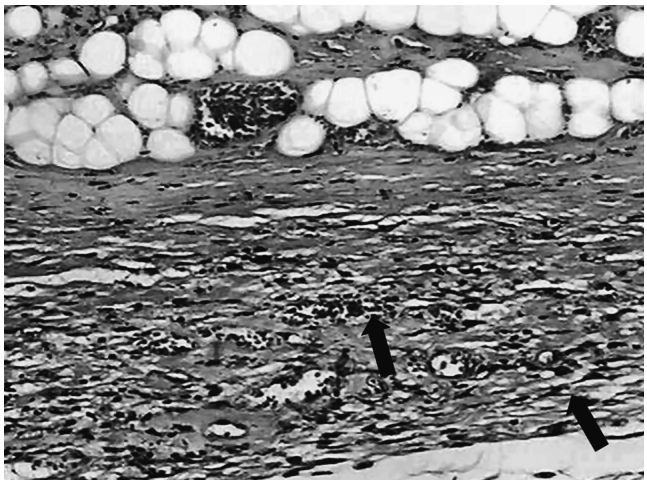

FIG. 11. Photomicrograph of a specimen treated with polarized light 5 d post-burn, showing a slight amount of granulation tissue with congested blood vessels (arrows). (hematoxylin and eosin, approximately 200×).

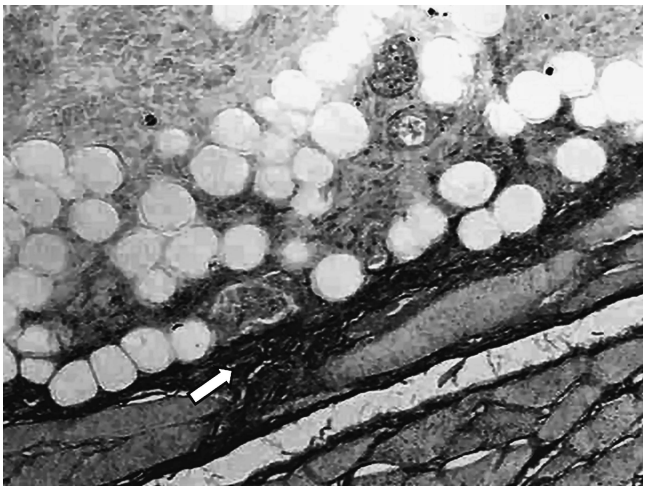

FIG. 12. Photomicrograph of a specimen treated with polarized light 5 d post-burn, showing a slight amount of mature collagen fibers (arrow) (Sirius red, approximately 400×).

A previous report from our team[17] evaluated the biomodulating effect of a PL source in a rodent model. Our results showed a larger concentration and better distribution of collagen fibers in the PL-illuminated groups compared to the controls. This was found be related to faster wound healing. Our findings in the present investigation also showed that, despite being present in both illuminated and non-illuminated specimens, both the amount and level of maturation of collagen fibers was better in the PL-illuminated subjects than the controls.

PL illumination also resulted in the deposition of a fibrinous crust on all specimens. Crusting is important, as the crust acts as a mechanical barrier between the environment and the injured tissues. This protection allows better conditions for cellular repair, and aids migration of the cells into the healing tissue.[32]

At the early stages of the repair process, we observed that subjects treated with 660-nm laser energy showed some crust formation; in the other groups no crusting was seen. Re-epithelialization was not observed in any group.

The acute inflammation was mostly slight in the specimens from animals treated with 780-nm laser or PL energy, and was slight in half of the specimens from animals treated with 660-nm laser energy. None of the controls showed this reaction. Chronic inflammation varied from slight to moderate in the subjects treated with 660-nm laser energy. In the 780-nm laser irradiated subjects chronic inflammation was slight in the half of the specimens. In the PL-illuminated group few of the animals showed this reaction.

In all groups the presence of fibroblasts and the amount of granulation tissue were both slight. The deposition of collagen was slight in the animals illuminated with PL and in the controls. In the group treated with 780-nm laser energy, most specimens showed a moderate deposition, while in the group irradiated with 660-nm laser energy it varied from slight to moderate. Neoangiogenesis, as seen in most of the animals treated with PL, was moderate. In the 660-nm laser and control groups it varied from slight to moderate; in the 780-nm laser group it was mostly slight. For this parameter, the 660-nm laser was the most effective. This is accordance with the findings of Meirelles,[32] who found that third-degree burns treated with 660-nm laser energy showed satisfactory repair early in the experimental period (at days 3 and 5).

On day 5, most of the specimens from all groups showed some crust formation. Re-epithelialization was not yet seen in any group. In the control animals, the acute inflammatory reaction was moderate. In the laser groups, in most cases re-epithelialization was slight, and in the PL-illuminated subjects it varied from slight to moderate. Chronic inflammation was mostly slight in all groups.

It is important that at this stage, the macrophage is critical to the inflammatory stage of wound healing and essential to new tissue growth through macrophage-derived growth factors, such as platelet-derived growth factor, transforming growth factor-α, transforming growth factor-β, interleukin-1, and tumor necrosis factor. Some reports have shown an influence of some wavelengths of light on these growth factors.[37,38]

In the groups treated with the 660-nm laser or PL illumination, the number of fibroblasts varied from slight to moderate, and in the animals treated with the 780-nm laser it was mostly slight. In the controls, the numbers varied from slight to intense. Fibroblasts are responsible for synthesizing collagen, and collagen makes up about 50% of scar tissue.

The amount of granulation tissue present varied from slight to intense. In the control group it was moderate, and it was slight in the PL-illuminated group. The deposition of collagen varied from slight to intense in the 780-nm laser group, and varied from slight to moderate in the other two treatment groups. In the most of the controls, amounts of collagen deposition were slight. Collagen, however, cannot be synthesized in the absence of an adequate oxygen supply, and clearly this was the direct result of the poor blood supply. Angiogenesis is then required.[39,40]

Neoangiogenesis varied widely in the 780-nm laser group, and it was moderate in the 660-nm laser group and the controls. In the PL group it varied from slight to intense. One

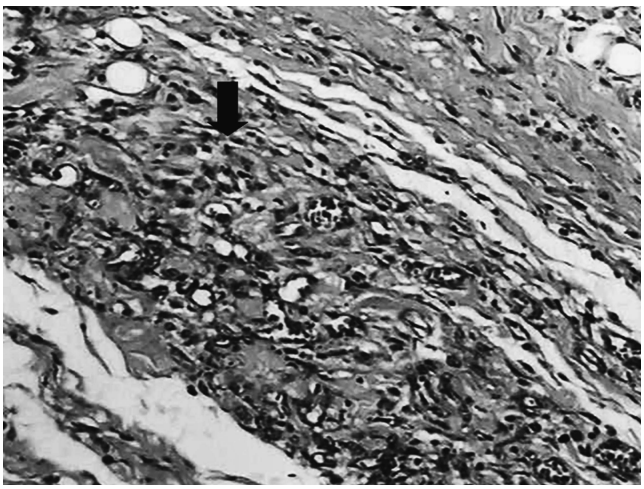

FIG. 13. Photomicrograph of a specimen treated with polarized light 7 d post-burn, showing a slight amount of granulation tissue (arrow) (hematoxylin and eosin, approximately 200×).

Table 2. Summary of the Histological Observations According to the Criteria Used in This Investigation

Criterion:	*Acute inflammation*			*Chronic inflammation*			*Neoangiogenesis*			*Granulation tissue*		
Day: / *Group*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*
Control	100% absent	33.3% slight; 66.6% moderate	100% slight	100% absent	33.3% absent; 66% slight	100% slight	66.6% slight; 33.3% moderate	100% slight	100% moderate	100% slight	100% moderate	33.3% slight; 66.6% moderate
660 nm laser	50% absent; 50% slight	75% slight; 25% intense	100% intense	25% absent; 25% slight; 50% moderate;	100% slight	75% slight; 25% moderate	50% slight; 50% moderate	75% slight 25% intense	100% moderate	100% slight	50% slight; 25% moderate 25% intense	50% moderate; 50% intense
780 nm laser	100% slight	75% slight; 25% moderate	100% slight	25% absent; 75% slight	100% slight	25% absent; 75% slight	25% absent; 75% slight	25% slight; 50% moderate 25% intense	25% absent; 25% slight; 25% moderate; 25% intense	50% absent; 50% slight	25% slight; 50% moderate 25% intense	25% absent; 75% moderate
PL	25% absent; 75% slight	50% slight; 50% moderate	50% slight; 25% moderate 25% intense	75% slight; 25% moderate	50% absent; 50% slight	25% absent; 75% slight	25% slight; 75% moderate	50% slight; 50% intense	25% slight; 50% moderate; 25% intense	100% slight	75% slight; 25% moderate	100% slight

Criterion:	*Fibroblasts*			*Collagen fibers*			*Crust formation*			*Re-epitheliazation*		
Day: / *Group*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*	*3*	*5*	*7*
Control	100% slight	33.3% slight; 33.3% moderate;	100%	100% slight	66.6% slight; 33.3% intense	66.6% absent; 33.3% moderate	100% absent	33.3% absent; 66.6% slight	100% absent	100% absent	100% absent	100% absent
660 nm laser	75% slight; 25% moderate	50% slight; 50% moderate	25% slight; 75% moderate	50% slight; 50% moderate	50% slight; 50% moderate	100% slight	50% absent; 50% slight	75% slight; 25% intense	25% slight; 75% moderate	100% absent	100% absent	100% absent
780 nm laser	100% slight	75% slight; 25% moderate	75% slight; 25% moderate	25% slight; 75% moderate	25% slight; 50% moderate 25% intense	25% slight; 75% moderate	100% absent	75% slight; 25% moderate	75% slight; 25% moderate	100% absent	100% absent	100% absent
PL	100% slight	50% slight; 50% moderate	50% slight; 50% moderate	100% slight	50% slight; 50% moderate	50% slight; 50% moderate	75% absent; 25% slight	25% absent; 75% slight	100% slight	100% absent	100% absent	100% absent

important aspect of the findings of this study was the wide difference seen in the amounts of neoangiogenesis in the test groups. The amounts of blood vessels seen in the PL-illuminated and laser-treated groups were generally larger than those seen in the controls.[34,41]

At the end of the experimental period crust formation was moderate in the group treated with 660-nm laser energy and slight in the groups treated with PL or the 780-nm laser. In control specimens, no crust formation was seen. No signs of re-epithelialization were seen in any of the groups within the experimental period of 7 d.

Acute inflammation varied from slight to moderate in the controls, from slight to intense in the PL-illuminated group, was intense in the group treated with the 660-nm laser, and slight in the group treated with the 780-nm laser. Chronic inflammation was slight in most specimens from all of the groups. This may be due to the fact that the repair of a second-degree burn initially requires the removal of the necrotic tissue via phagocytosis. This phenomenon is followed by the migration of epithelial cells to the wounded site. As this takes a long time to occur in second-degree burns, it is likely that the time period of this study was too short to detect any differences between the groups.

The number of fibroblasts was moderate in the group treated with the 660-nm laser, and slight in the 780-nm laser group. In the PL-illuminated group, it varied from slight to moderate, and it was also moderate in the controls. The amount of granulation tissue was moderate in the 780-nm laser group, and varied from moderate to intense in the 660-nm laser-treated group. In the PL-illuminated group, the amount of granulation tissue was slight, and it varied from slight to intense in the controls. The deposition of collagen was moderate in the 780-nm laser-treated group, and slight in the 660-nm laser-treated group. In the PL-illuminated group it varied from slight to moderate, and it was moderate in the controls.

Different parameters for LPBM were used in previous studies of wound healing, which explains the conflicting results reported. Previous reports from our group have pointed out that at least two threshold parameters, energy density and intensity, strongly influence the final results of LPBM.[43,44] We have found that LPBM improves cutaneous wound repair, and that the effect is inversely proportional to the wavelength and intensity.[43] The treatment is most effective with higher intensity and short wavelength, or lower intensity and higher wavelength.

It should be noted that light penetration into skin results in a reduction of the amount of energy that reaches the subcutaneous tissue. Most studies of wound healing showed better results when shorter wavelengths were used. However, we have found good results with the use of an IR laser, alone or in association with a shorter wavelength.[44] This aspect was further confirmed in the present investigation, and it may be a result of the deeper penetration of IR laser energy, which resulted in the stimulation of the cells located in the deeper portions of the wounds, as well as the effect of heating, which is usually seen when high fluencies are used at this wavelength. The combination of wavelengths with different levels of absorption and penetration may further improve wound healing, as LPBM may stimulate repair at both at the surface and at deeper levels. It is also important to note that the choice of optical parameters used for improved wound healing depend on wavelength, dose per session, the model used, the type of wound, and the treatment conditions, among other factors.[43,44]

Despite our positive findings on the healing of second-degree burns treated with different light sources, some previous researchers have failed to do so.[27,42] However, it must be considered that in both of those other studies contralateral wounds acted as controls.

Conclusion

The findings of this investigation suggest that laser or polarized light could be useful for treating severe burns, especially if it is begun at an early stage of healing. The results of this investigation demonstrate that damaged tissues are able to efficiently absorb and process the light at all tested wavelengths. It appears that LPBM at 660 nm showed better results during the early stages of wound healing, and that the use of the 780-nm laser showed beneficial effects throughout the experimental period.

Despite our findings that indicate that the use of both types of light sources improved the healing of second-degree burns at the early stages, longer-term assessments are needed to verify if these improvements result in improved success rates in those with second-degree burns.

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