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ORIGINAL ARTICLE

LED phototherapy in full-thickness burns induced by CO_2 laser in rats skin

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Abstract

Many studies have been conducted on the treatment of burns because they are important in morbidity and mortality. These studies are mainly focused on improving care and quality of life of patients. The aim of this study was evaluate the LED phototherapy effects in rats skin full-thickness burns induced by CO_2 laser. The animals were divided in NT group that did not received any treatment and LED group that received LED irradiation at 685 nm, 220 mW, and 4.5 J/cm^2 during 40 s by burned area. Biopsies were obtained after 7, 14, and 21 days of treatment and submitted to histological and immunohistochemical analysis. The LED phototherapy shows anti-inflammatory effects, improves angiogenesis, and stimulates the migration and proliferation of fibroblasts. The T CD8+ lymphocytes were more common in burned areas compared to T CD4+ lymphocytes since statistically significant differences were observed in the LED group compared to the NT group after 7 days of treatment. These results showed that LED phototherapy performs positive influence in full-thickness burns repair from the healing process modulated by cellular immune response. The obtained results allowed inferring that burns exhibit a characteristic cell immune response and this cannot be extrapolated to other wounds such as incision and wounds induced by punch, among others.

Keywords CO_2 laser · Full-thickness burns · LED phototherapy · Wound healing · Histology · Immunohistochemistry · Lymphocytes T CD4+ and T CD8+

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Introduction

Burns are lesions caused by thermal energy that cause partial or total destruction of the cutaneous tissue and attachments, reaching deeper portions such as the subcutaneous tissue, muscles, tendons, and bones [1, 2], and can trigger several local and systemic reactions that cause severe complications for the patient [3, 4], among which infections that frequently lead to septicemia and death [1, 2, 5, 6].

Despite progress in burn treatment, mortality and morbidity rates are still significant [1, 4–7]. According to the World Health Organization (WHO), burns cause 300,000 deaths in the world annually [8, 9]. In Brazil, there are one million new cases per year, which generates an average cost of 25 million reais for the government [1, 2, 10].

Chiarotto et al. [2014] report that different therapeutic approaches have been used to achieve an acceptable outcome

for the treatment of burns, which include the use of dressings, drugs, polymers, stem cells, and phototherapy [11].

Phototherapy using light-emitting diode (LED) has recently been investigated as therapeutic modality for burns due to its photobiomodulation effects on the tissue repair process. Authors claim that LED phototherapy accelerates wound healing as it promotes angiogenesis and local circulation and stimulates cell proliferation of epithelial, endothelial, keratinocyte, macrophage, lymphocyte, and fibroblast cells, as well as the synthesis of collagen, elastin, and ATP [12–17]. They also report that LEDs can provide an optional therapeutic resource to conventional treatments or used in combination with the latter, offering the advantage of low cost and proven efficiency in the treatment of ulcers and other diseases [13, 14, 18].

The purpose of the present study was to perform LED phototherapy on CO_2 laser-induced full-thickness burns on rat skin and observe tissue changes in lesions during the tissue repair process.

Materials and methods

Animals

Thirty-three male Wistar strain rats (*Rattus norvergicus albinus*), aged 90 days and weighing 250–300 g, were used. During the experimental phase, the animals were kept in separate boxes under natural conditions of illumination and room temperature and fed with laboratory standard diet and water ad libitum.

Induction of burns

A CO_2 laser (Synrad Model J48-5-2848, USA) with wavelength of 10.600 nm in continuous mode was used to induce three full-thickness burns in each animal according to a previously published protocol [19]. The optical power was 1.3 W; the laser beam diameter was 3.5 mm and the irradiated surface was 0.096 cm^2. The animals were anesthetized with intraperitoneal injection of ketamine hydrochloride (Quetamina Vetnil Rhobifarma, Hortolandia, SP, Brazil; 125 mg/kg) and xylazine hydrochloride (Xilazin Syntec Rhobifarma, Hortolandia, SP, Brazil; 7 mg/kg) and then submitted to the trichotomy of the dorsal region where the target areas were demarcated which were continuously irradiated by the CO_2 laser during 120 s.

Experimental groups

The animals were divided into two groups: NT group (no treatment, $n = 16$) and LED group (treated with LED phototherapy, $n = 17$).

The NT group received no treatment and was sacrificed after 7, 14, and 21 days. A LED device (StarLaser Microdont®, PM100D, ThorLabs, Newton, NJ, USA, emission at $\lambda = 685$ nm, power of 220 mW, energy density of 4.5 J/cm^2, $\varnothing = 1.58$ cm) was used to treat burns of the LED group. Contact irradiation was applied on each burn for 40 s being that light beam covered the burned area as well as surrounding margin. The irradiated surface was 1.95 cm^2 and any movement was performed during irradiation. The sessions were performed on a daily basis for 21 days and the animals were sacrificed after 7, 14, and 21 days.

Biopsies were obtained immediately after the animals were sacrificed, fixed in 4% paraformaldehyde (Sigma Aldrich Chemical, Saint Louis, MO, USA) for 48 h, and mounted in paraffin (Paraplast Plus® Sigma-Aldrich Chemical, St. Louis, MO, USA) for histological and immunohistochemical analyses.

Histological analysis

For each biopsy, four 5-μm-thick histological sections were obtained and stained with hematoxylin and eosin (H.E) for histomorphological and histomorphometric assessments.

A descriptive histomorphological analysis was performed from light microscopy (Leica Microsystems, Model DM LB2, Wetzlar, Germany). For the histomorphometric analysis, photomicrographs of the histological sections (×40 magnification) were obtained with the aid of the microscope coupled to a photographic camera (Leica Microsystems, Model DFC280, Cambridge, UK). The recorded images were analyzed with the aid of the Image J software (National Institutes of Health, Bethesda, MD, USA) from the count of the mononuclear inflammatory cells, blood vessels, and fibroblasts present in the burn healing areas at the different periods (7, 14, and 21 days). Six photomicrographs were obtained from each histological section (superficial, intermediate, and deep regions) in which fields of study measuring 100 μm × 100 μm were delimited for the count.

Immunohistochemical analysis

For each biopsy, four 3.5-μm-thick histological sections were obtained and distributed onto silanized slides. The indirect method with peroxidase-biotin-streptavidin amplified systems was used for immunohistochemical reaction [20, 21]. Antigen retrieval was performed in a microwave using Tris buffer, pH 7.6, while peroxidase inhibition was performed using hydrogen peroxide (20 volumes) added to absolute methanol (1:1). Anti-rat monoclonal CD4 antibody (C2255-02R1, USBiological, Swampscott, MA, USA) incubated for 12 h and anti-rat CD8 antibody (C2259-14P, USBiological, Swampscott, MA, USA) incubated for 2 h were used. The secondary antibody LSAB+Sys-HRP (K069011-2, Dako, Glostrup, Denmark) and liquid DAB substrate-chromogen system

(K346811-2, Dako, Glostrup, Denmark) were used for immunohistochemical development. The sections were counterstained with Mayer's hematoxylin (Merck, Darmstadt, Germany).

For the histomorphometric analysis, photomicrographs of the histological sections (×40 magnification) were obtained with the aid of the microscope coupled to a photographic camera (Leica Microsystems, Model DFC280, Cambridge, UK). The recorded images were analyzed with the aid of the Image J software (National Institutes of Health, Bethesda, MD, USA) from the count of the CD4+ and CD8 T lymphocytes present in the burn healing areas at the different periods (7, 14, and 21 days). Six photomicrographs were obtained from each histological section (superficial, intermediate, and deep regions) in which fields of study measuring 100 μm × 100 μm were delimited for the count.

Statistical analysis

The results obtained from the histomorphometric analysis were performed using the Instat software® (GraphPad Software, Inc., CA, USA), analysis of variance (ANOVA), and Tukey-Kramer post-test, with a critical value of $P < 0.05$.

Results

Histomorphological analysis

The histomorphological observations of the burned areas of the NT and LED groups are summarized in Table 1.

In the NT group, the presence of a large amount of necrotic tissue and mild epithelial proliferation under it after 7 days was observed. Coagulation necrosis of the skin, congestive vessels followed by small areas of edema, and dispersed inflammatory cells were also observed. After 14 days, the epithelium was more pronounced under the crust and a large number of inflammatory cells close to this region were observed. A large number of inflammatory cells were found in the dermis and the presence of neoformed blood vessels was moderate. Inferiorly, hypertrophied muscle fibers with vacuolated appearance, cytoplasmic breakdown, and edema were observed. After 21 days, the crust was still present and, under it, the epithelium was thin and with few cells. In the dermis, blood vessels were present and the amount of inflammatory and fibroblast cells was moderate. Inferiorly, the muscle fibers were few in number and below them, there was a large amount of loose connective tissue. Figure 1 shows the histomorphological aspects of the burned areas of the NT group after 7 (a and b), 14 (c and d), and 21 days (e and f).

In the LED group, after the 7-day period, a large amount of necrotic tissue and, under it, moderate epithelial proliferation from the margin of the burn were observed. Inflammatory cells were observed in the dermis underlying epidermis. In the dermis, numerous congestive blood vessels, intense edema, and numerous inflammatory cells were noted. Very thin or small-diameter muscle fibers were observed in the panniculus carnosus. After 14 days, the epithelial proliferation was more pronounced, although necrotic tissue was still present. The superficial and deep dermis was rich in inflammatory and fibroblast cells and numerous blood vessels were also observed. In the panniculus carnosus, fine muscle fibers were

Table 1 Histomorphological observations of the burned areas of the NT and LED groups after 7, 14, and 21 days

Periods (days)	NT	LED
7	Presence of necrotic tissue Mild epithelial proliferation Few congestive blood vessels Mild edema Dispersed inflammatory cells Coagulation necrosis	Presence of necrotic tissue Moderate epithelial proliferation Numerous congestive blood vessels Intense edema Numerous inflammatory cells Neoformed muscle fibers thin or small-diameter
14	Presence of necrotic tissue More pronounced epithelial proliferation Numerous inflammatory cells in superficial and deep dermis Moderate neoformed blood vessels Hypertrophied muscles fiber with cytoplasmic breakdown	Presence of necrotic tissue More pronounced epithelial proliferation Numerous inflammatory and fibroblasts cells in superficial and deep dermis Numerous neoformed blood vessels Fine muscle fibers
21	Presence of necrotic tissue Thin epithelium with few cells Moderate blood vessels Moderate inflammatory and fibroblast cells Neoformed connective tissue Few neoformed muscle fibers	Absence of necrotic tissue Keratinized epithelium with thin thickness layers Presence of epithelial crests and conjunctival papillae Neoformed connective tissue Few blood vessels Numerous muscular fibers with thin aspect and homogenous cytoplasm

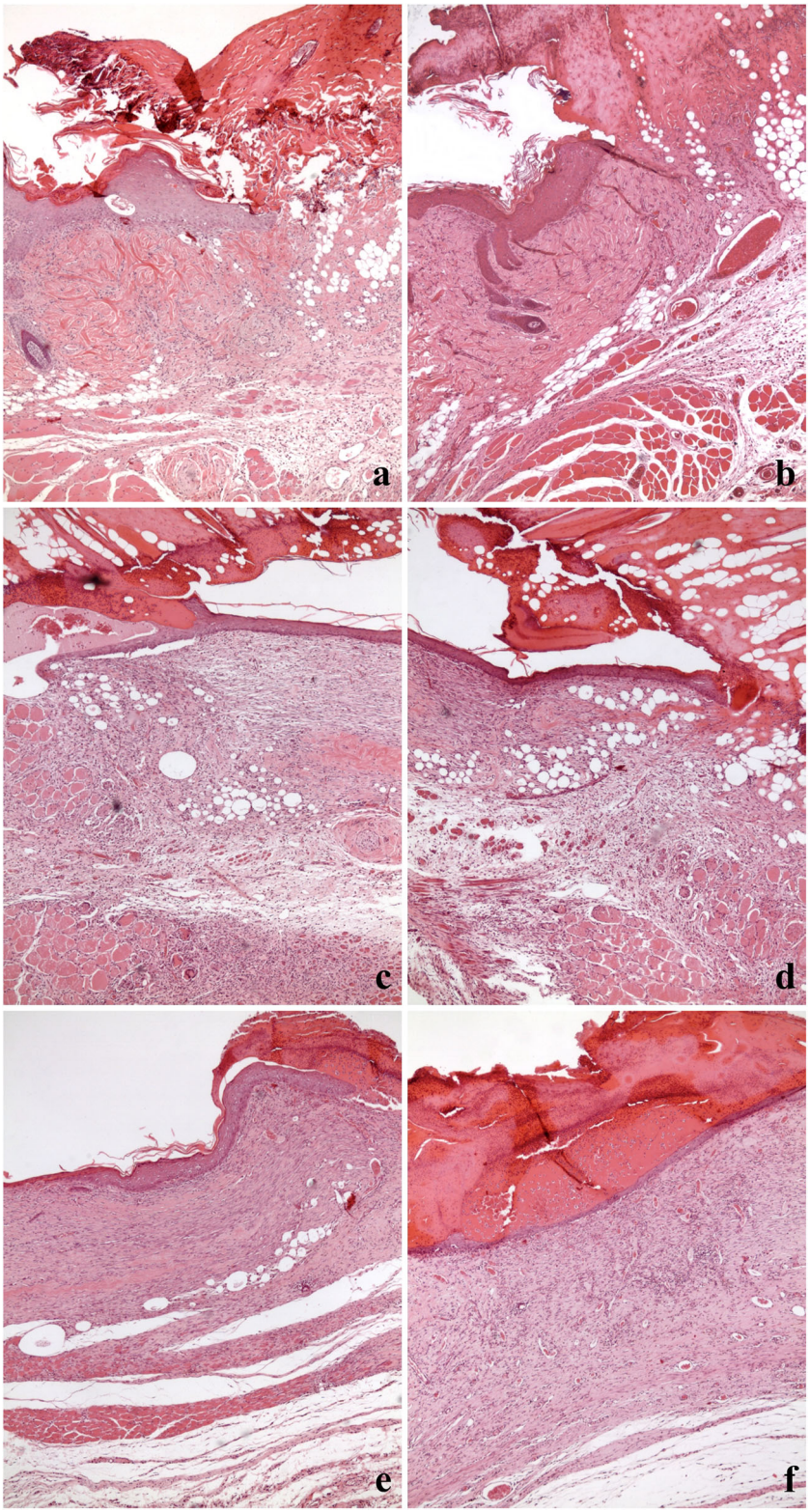

Fig. 1 Photomicrographs of burned areas in animals that did not receive treatment (NT Group). **a** and **b** show morphological aspects after 7 days, **c** and **d** after 14 days, and **e** and **f** after 21 days (H.E., 87.5×)

noted. After 21 days of treatment, the crust was absent and the continuous coating of the keratinized epithelium was observed with thin thickness layers. At the interface with neoformed connective tissue, the epidermis was rectilinear and in other regions was slightly sinuous with an early formation of epithelial crests and conjunctival papillae. In the connective tissue, few blood vessels were observed. The presence of numerous muscular fibers with thin aspect and homogenous

cytoplasm was noticed inferiorly. Figure 2 shows the histomorphological aspects of the burned areas in the LED group after 7 days (a and b), 14 (c and d), and 21 days (e and f) of LED phototherapy.

Histomorphometric analysis

Table 2 shows the values obtained from the counts in the burned areas (mean ± standard deviation) at the different study periods of the NT group and LED group.

The number of mononuclear inflammatory cells in the burn healing areas was higher in the LED group than in the NT group after 7 days of study ($P < 0.01$). In the NT group, there was an increase in the number of mononuclear inflammatory cells during the study periods and this number of cells was maintained after 14 and 21 days. On the other hand, in the LED group, there was a gradual reduction in the number of cells throughout the study periods.

The number of blood vessels was higher in the LED group in all study periods and a higher number of vessels were observed after 14 days when compared to 7-day period ($P < 0.01$). Although the number of blood vessels in the NT group also increased during all study periods, no significant statistical differences were found.

The number of fibroblasts was higher in the LED group than in the NT group at all study periods and statistically significant after 7 days ($P < 0.01$). The fibroblasts presented the same pattern of behavior in the NT group and LED group, that is, they were present in a large number after 7 days, a greater number after 14 days, and a reduced number after 21 days.

The number of fibroblasts was statistically higher in the 14-day period than in the 7-day period in the NT group ($P < 0.05$) and significantly lower within 21-day period ($P < 0.01$). In the LED group, significant statistical differences showing a reduction in the number of fibroblasts were observed between 14 and 21 days ($P < 0.05$).

Immunohistochemical analysis

Table 3 shows the values obtained from the counts of CD4+ T lymphocytes and CD8+ T lymphocytes in the burned areas (mean ± standard deviation) in the NT group and LED group at the different study periods.

Regarding the CD4+ T lymphocytes, it was possible to observe that the increase in the number of cells was gradual during the periods of study in the NT and LED groups. However, no statistically significant differences were observed between the groups.

In the NT group, there were no significant differences in the number of cells between 7 and 14 days after induced burn wounds. However, the number of CD4+ T lymphocytes significantly increased after 21 days when the two time intervals were compared (7 vs 21 days $P < 0.001$; 14 vs 21 days $P < 0.01$).

The number of CD4+ T lymphocytes in the LED group significantly increased during the study period (7 vs 21 days $P < 0.001$) and a significant increase was observed between the time intervals of 7 and 14 days ($P < 0.05$).

Figure 3 shows the presence of CD4+ T lymphocytes in the burned areas in the NT and LED groups during the different study periods.

Comparing both groups regarding the study periods, the number of CD8+ T lymphocytes was higher and statistically significant after 7 days in the LED group when compared to the NT group ($P < 0.01$). In addition, there was a gradual increase of these cells throughout the periods after induced burn wounds in the NT group (7, 14, and 21 days) whereas a reduction was observed in the LED group between 7 and 14 days.

Figure 4 shows the presence of CD8+ T lymphocytes in the burned areas of the NT and LED groups at different study periods (7, 14, and 21 days).

Discussion

The number of mononuclear inflammatory cells was statistically significant ($P < 0.01$) in the LED group in comparison to the NT group in the 7-day period (11 ± 7 vs 45 ± 2). Thus, it can be affirmed that the LED stimulated the migration and proliferation to the burn healing areas, which is in agreement with the studies conducted by Fiório et al. [2011] and Neves et al. [2014] [22, 23]. This may have occurred due to a large number of CD8+ T lymphocytes (6 ± 3 vs 23 ± 7), stimulated from the LED irradiation, that were observed in the same study period; the presence of these in the inflammatory focus increases the activation of macrophages [24].

An increase in the number of mononuclear inflammatory cells in the NT group was observed during the study periods, whereas a reduction of these cells was found in the LED group indicating an anti-inflammatory effect of LED. This result is in agreement with the observations of Catão et al. [2016] who induced burns on the dorsum of rats and found an anti-inflammatory effect after treatment with LED [25].

A significant increase in the number of CD4+ T lymphocytes was observed during study periods in the NT group (7 vs 21 days $P < 0.001$), which was predominant in the 21-day period compared to the previous period ($P < 0.01$). The same condition was observed in the LED group (7 vs 21 days $P < 0.001$); however, the predominance of CD4+ T cells occurred within the 14-day period compared to the 7-day period ($P < 0.05$), suggesting that the LED accelerated the migration, proliferation, and differentiation of these cells.

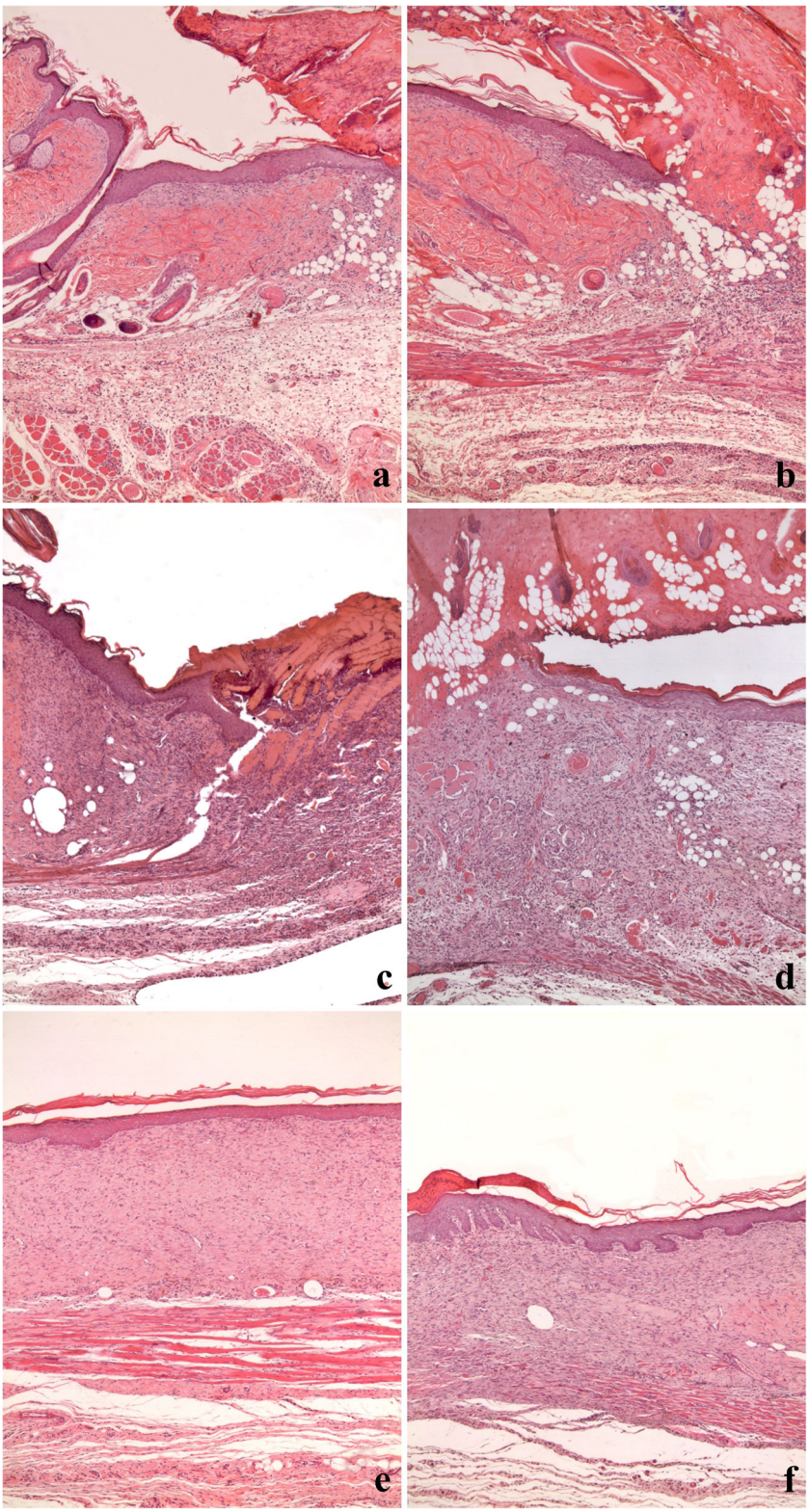

Fig. 2 Photomicrographs of burned areas in animals that received LED phototherapy after 7 days (**a**, **b**), 14 (**c**, **d**), and 21 days (**e**, **f**) (H.E; 87.5×). λ = 685 nm; power 220 mW; energy density 4.5 J/cm^2; time 40 s/area

The immunohistochemical analysis of CD4+ and CD8+ T lymphocytes suggested that the LED promoted the biomodulation of the cellular immune response and that this effect was more significant within the 7-day period after treatment as there was a statistically significant increase in the number of CD8+ T lymphocytes in the LED group when compared to the NT group ($P < 0.01$).

Table 2 Analysis of mononuclear inflammatory cells, blood vessels, and fibroblasts of the burned areas in the NT group and LED group

Periods (days)	Mono inflam cells		Blood vessels		Fibroblasts	
	NT	LED	NT	LED	NT	LED
7	11 ± 7	45±21[a]	5 ± 4	11 ± 6	375 ± 89	545±54[c]
14	24 ± 10	38 ± 24	16 ± 11	26±9[b]	524±92[d,e]	606±87[f]
21	26 ± 12	27 ± 4	12 ± 5	15 ± 4	344 ± 37	448 ± 44

Values are expressed as mean ± standard deviation of the mean

[a] NT group 7 days vs LED group 7 days ($P < 0.01$)

[b] LED group 7 days vs LED group 14 days ($P < 0.01$)

[c] NT group 7 days vs LED group 7 days ($P < 0.01$)

[d] NT group 7 days vs NT group 14 days ($P < 0.05$)

[e] NT group 14 days vs NT group 21 days ($P < 0.01$)

[f] LED group 14 days vs LED group 21 days ($P < 0.05$)

The cellular immune response is based on the principle that antigen-presenting cells (in this case, macrophages and epidermal dendritic cells—Langerhans) contain epitopes (MCH II) to the CD4+ T cells; the IL-12 released by antigen-presenting cells promote the differentiation of CD4+ T cells in Th1, which in turn activate macrophages and CD8+ T lymphocytes. The CD8+ T lymphocytes recognize epitopes (MCH I) and eliminate the target cell after recognition, especially since they have been activated by Th1 cells through IFNγ (also released by NKC). Another important cytokine possibly involved in this process is IL-2 (released by Th0 and Th1) that exerts a mitogenic effect and activator of Th1 and T CD8+, which would justify the significant increase of CD8+ T cells after 7 days of LED phototherapy in comparison to the group that did not receive treatment [25–27].

In the present study, there were no statistically significant differences between the NT and LED groups regarding the number of blood vessels at the different study periods. Despite this, a higher frequency of blood vessels was observed in the animals that received LED phototherapy and this was statistically significant in the 14-day period when compared to 7-day period ($P < 0.05$). These results are in accordance with previous studies that found that the increase in the number of blood vessels in the LED-treated groups was higher than in the groups that received no treatment [28, 29].

In the study of Neves et al. [2014], significant differences were observed in the number of blood vessels in the animals treated with LED in comparison to the untreated group after induced full-thickness burns within the 14-day period and no significant differences were observed between the different study periods in the same group [23]. Corazza et al. [2007] compared the number of blood vessels present in punch-induced skin wounds in untreated animals and treated with LED phototherapy and observed that the number of blood vessels was statistically significant at periods 3, 7, and 14 days in the group that received phototherapy [29] The relative divergence of results between these two studies is probably related to the etiology of the lesion, since a statistically significant increase in the number of blood vessels in punch-induced LED-treated skin wounds was found in the study of Souza et al. [2013] when compared to the control group [30].

As previously mentioned, Neves et al. [2014] [23] did not observe significant differences in the number of blood vessels in the animals that received LED phototherapy between the study periods. In their experiment, the LED phototherapy sessions were performed at 48 h intervals, so the results obtained in the present study indicate that the 24-h interval between sessions was more effective for the formation of new blood vessels in the initial healing/repair of full-thickness burns since the wavelength and dosimetry protocols were similar in both studies.

In addition, angiogenesis was favorably stimulated by LED irradiation during the study periods and, based on results, it is suggested the active participation of macrophages and CD4+ T cells (Th1) that produce important cytokines for the process, among them, EGF, VEGF, and TGFα [24].

Table 3 Analysis of CD4+ and CD8+ T lymphocytes of the burned areas in the NT group and LED group

Periods (days)	CD4+ T lymphocytes		CD8+ T lymphocytes	
	NT	LED	NT	LED
7	0.5 ± 0.5	0.5 ± 8	6 ± 3	23±7[e]
14	3 ± 2	7 ± 6[c]	11 ± 4	15 ± 7
21	15 ± 8[a,b]	13 ± 2[d]	17 ± 5	19 ± 9

Values are expressed as mean ± standard deviation of the mean

[a] NT group 7 days vs NT group 21 days ($P < 0.001$)

[b] NT group 14 days vs NT group 21 days ($P < 0.01$)

[c] LED group 7 days vs LED group 14 days ($P < 0.05$)

[d] LED group 7 days vs LED group 21 days ($P < 0.001$)

[e] NT group 7 days vs LED group 7 days ($P < 0.01$)

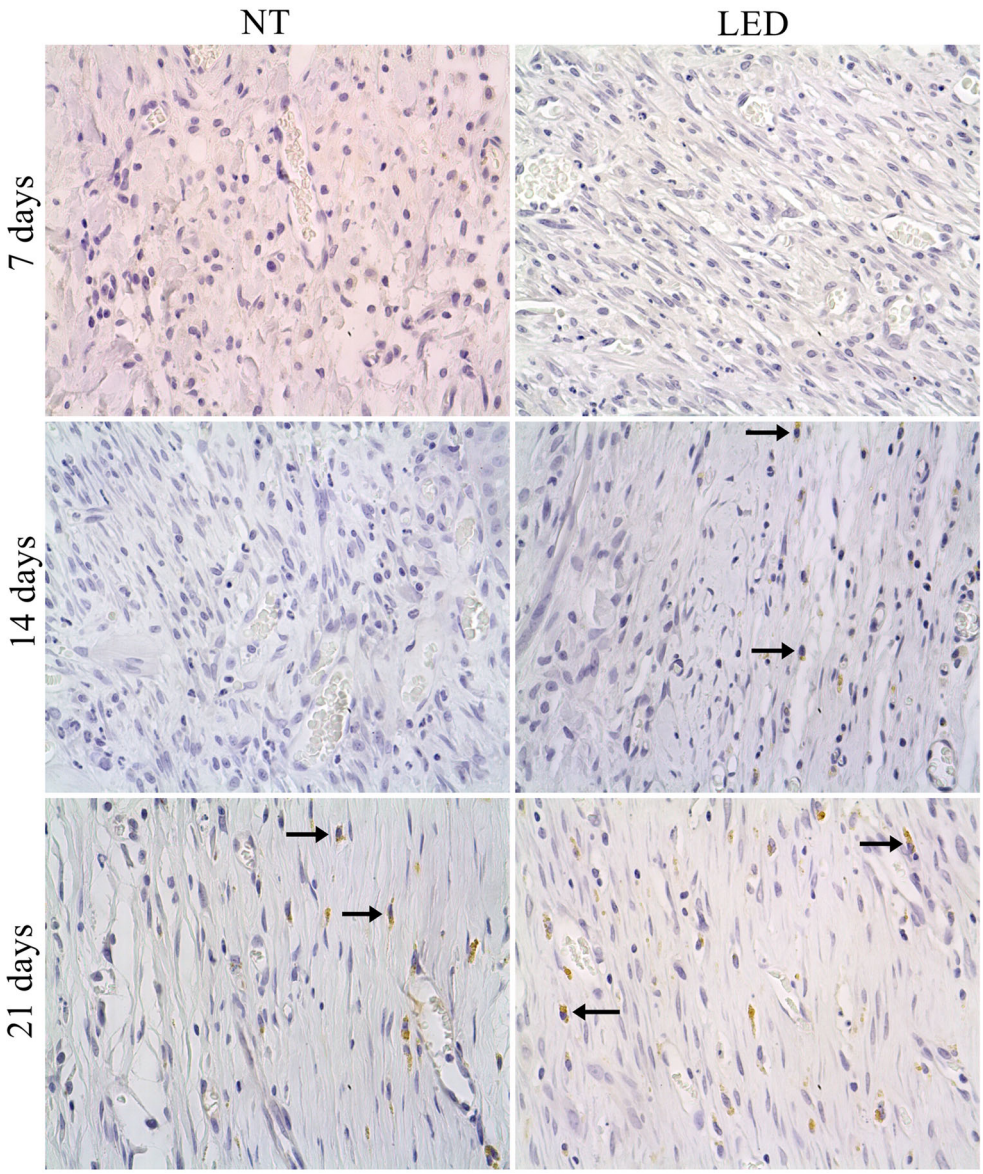

Fig. 3 Photomicrographs showing the immunolabeling of CD4+ T lymphocytes (arrows) in the burned areas of the NT and LED groups after 7, 14, and 21 days (700×)

Thus, the results of the present study indicate that LED improved microcirculation, as well as improved nutritional support in the burned areas during inflammatory and tissue repair processes.

LED phototherapy increased the number of fibroblasts in all study periods compared to the group that did not receive treatment, indicating that LED potentiates the synthesis of the connective tissue matrix. This evidence was statistically significant after 7 days of treatment ($P < 0.01$). Thus, it is suggested that the activity of macrophages and CD4+ T lymphocytes for release of FGF-like cytokines during this period was intense [24].

Significant reductions in the number of fibroblasts were observed in the NT and LED groups ($P < 0.01$ and $P < 0.05$, respectively) between 14 and 21 days periods. This observation indicates the beginning of the maturation phase of the tissue as mononuclear inflammatory cells, CD4+ and CD8+ T lymphocytes, and blood vessels are still observed moderately. These results are in agreement with those obtained by Catão et al. [2015], who applied daily LED phototherapy ($\lambda = 520–550$ nm) on full-thickness burns induced on the dorsum of rats and observed that the LED irradiation significantly stimulated the proliferation and maturation of collagen within the 3, 7, 14, and 21-day periods, thus favoring the tissue repair process [31].

Neves et al. [2014] and Dall Agnol et al. [2009] also found that LED increased the number of fibroblasts in full-thickness burns and in punch-induced skin wounds, respectively [23, 28]. However, both studies did not observe statistical significance in this regard. In the first study, the burns were treated at 48-h intervals and in the second, only one LED phototherapy session was performed, 30 min after the surgical incision. This

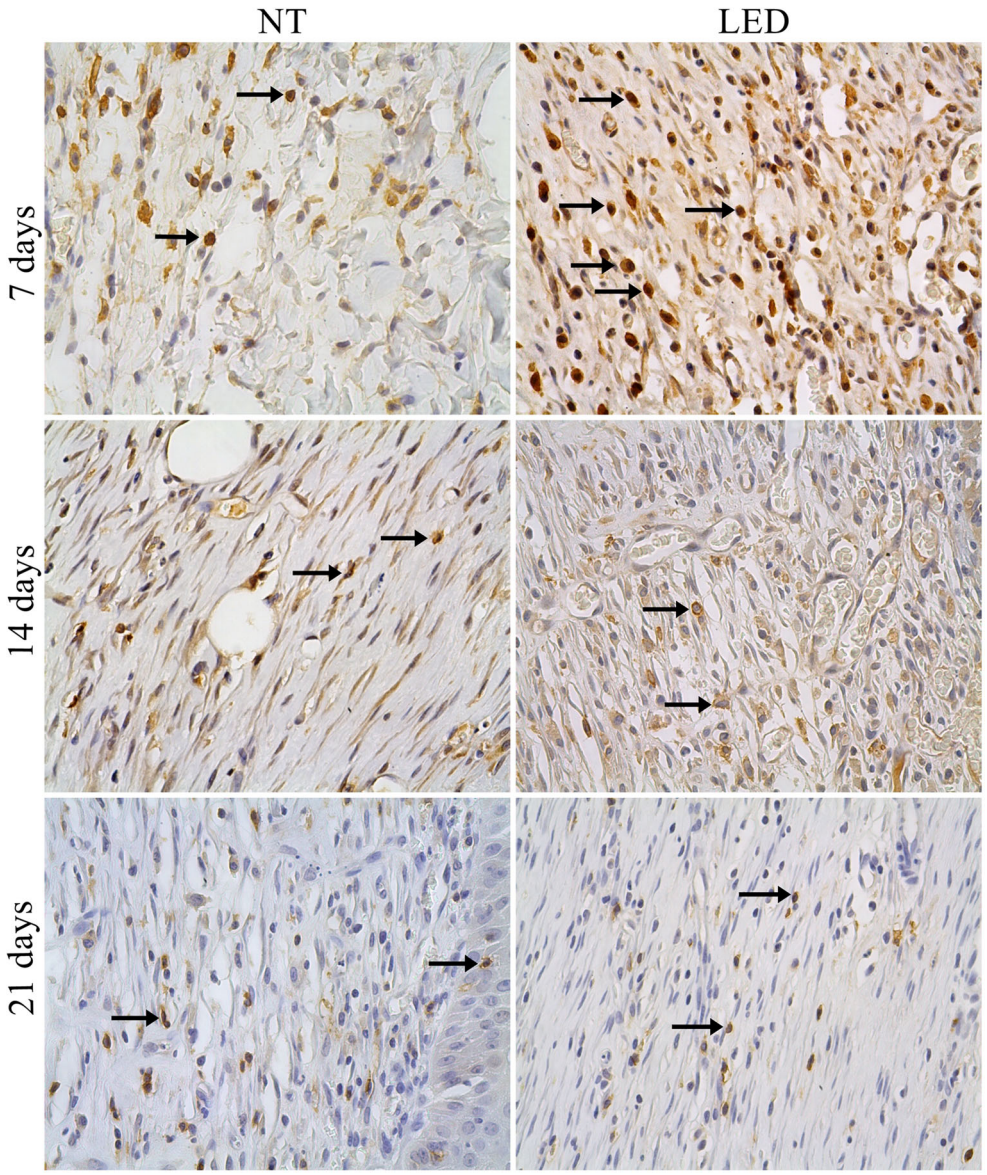

Fig. 4 Photomicrographs showing the immunolabeling of CD8+ T lymphocytes (arrows) in the burned areas of the NT and LED groups after 7, 14, and 21 days (700×)

shows that the daily LED phototherapy sessions used in the present study had beneficial effects on the fibroblast response to full-thickness burns.

In the present study, the LED phototherapy effect on the tissue repair of full-thickness burns induced by CO_2 laser was evaluated. The results showed that the LED exerted a beneficial influence on the different phases of cicatrization and that biomodulation occurred due to the anti-inflammatory effect on the mononuclear inflammatory cells as well as the increase in the number of blood vessels and fibroblasts. These results are in agreement with previous studies that used LED phototherapy on burns [22, 23, 28–31] and studies that used laser for the same purpose [2, 11, 29–32]. Within this context, some studies have shown that both non-coherent light (LED) and coherent light (LASER) are able to stimulate angiogenesis evenly during burn healing [17, 28–30].

It was also found that LED exerted a beneficial influence on the immune response as it significantly increased the number of T lymphocytes, particularly those of the CD8+ type. These results are in agreement with those obtained in studies that evaluated the frequency of CD4+ T and CD8+ T cells after burns. Buchanan et al. [2006] induced skin burns on rats and found that CD8+ T lymphocytes proliferated more rapidly than CD4+ T cells whose proliferation was radically inhibited by the burn; they suggested that there may be inherent fragility of CD4+ T lymphocytes after burns or a high dependence on their cytokine-associated proliferation when compared to CD8+ T cells; they concluded that CD8+ T lymphocytes play an important role in the immune response in burn wounds [33]. In another study conducted in patients by Kokhaei et al. [2014], significant increase of CD8+ T lymphocytes and reduction of CD4+ T lymphocytes when burns (20–60%

total body surface area) were treated with cimetidine, a drug recognized for its property of potentiating the immune response, were observed [34].

The results of the present study are not in accordance with studies that assessed the participation of CD4+ T and CD8+ T lymphocytes in other types of wounds. Davis et al. [2001] investigated the frequency of CD4+ T and CD8+ T lymphocytes in laparomized rats and found that the number of CD4+ T lymphocytes was statistically higher than CD8+ T lymphocytes between the pre-laparotomy and sacrifice periods (70 days) [35]. Chen et al. [2014] found that the number of CD4+ T cells at 5, 7, 10, and 21 days was significantly higher than CD8+ in punch-induced wounds on rat skins [36]. Therefore, it is suggested that burn wounds have a characteristic immune response profile, which makes it impossible to compare them with wounds of different etiologies.

These considerations allow to infer that the present study showed that it is not possible to extrapolate the results obtained in the treatment of burns for other types of wounds, as the burn wounds present a characteristic profile regarding the immunological response that differs from the other wounds, such as incisional wounds and experimental punch wounds. Consequently, tissue healing and repair processes are likely to have peculiar characteristics among each other.

Conclusion

Phototherapy with LED reduced the number of mononuclear inflammatory cells and increased the number of blood vessels and fibroblasts in full-thickness burns. In addition, the number of CD4+ T lymphocytes and, more significantly, the number of CD8+ T lymphocytes increased, showing the effect of photobiomodulation on the cellular immune response.

Further studies involving induced burn wounds are important to better understand the biomodulation of the effect of LED phototherapy on these lesions.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures in the animals of this study were in accordance with the ethical standards and were carried out with the approval of the Research Ethics Committee of the São José dos Campos Dental School, Institute of Science and Technology of the Paulista State University (ICT-UNESP), São José dos Campos, SP, Brazil (Protocol No 10/2011-PA/CEP).

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