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A Comparative Study of the Effects of Laser Photobiomodulation on the Healing of Third-Degree Burns: A Histological Study in Rats

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ABSTRACT

Objective: **The aim of this investigation was to compare by light microscopy the effects of laser photobiomodulation at wavelengths of 660 and 780 nm on third-degree burns in Wistar rats.** ***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. Laser energy has been suggested as an effective method to improve wound healing.** ***Materials and Methods:*** **Fifty-five animals were used in this study. A third-degree burn measuring 1.5 × 1.5 cm was created on the dorsum of each animal. The animals were divided into three subgroups according the type of laser photobiomodulation they received (wavelength of 660 or 780 nm, 35 mW, θ = 2 mm, and 20 J/cm^2). In the animals receiving treatment, it was begun immediately post-burn at four points around the burn (5 J/cm^2) and repeated at 24-h intervals for 21 d. The animals were humanely killed after 3, 5, 7, 14, and 21 d by an intraperitoneal overdose of general anesthetic. The specimens were routinely cut and stained, and then were analyzed by light microscopy.** ***Results:*** **The results showed more deposition of collagen fibers, larger amounts of granulation tissue, less edema, a more vigorous inflammatory reaction, and increased revascularization on all laser-treated animals. These features were more evident at early stages when the 660-nm laser was used, and were more evident throughout the experimental period for the animals receiving 780-nm laser therapy.** ***Conclusion:*** **We concluded that laser photobiomodulation using both wavelengths improved healing of third-degree burns on Wistar rats.**

INTRODUCTION

WOUND HEALING is a complex and highly organized biological response to injury that results in the loss of tissue integrity. Open wounds such as burns heal by secondary intention, and factors such as size and location affect healing, and coexistent systemic disease such as diabetes may impair the process.[1]

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.[2,3]

Most histological studies show similar mechanisms of wound healing in both burns and other types of cutaneous injuries,[4] and several studies have been performed to assess the effect of laser photobiomodulation (LPBM) on burns. Most studies seem to indicate that LPBM may promote quicker healing of burns.[5–7]

The use of LPBM may result in reduced pain due to its effects on cyclooxygenase. The response to laser therapy depends on the characteristics of the organism and on the condition being treated. Some symptoms resolve quickly after the application of laser energy, but others require several treatment sessions before a response is seen.[8] The present study was designed to examine the effects of LPBM on healing of burns on the dorsum of Wistar rats.

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Table 1. Criteria Used for Light Microscopy Analysis

Criterion	*Score*		
Re-epithelialization	Absence	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	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 inflammation	Discrete: Presence of <25% chronic inflammatory cells on the field	Moderate: Presence of <25%–50% chronic inflammatory cells on the field	Intense: Presence of >50% chronic inflammatory cells on the field
Granulation tissue	Discrete: At 400× magnification, discrete presence of fibroblasts; collagen fibers and inflammatory cells present	Moderate: At 400× magnification, moderate presence of fibroblasts; collagen fibers and inflammatory cells present	Intense: At 400× magnification, intense presence of fibroblasts; collagen fibers and inflammatory cells present
Amount of fibroblasts	Discrete: Presence of <25% young and less differentiated fibroblasts among other cell types	Moderate: Presence of <25%–50% young and less differentiated fibroblasts among other cell types	Intense: Presence of >50% young and less differentiated fibroblasts among other cell types
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 that seen on healthy adjacent tissue	Intense: More than that seen on healthy adjacent tissue

MATERIALS AND METHODS

Following the approval of the Animal Experimentation Ethics Committee of the School of Dentistry of the Federal University of Bahia, 55 young adult male Wistar rats weighing 200–230 grams 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 divided into three groups as follows: group 1: controls (n = 15); group 2: LPBM (660 nm, n = 20); and group 3: LPBM (780 nm, n = 20). The groups receiving treatment were then divided into five subgroups according to the time of sacrifice of the animals. Each group was comprised of three control and four experimental animals. The animals were humanely killed 3, 5, 7, 14, and 21 d after the procedure. Under intraperitoneal general anesthesia (0.10 mL/100 g of ketamine and 0.25 mL/100 mg of xylazine), each animal had its dorsum shaved and cleaned. While under anesthesia, a specially-designed instrument[9] measuring 1.5 × 1.5 cm was heated until it was red hot and was applied to the skin for 20 s, inducing a third-degree burn. If the animals had shown any signs of pain, they were to be given nonsteroidal anti-inflammatory drugs, but none of the rats showed any such signs. Using a laser (Kondortech, São Carlos, SP, Brazil), LPBM was performed on group 2 (660 nm, 20 J/cm^2, 35 mW, θ = 2 mm,) and group 3 (780 nm, 20 J/cm^2, 35 mW, θ = 2 mm). We chose these two types of treatment because we found conflicting results in the literature regarding the differing effects of various treatment parameters on the outcome of LPBM. We used two different wavelengths because of differences in the absorption and penetration of laser energy, and because both superficial and deeper tissues were affected by the burns. LPBM was begun immediately post-burn, and was repeated daily until the day before the animal was killed. The laser energy was applied transcutaneously at four equidistant points on the wound margin. The dose per point was 5 J/cm^2, and the dose per session was 20 J/cm^2. At the time points cited above, each animal was killed by an overdose of general anesthetic. Specimens were taken and kept in 10% formalin for 24 h. The specimens were then routinely cut and embedded in wax, and the slides were stained with hematoxylin and eosin (H&E) and Sirius red. The specimens were analyzed under light microscopy by an experienced pathologist. The criteria used for analysis are shown in Table 1.

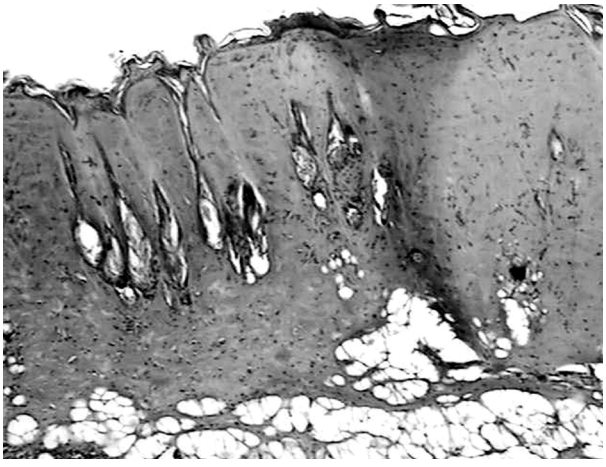

FIG. 1. Photomicrograph of a specimen from an animal receiving LPBM at 780 nm that was killed 3 d post-burn, showing coagulation necrosis extending deeply into the wound, and cells exhibiting pyknotic nuclei (H&E, approximately ×40).

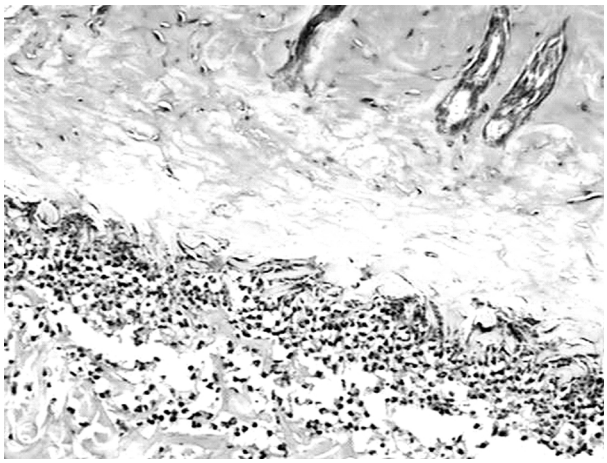

FIG. 2. Photomicrograph of a 740 nm specimen at day 5 showing the persistence of necrosis extending deeply into the dermis and the presence of inflammatory cells (H&E, approximately ×100).

RESULTS

Three days post-burn, the control specimens showed necrotic skin fragments down to the muscle. Small amounts of granulation tissue with variable inflammation and collagen deposition were seen. In animals receiving LPBM at 660 nm, the necrosis extended down to the epidermis and crusting was seen in all specimens. Inflammation was moderate or marked in most specimens. Small amounts of fibroblasts, collagen fibers, and neoangiogenesis were seen in all specimens. In animals receiving LPBM at 780 nm, the necrosis extended down to the subcutaneous tissue (Fig. 1) and inflammation was variable. The amounts of granulation tissue such as fibroblasts and neoangiogenesis were also small in most of the specimens (Fig. 2). At day 5, the number of fibroblasts remained small in control specimens, and collagen deposition and neoangiogenesis were rarely seen at this point. In animals receiving LPBM at 660 nm, the subcutaneous tissue was still necrotic in all specimens, and damage extended down to the subcutaneous tissue. Crusting and moderate to marked inflammation were seen in most specimens. Moderate amounts of granulation tissue exhibiting few fibroblasts, and some collagen deposition and newly formed blood vessels were seen in all cases. In animals receiving LPBM at 780 nm, the necrosis affected the tissue down to the subcuta-

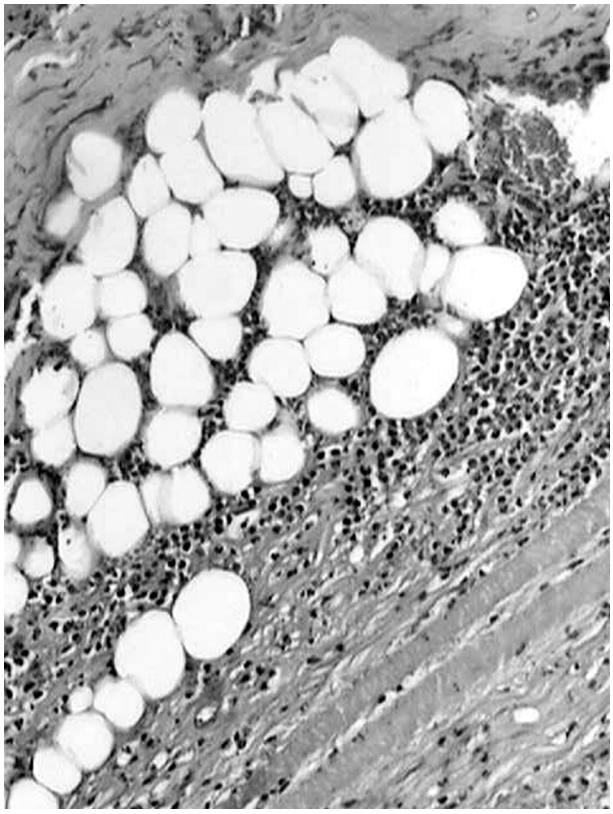

FIG. 3. Photomicrograph of a specimen from an animal receiving LPBM at 780 nm killed 5 d post-burn. Acute inflammatory cells are seen infiltrating the adipose tissue (H&E, approximately ×100).

neous tissues; crusting was rarely seen. Small amounts of granulation tissue composed of fibroblasts and collagen fibers, along with varying levels of inflammatory reaction and neovascularization were seen in all specimens (Fig. 3). Seven days post-

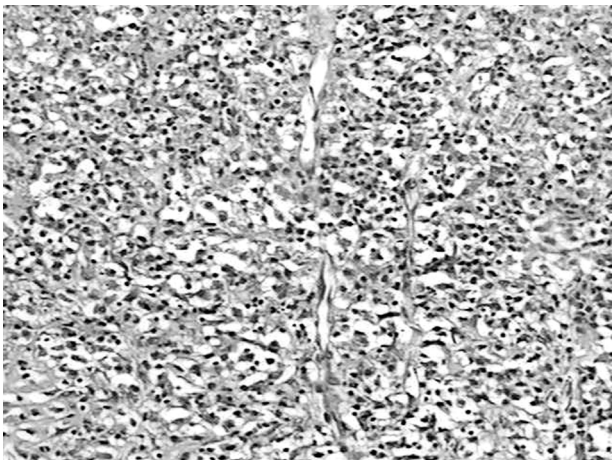

FIG. 5. At day 14, the control specimens exhibited granulation tissue with a mononuclear inflammatory infiltrate, some neutrophils and young fibroblasts, and formation of a small amount of collagen matrix and new blood vessels (H&E, approximately ×100).

burn, thermal necrosis was seen down to the subcutaneous tissues, and adipocytes were seen in the granulation tissue of control specimens; crusting was rarely seen. Inflammation varied from slight to moderate (Fig. 4). Collagen deposition and neovascularization were also slight at this point. In animals receiving LPBM at 660 nm, ulceration remained in most specimens, and crusting was slight to moderate. Moderate amounts of neutrophilic inflammatory infiltrate were seen, and a moderate number of young fibroblasts organizing into strands were seen in most specimens. A moderate number of immature collagen fibers were seen in half the specimens, and small numbers in others. Tortuous, congested newly formed blood vessels were markedly or moderately seen in all specimens. In animals receiving LPBM at 780 nm, the necrosis was still pres-

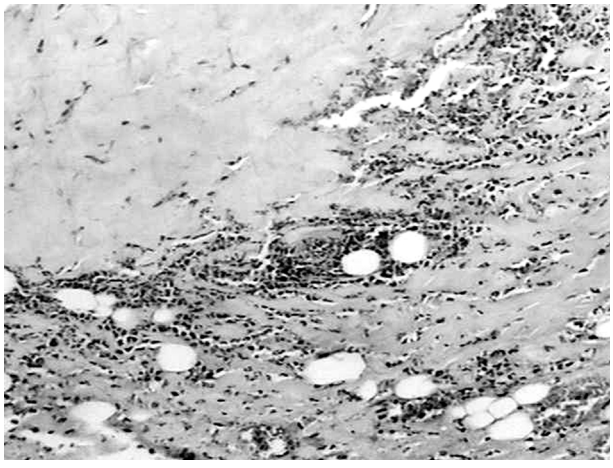

FIG. 4. Photomicrograph of a control specimen 7 d post-burn, showing that necrotic tissue is still visible deeply in the wound, and that this area also has some inflammatory infiltrate (H&E, approximately ×100).

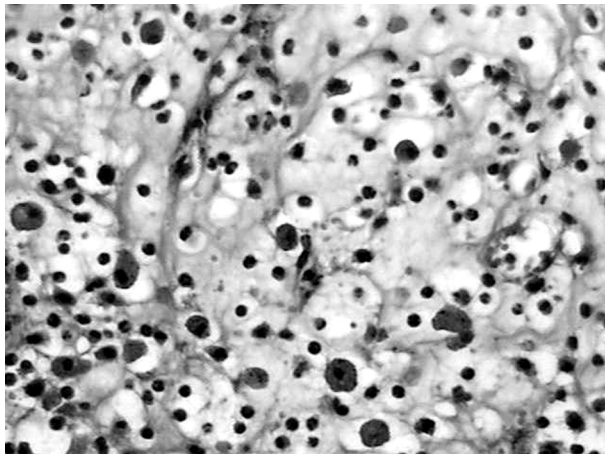

FIG. 6. Photomicrograph of a specimen from an animal receiving LPBM at 660 nm on day 14 post-burn, showing macrophages, lymphocytes, and a few neutrophils scattered in edematous tissue (H&E, approximately ×100).

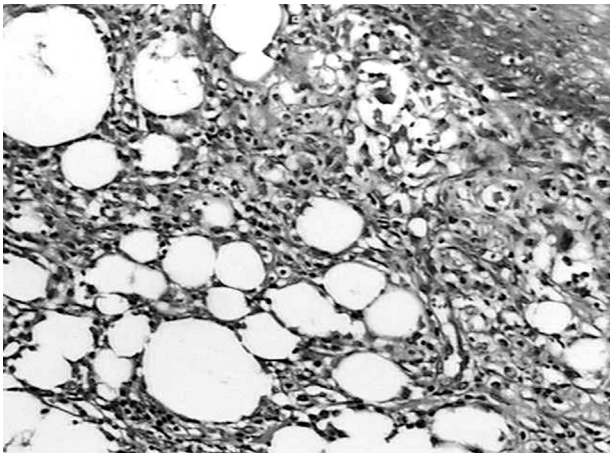

FIG. 7. Photomicrograph of a specimen from an animal receiving LPBM at 780 nm on day 14 post-burn, showing large amounts of granulation tissue with numerous adipose cells superficially, along with some skin repair. In the upper right corner, stratified epithelium can be seen (H&E, approximately ×100).

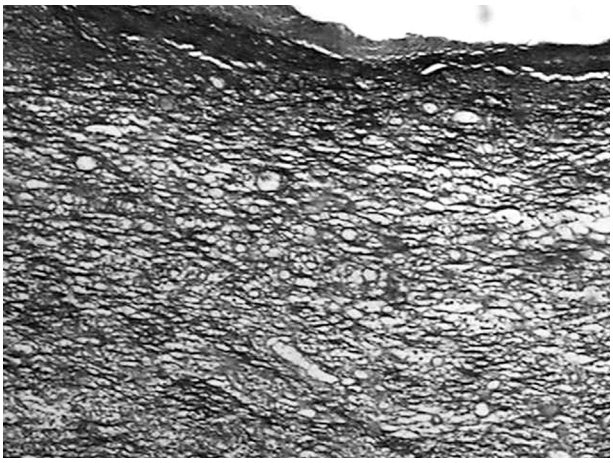

FIG. 9. At the end of the experimental period (day 21 post-burn), granulation tissue covered by a fibrinous crust and deposition of mature collagen fibers parallel to the wound surface were seen in control specimens (Sirius red, approximately ×40).

ent down to the subcutaneous tissues, and some inflammatory cells were also seen. Moderate amounts of granulation tissue with some fibroblasts, along with slight to moderate amounts of collagen deposition were seen in all specimens. All specimens also showed some newly formed blood vessels. At day 14, crusting was seen on nearly all control specimens, and levels of inflammation varied from slight to severe. Marked granulation tissue (Fig. 5), with a moderate number of fibroblasts and small amounts of immature collagen fibers were seen in all control specimens. Neovascularization was moderate. In animals receiving LPBM at 660 nm, ulceration covered by a thin to thick crust remained in most specimens. A marked amount of granulation tissue and moderate amounts of inflammatory infiltrate were also observed (Fig. 6). A moderate number of young fibroblasts forming into strands, and producing a moderate number of immature collagen fibers was seen in most specimens. Tortuous, congested newly formed blood vessels were markedly to moderately present in all specimens. In animals receiving LPBM at 780 nm, extensive ulceration covered by a crust was seen in most specimens. Granulation tissue with newly formed tortuous, congested blood vessels were evident in most specimens. The number of young fibroblasts seen was moderate in most specimens. These were seen in subcutaneous tissues (Fig. 7), and were dispersed in a matrix of collagen fibers in most specimens. These fibers were thick and mature. Acute inflammation was moderately to markedly present in most specimens. Twenty-one days post-burn, wounds in control animals showed extensive ulceration covered by a thin crust, and the dermis showed marked amounts of granulation tissue in all specimens (Fig. 8). The number of young fibroblasts was mod-

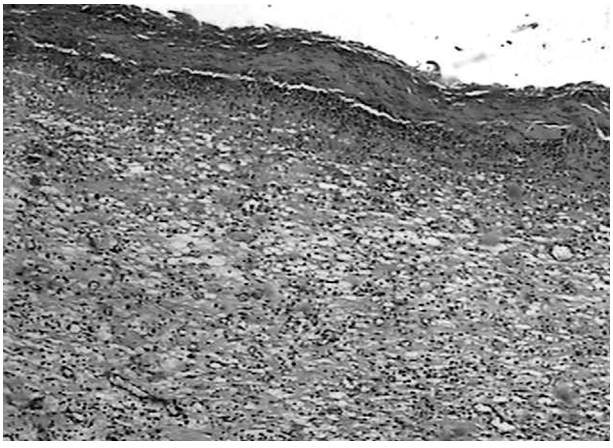

FIG. 8. Photomicrograph of a control specimen at day 21 post-burn, showing granulation tissue covered by a fibrin crust at the wound surface. In the dermis, inflammatory cells can be seen, as well as collagen fibers and newly formed blood vessels (H&E, approximately ×100).

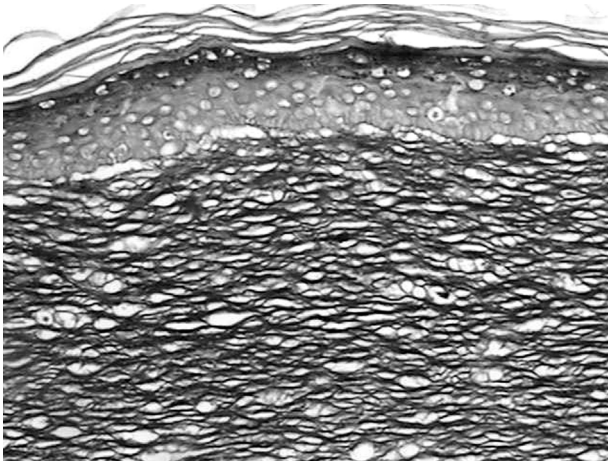

FIG. 10. Photomicrograph of a specimen from an animal receiving LPBM at 660 nm on day 21 post-burn, showing that the collagen fibers were mature and organized, and parallel to the wound surface (Sirius red, approximately ×100).

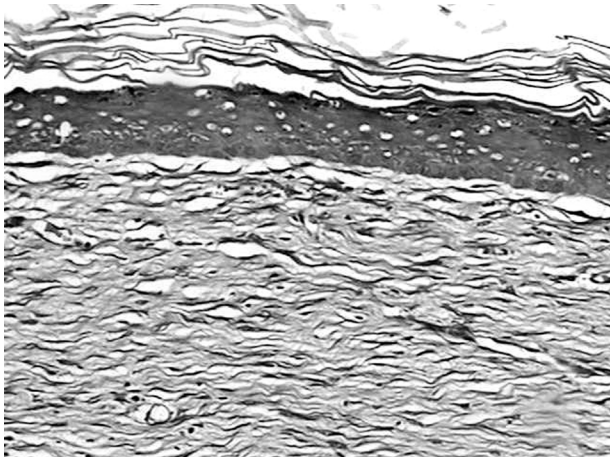

FIG. 11. Photomicrograph of a specimen from an animal receiving LPBM at 660 nm on day 21 post-burn, showing that the dermis was completely covered by epithelium with marked deposition of collagen and low cellularity, along with many newly formed blood vessels (H&E, approximately ×40).

erate, and these were seen parallel to the wound's surface (Fig. 9). In all specimens there were many new capillaries that were sometimes congested, along with varying amounts of mixed inflammatory infiltrate scattered among collagen fibers. In animals receiving LPBM at 660 nm, the ulceration was covered by a crust of varying thickness, along with a large band of tissue containing variable numbers of neutrophils. The amount of granulation tissue and neovascularization was moderate to marked in most specimens, and the number of collagen fibers was variable, as was their thickness (Fig. 10). Complete re-epithelialization was rarely seen (Fig. 11). In animals receiving LPBM at 780 nm, the ulceration was covered by a crust in most cases and re-epithelialization was seen in a few. The dermis showed a large amount of granulation tissue that was rich in newly formed congested blood vessels in all specimens. The number of fibroblasts parallel to the surface was moderate in all specimens. A variable amount of mixed inflammatory exudate was also seen at this stage. These were distributed among a large amount of collagen matrix in all cases (Fig. 12). A summary of the results is presented in Table 2.

DISCUSSION

There are several reports in the literature about the beneficial effects of LPBM on local re-vascularization, edema, pain, and inflammation, as well as on extracellular matrix and collagen deposition and organization.[10,11] In the present study, LPBM was used to treat third-degree burns that deeply affected the skin, and that presented several treatment challenges.

The use of 660-nm laser energy resulted in the appearance of increased amounts of both acute and chronic inflammatory cells. This hastened the inflammatory response, and was effective in stimulating development of granulation tissue, and increased numbers of fibroblasts and collagen fibers, as well as improving neovascularization. These features were seen at early stages of the healing process, and were probably due to the effects of laser energy at a cellular level, as has previously been suggested.[5,6] Later, the effect was more evident on re-epithelialization and on collagen matrix deposition and organization. At day 14, re-epithelialization had already begun in the laser-treated animals, but not in controls. Complete re-epithelialization was seen in irradiated animals at day 21, but not in controls. Re-epithelialization is important, as it restores the integrity of the skin, making it less vulnerable to infection. These features corroborate results of a previous report[12] that assessed several protocols for the treatment of burns in rats. It showed the benefits of 670-nm laser energy at doses ranging from 1–5 J/cm^2. However, doses above 38 J/cm^2 had inhibitory effects.

The use of 780-nm laser energy had positive effects on the granulation process, collagen matrix deposition, fibroblastic activity, and proliferation and neovascularization at days 3, 5, and 7. Positive effects on inflammation could be seen by days 5–7. At the end of the experimental period, the effects on re-epithelialization were quite evident. Maturation of the granulation tissue could clearly be seen on day 21.

One uncommon aspect was seen in our study, namely the presence of adipocytes in more superficial areas of the wounds in subjects irradiated with either wavelength of laser energy. This feature was not seen in control specimens. This phenomenon may be due to transformation of fatty cells into fibroblasts in granulation tissue. This has been suggested by results of a previous study,[13] that suggested that myofibroblasts, fibroblasts, osteoblasts, chondroblasts, and adipocytes have a common origin and share an ability to differentiate into other cell types of the same origin. Another group of researchers[14] pointed out that acute trauma to fatty tissue resulted in structural changes in adipocytes, making them more similar to fibroblasts. Furthermore, in another cellular study,[15] in which adipocytes and fibroblasts were cultured, the lipid components of the cells gradually became similar to those seen in fibroblasts. On the other hand, the fibroblasts were able to produce and retain their lipid components, and at the end of the experiment they had changed into adipocytes. To

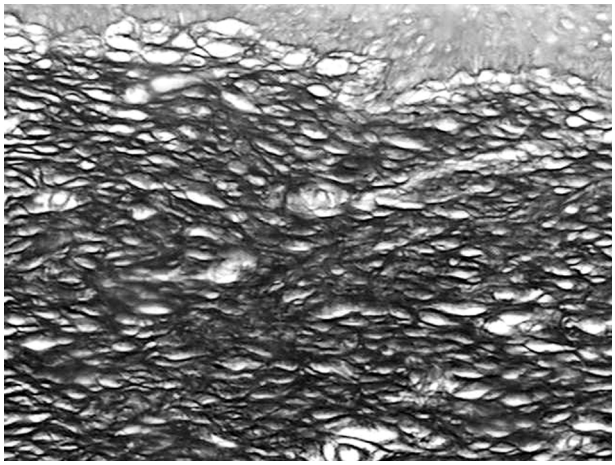

FIG. 12. Photomicrograph of a specimen from an animal receiving LPBM at 780 nm on day 21 post-burn, showing a large amount of mature collagen fibers organized parallel to the surface. The wound was completely covered by epithelium (Sirius red, approximately ×100).

Table 2. Summary of the Results

	3 Days			5 Days			7 Days			14 Days			21 Days		
Group	*G1*	*G2*	*G3*	*G1*	*G2*	*G3*	*G1*	*G2*	*G3*	*G1*	*G2*	*G3*	*G1*	*G2*	*G3*
Acute inflammation	50% slight 25% moderate	50% moderate; 50% intense	25% slight; 25% moderate	25% slight; 50% moderate	75% slight; 25% moderate	50% slight; 50% moderate	25% slight; 50% moderate	75% moderate	25% slight; 50% moderate	25% slight; 50% moderate	50% moderate; 50% intense	75% moderate; 25% intense	25% slight; 50% moderate	50% slight; 25% moderate	100% moderate
Chronic inflammation	50% slight 50% moderate	slight (1) moderate (3)	100% slight	25% slight 25% moderate	50% moderate 50% intense	25% slight 50% moderate 25% intense	75% moderate	75% moderate	50% moderate 50% intense	50% slight; 25% moderate	50% slight; 25% moderate	100% slight	50% moderate; 25% intense	50% moderate; 25% intense	50% moderate; 50% intense
Re-epithelization	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	75% partially present	Absent	<50%–75%	75% covering >50%; 25% present completing covering	75% present; 25%–50% completely covering
Fibroblasts	25% slight	100% slight	100% slight	50% slight	100% slight	75% slight	75% slight	75% slight	75% moderate; 25% intense	75% moderate	75% moderate	75% moderate	75% moderate	75% moderate	100% moderate
Granulation tissue	25% slight	100% slight	75% slight	25% slight	100% moderate	25% intense	75% slight	100% moderate	50% moderate 25% intense	75% intense	100% intense	100% intense	75% intense	50% moderate; 25% intense	100% intense
Collagen	25% slight	100% slight	100% slight	50% slight	100% slight	100% slight	75% slight	100% slight	50% slight 50% moderate	75% slight	25% slight 50% moderate	75% moderate	75% moderate	25% slight; 25% moderate; 50% intense	100% moderate
Neoangiogenesis	25% slight	100% slight	100% slight	25% slight	100% slight	100% slight	75% slight	100% slight	100% slight	50% moderate; 25% intense	25% moderate; 50% intense	75% intense	75% intense	50% moderate; 25% intense	100% intense

the best of our knowledge, this was the first report of the ability of LPBM to induce fat cells to change into fibroblasts. This is significant, because fibroblasts are important for healing due to their ability to secrete collagen.

CONCLUSION

Analysis of the results presented here demonstrates that damaged tissues are able to efficiently absorb and utilize laser energy of both wavelengths tested. It appears that LPBM at 660 nm can achieve better results at earlier stages of healing. On the other hand, the use of 780-nm laser energy had beneficial effects throughout the experimental period, and transformed the newly formed tissue rapidly into new dermis.

We conclude that the use of LPBM at both 660 and 780 nm was effective in stimulating wound healing in third-degree burns in rats.

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