# Modulation of fibroblast proliferation and inflammatory response by low-intensity laser therapy in tissue repair process*

# *Modulação da proliferação fibroblástica e da resposta inflamatória pela terapia a laser de baixa intensidade no processo de reparo tecidual**

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**Abstract:** BACKGROUND - Several studies have been performed to understand the tissue repair process as well as the possible effects of laser therapy in wound healing.
OBJECTIVES - To study the behavior of skin wounds induced in the dorsal region of Wistar rats (Rattus norvegicus), which were submitted to the low-intensity laser therapy at 3.8 J/cm² dosage, 15mW potency, during 15 seconds.
MATERIAL AND METHODS - The animals (n=12) were divided into two groups - control and laser-treated. The latter comprised three applications (immediately after surgery, 48 hours and 7 days after induction of surgical wounds). Ten days after surgery samples of the wounds were collected and submitted to histopathological and histomorphometric studies.
RESULTS - Neovascularization, fibroblast proliferation and reduced inflammatory infiltrate in surgical wound submitted to laser therapy were demonstrated.
CONCLUSION - Taken together, the results suggest that low-intensity laser therapy is an effective method to modulate tissue repair, thus significantly contributing to a faster and more organized healing process.
Keywords: Laser therapy, low-level; Rats; Wound healing

***Resumo:*** *FUNDAMENTOS - Ao longo dos anos, diversos estudos têm sido realizados para compreender o processo de reparo tecidual, bem como os possíveis efeitos da terapia a laser no processo de cicatrização de feridas.*
*OBJETIVOS - Investigar o comportamento de feridas cutâneas provocadas na região dorsal de ratos Wistar (Rattus norvegicus), que foram submetidos ao tratamento com laser de baixa intensidade, com 3,8 J/cm² de dosagem, 15mW de potência e tempo de aplicação de 15s.*
*MÉTODOS – Os animais (n = 12) foram divididos em dois grupos, um controle e outro tratado com laser. Foram realizadas, no grupo tratado, três aplicações (imediatamente após o ato cirúrgico, 48 horas e sete dias após a realização das feridas cirúrgicas). Dez dias após o ato cirúrgico foram colhidas amostras das lesões de ambos os grupos para realização de estudo histopatológico e histomorfométrico.*
*RESULTADOS - Foram evidenciados aumentos da neovascularização e da proliferação fibroblástica, e diminuição da quantidade de infiltrado inflamatório nas lesões cirúrgicas submetidas à terapia com laser.*
*CONCLUSÃO - Os resultados em conjunto sugerem que a terapia a laser de baixa intensidade é um método eficaz no processo de modulação da reparação tecidual, contribuindo significativamente para a cicatrização tecidual mais rápida e organizada.*
*Palavras-chave: Cicatrização de feridas; Ratos; Terapia a laser de baixa intensidade*

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Conflict of interest: None

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## INTRODUCTION

Tissue repair is a dynamic state that encompasses different processes, among which inflammation, cell proliferation and synthesis of elements which constitute the extracellular matrix, such as collagen, elastin and reticular fibers.[1] Collagen synthesis is a fast and harmonic process that starts with the interstitial lesion and extends up to the end of the healing phase, when the remodeling of the tissues occurs.[2]

The scarring and tissue repair processes occur after trauma or disease.[3] Wound repair and restructuring constitute a complex mechanism, in which several factors contribute to a variety of scarring types, such as hypertrophy, atrophy or normotrophy of the injured area. These processes comprise three phases: inflammation, granulation and formation of the extracellular matrix.[4] Normally, in the wound healing process, after the granulation phase starts, there is a slight predominance of macrophages and an increase in the number of fibroblasts, with synthesis of a new extracellular matrix, and remodeling of these tissues occurs with a contraction of the granulation tissue. During the matrix formation phase, the fibroblasts produce great amounts of extracellular matrix. Collagen synthesis occurs on the 21st day after the lesion and the return of the skin to its normal aspect on the 26th day.[5] Once the wound is resolved and involved by granulation tissue, a significant decrease in macrophages and fibroblasts occurs, and the maturation of the scar becomes relatively acellular.[6]

Radiation and is based on a theory developed by physicist Albert Einstein who, in his article "Zur Quantumtheorie der Strahlung", of 1917, presented the physical principles of stimulated emission (the laser phenomenon), which is classified as "high-power" (with destructive potential) or "low-power" (without destructive potential).[7] This therapy was first used by Mester et al., who used 488 and 515nm argon laser. Subsequently, the helium-neon (He-Ne) laser was introduced, which emits red light with a wavelength of 632.8nm, and was replaced by a less expensive and more powerful device, the diode laser, with a wavelength of 660-950nm.[8] Experimental treatments in patients started in the 1970's, following reports of positive results obtained by irradiation with low-intensity laser therapy (LILT) of cell cultures and in animal experiments. The studies performed were insufficient to confirm the beneficial effects of LILT.[9,10] effects appeared,[11] but failed due to the great number of interventions and to unsatisfactory quality of methodology.

Several studies have been conducted in order to understand the wound healing process, aiming to clarify the different aspects of the granulation tissue, of tissue epithelization and neoformation, as well as the possible effects of LILT in the tissue repair process. This study had the objective of evaluating, by means of a histopathologic and histomorphometric analysis, the clinical-biological behavior of skin wounds induced in the dorsal area of Wistar rats (*Rattus norvegicus*) submitted to LILT. In particular, the effects of LILT on angiogenesis, fibroblast proliferation and the inflammatory infiltrate were analyzed.

## MATERIAL AND METHODS

### Animals

Twelve male Wistar rats (*Rattus norvegicus*), six to eight weeks old, weighing 160 to 220 g, provided by the Animal House of the Centro de Biologia da Reprodução – CBR [Reproduction Biology Center] of the Universidade Federal de Juiz de Fora (UFJF/MG), were used. The rat housing at CBR-UFJF has large dumping containers with wire screens and two exhausters, besides room heaters. The temperature is kept at around 22ºC, by natural ventilation in summer and with the help of heaters in winter. The lighting is mixed – natural light and fluorescent light bulbs, automatically controlled to get on at 6:00 a.m. and off at 6:00 p.m. The animals were kept in individual polypropylene cages, equipped with beds of selected wood shavings, baby bottles with water and troughs for palletized chow, under maintenance conditions which are in agreement with the criteria of the Colégio Brasileiro de Experimentação Animal [Brazilian College of Animal Experimentation], and submitted to daily macroscopic evaluations in order to observe signs indicating secondary infection.

The animals were randomly divided into two groups, one (I) submitted to surgical skin wounds (n=6), and the other (II) to surgical skin wounds and low-intensity laser therapy (n=6).

In the treatment protocol used for this study, group I was kept as control and, in group II, laser treatment was performed; the whole experiment lasted 10 days.

### Experimental modelc

The dorsal area of the animals was shaved after anesthesia with intraperitoneal ketamine (100ml/kg) + xylazine (10mg/kg) and, by means of a punch of approximately 10mm in diameter, a circular fragment of skin tissue was removed.

The treatment was carried out using low-intensity laser (Twin Laser) with the following characteristics: infrared emission laser, pulsatile, arsenium and gallium semiconductors, wavelength of 870nm, peak power of 70mW, mean exit power of 0.5 to 3.5mW and application through fiber optics. The application was done by the scanning method in the central area of

the wound, thus allowing its uniform treatment. In the control group, no kind of treatment was used. In group II, the experimental wounds were submitted to LILT with the following parameters: 15mW of power, a 3.8J/cm$^2$ dose for 15s on each one of three applications, the first one immediately after surgery, the second one 48 hours after the surgical procedure, and the third one seven days after performing the surgical lesion.

After 10 days from the surgery and after laser application, the animals were sacrificed with an overdose of anesthesia with intraperitoneal ketamine (100ml/kg) + xylazine (10mg/kg). The samples from the skin lesions were collected so that part of the skin adjacent to the wound rims and the entire scar tissue in all its depth were included.

**Histopathology and histomorphometry**

All skin lesion samples obtained were fixed in 10% buffered formalin (pH 7) for at least 24 hours. After fixation, the samples were gradually dehydrated in increasing concentrations of ethanol (70% to 100%), cleared in xylene, soaked and embedded in paraffin, according to routine histological methods. The paraffin-embedded fragments were cut with an "820" Spence microtome and 6?m thick sections were obtained. The histological slides were kept in an incubator to dry, and then the sections were stained with hematoxylin and eosin for histological analysis.

Histomorphometry was performed using images captured and evaluated by a computerized Axion Vision (Zeiss, Berlin, Germany) image capture system. Images were captured from four randomly chosen microscopic fields for each histological slide, using the digital camera (total enlargement 400x) of an Axiostar Plus microscope (Zeiss, Berlin, Germany). The images were stored and submitted to a count of inflammatory cells, evaluation of fibroblast proliferation, analysis of the local angiogenesis and of the diameter of the ulcerated areas of the wounds at the end of the experiment, using digital marking.

**Statistical analysis**

The statistical analysis of the several parameters evaluated in the two groups was made by a nonparametric method, the Mann-Whitney test, with a significance level of $p<0.05$.

**RESULTS**

The clinical observation of skin lesion samples of the animals showed a small amount of clot on the surface, with clear presence of blood vessels in the deep hypodermis. These vessels, when accidentally ruptured in the dermis or hypodermis region, filled the lesions partially or totally with clots.

On the 10$^{th}$ postoperative day, the skin lesions of group I (control) exhibited an early-phase tissue repair pattern, with formation of a whitish crust, with slightly elevated rims and a reddish core due to the accentuated presence of blood irrigation in that area, indicating the presence of granulation tissue. On the other hand, the wounds of group II, which had been submitted to low-intensity laser treatment, showed complete tissue repair, exhibiting scars with evident rims and a central portion slightly unleveled, but presenting an advanced morphological and functional recovery of the involved tissues.

The animals, placed in individual cages under maintenance conditions which are in agreement with the criteria of the Colégio Brasileiro de Experimentação Animal, were submitted to daily macroscopic evaluations and presented no signs indicating secondary infection during the experiment.

In the analysis of the histological results, two distinct areas were evaluated, one more superficial, involving epithelial proliferation, and the area of connective tissue beneath the more superficial portion of the lesion. In the control group, next to the rim of the surgical wound, a discrete epithelial proliferation was observed, and virtually all over its extension the presence of tissue exhibiting a broad ulceration area and fibrinonecrotic material over granulation tissue was seen (Figure 1). However, in group II, the one submitted to laser therapy, the histopathological study evidenced material showing undamaged epidermis covering well-developed granulation tissue (Figure 2), with connective tissue rich in collagen fibers with a parallel orientation with regard to the surface of the wound, determining a more organized tissue repair process.

Graph 1 presents the diameter of the ulcerated areas on the 10th postoperative day, the treated animals having shown a significant reduction in the diameters of the ulcerated areas at the end of the experiment, as compared to the control group ($p<0.05$). As for vascularization, the treated animals presented a significantly greater number of blood vessels per microscopic field (Graph 2).

The histomorphometric analysis also demonstrated that the animals treated with LILT presented a greater number of fibroblasts per microscopic field (Graph 3), where the identification of fibroblasts, based on morphological criteria, was made by two different researchers. Moreover, it was observed that LILT significantly reduced the intensity of the inflammatory infiltrate present in the lesions submitted to treatment (Graph 4).

**DISCUSSION**

Low-intensity laser treatment is a method that is accepted by the Food and Drug Administration

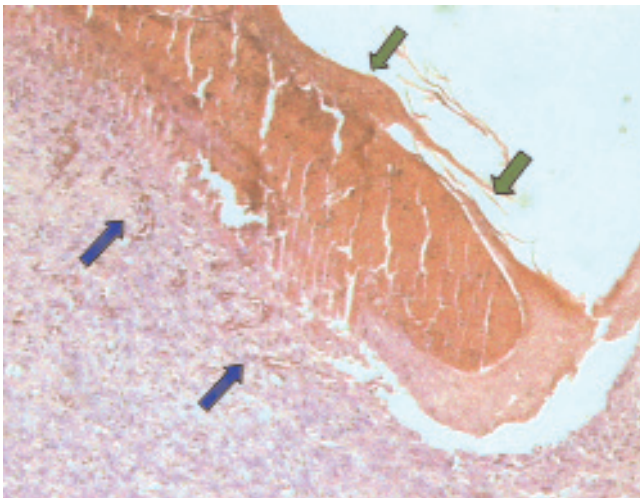
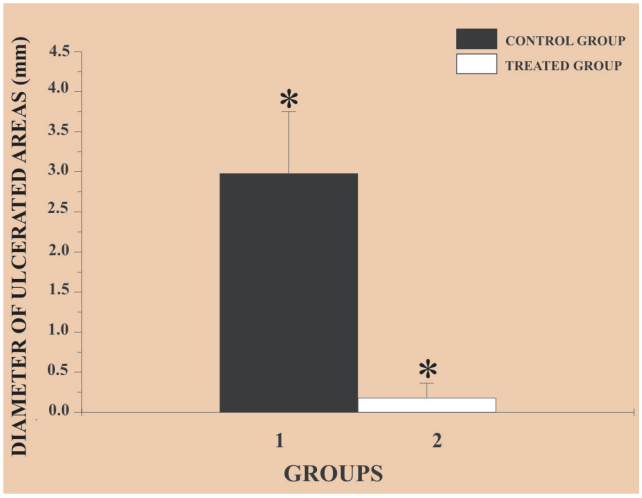


**Figure 1**: Skin sample of animal of the control group exhibiting a broad ulceration area and fibrinonecrotic material (green arrows) over granulation tissue (blue arrows). HE staining, total magnification 50 x

**Graph 1**: Diameter of ulcerated areas in skin lesions (10th postoperative day)




Results expressed in means ± standard deviation - $p<0.05$

(FDA) as an effective clinical treatment for tissue healing, as it has already been widely studied.[12,13] *In vitro* studies suggest that LILT favors collagen synthesis,[14] increases the motility of keratinocytes,[15] releasing growth factors,[16] besides transforming fibroblasts into myofibroblasts.[17]

Several studies used superficial wounds for evaluating the effects of LILT on wound healing. Some of them used ulcers of different sizes and depths,[12,18,19] and others developed superficial wound models in animals.[20-22] These different methods produced a variety of results and conclusions about the effects of LILT. In this study, we observed that the surgical lesions submitted to low-intensity laser treatment, when compared to the lesions in the control group, showed a better developed tissue repair process, with greater wound contraction and higher epithelial migration speed.

Several authors reported on clinical trials which showed the benefits of LILT in tissue healing, but others found none of these effects.[20,23] The conflicting data from the literature give rise to plausible doubts about variations in the treatment factors and limitations in experimental designs, including the comparison between clinically heterogeneous wounds, the need for control groups and the limita-

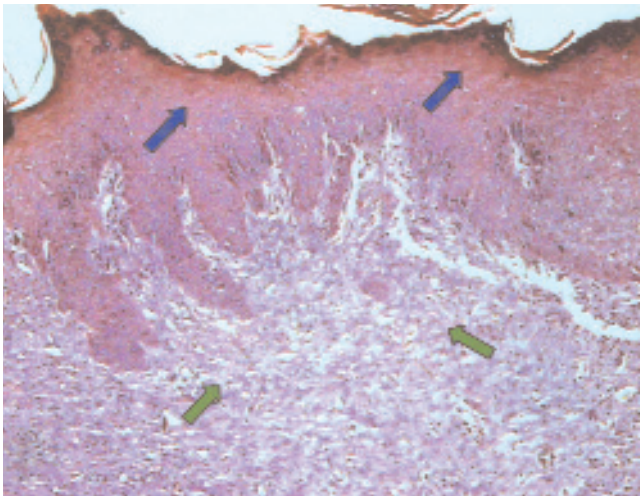
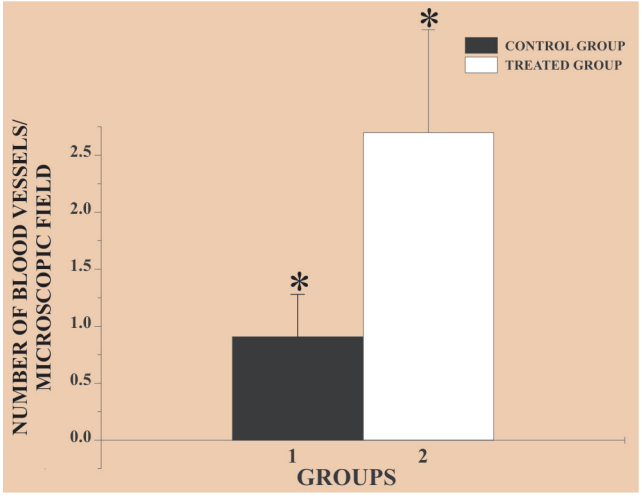


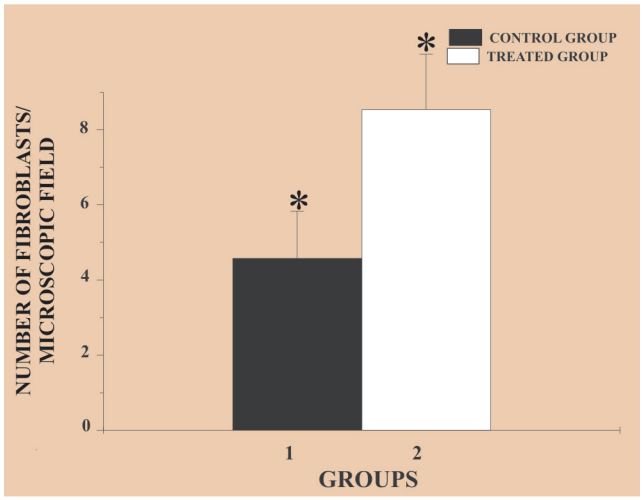
**Figure 2**: Skin sample of a treated animal showing undamaged epidermis with hyperplasia (blue arrows), covering well developed granulation tissue (grren arrows). HE staining, total magnification 50 x

**Graph 2**: Number of blood vessels (10th postoperative day)




Results expressed in means ± standard deviation - $p<0.05$

**Graph 3**: Total number of fibroblasts (10th postoperative day)




Results expressed in means ± standard deviation - $p<0.05$

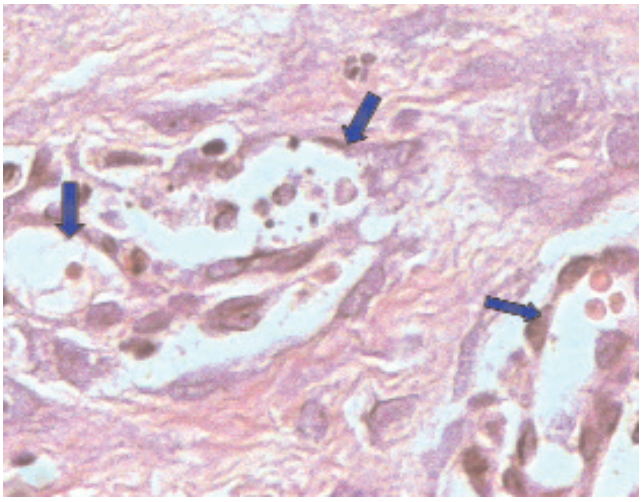


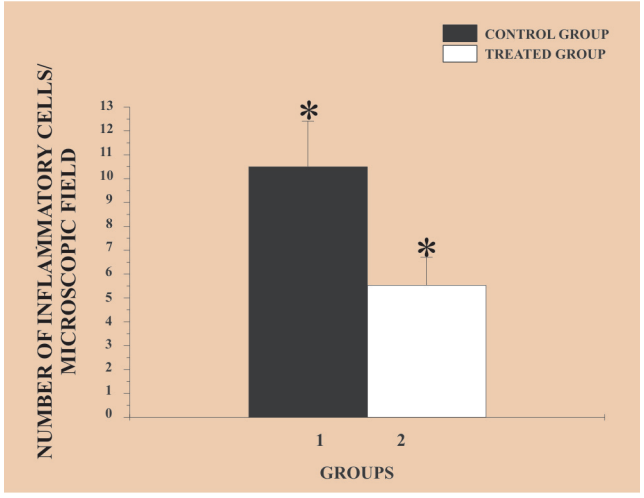
**Figure 3**: Connective tissue showing few blood vessels in animal of the control group (blue arrows). HE staining, total magnification 400 x

tions faced in the investigation of such contradictory results. LILT is still a controversial method, as can be seen in numerous publications,[8,24,25] mainly because its mechanism of action is unknown.

The field of action of laser is very broad and the studies showed its contribution to the tissue repair process, particularly regarding its influence on the modulation of certain cell types in the wound healing process. Gómez-Villamandos et al. reported an increase in wound healing after laser therapy, with increased mitotic activity, number of fibroblasts, collagen synthesis and neovascularization of the injured tissues.[26] Other authors[16,27] observed that the production of fibroblast growth factors (FGF) and the predominance of fibroblasts in the culture increased considerably after low-intensity laser irradiation. Furthermore, Bisht et al. reported the development of granulation tissue and epithelization of wounds in Wistar rats treated with He-Ne laser.[28]

In the present study, we evaluated vascular proliferation and observed a smaller amount of blood vessels in the surgical lesions of the control group (Figure 3) compared to those of the treated group (Figure 4), where an increase in vascular proliferation was found in the surgical wound samples, which was also observed when fibroblast proliferation in both

**Graph 4**: Number of inflammatory cells (10th postoperative day)



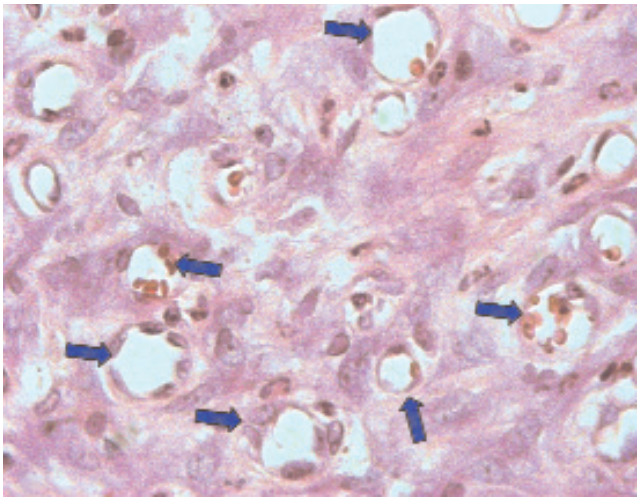



**Figure 4**: Connective tissue showing many blood vessels in animal of the treated group (blue arrows). HE staining, total magnification 400 x

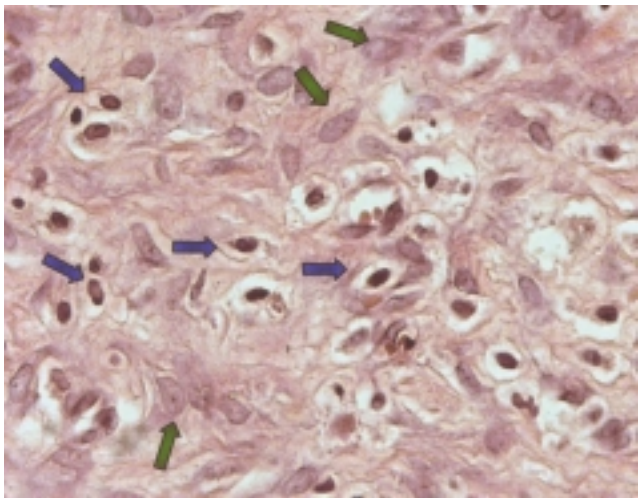


**Figure 5**: Connective tissue showing a smaller number of fibroblasts (green arrows) and a large amount of mononucleated inflammatory cells in the surgical wound of an animal of the control group (blue arrows). HE staining, total magnification 400 x

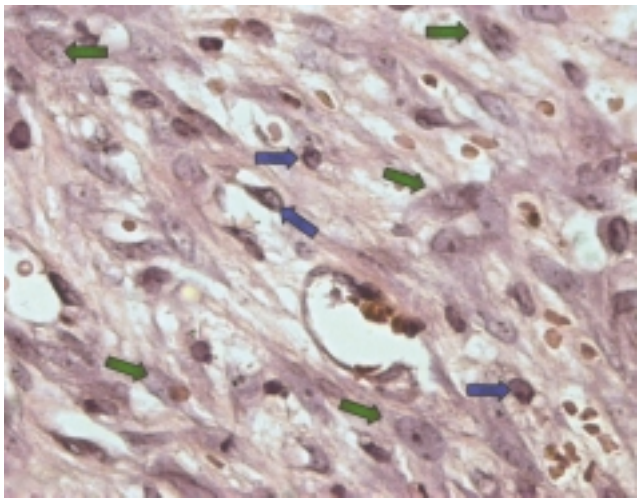


**Figure 6**: Connective tissue showing a large number of fibroblasts (green arrows) and few mononucleated inflammatory cells in the surgical wound of animal of the treated group (blue arrows). HE staining, total magnification 400 x

groups was evaluated. In the control group samples, a smaller number of fibroblasts were observed (Figure 5) compared to those from the treated group, where a significant increase in fibroblast proliferation was found (Figure 6).

Moreover, when the amount of inflammatory cells present in the lesions of both groups was analyzed, a greater number of such cells were observed in the control group samples (Figure 5) compared to those from the treated group (Figure 6), which exhibited a significant decrease of inflammatory infiltrate.

These findings suggest a faster tissue repair in the group submitted to LILT, expressed by an increase in cell proliferation and vascularization, besides an expressive decrease in the number of inflammatory cells.

## CONCLUSION

Taken together, the results suggest that LILT is effective in tissue wound healing, as observed in an experimental model of surgical wounds produced in rats. This type of laser therapy showed positive effects, speeding up tissue proliferation, increasing local vascularization and forming a more organized granulation tissue. ❑

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