Effect of low level laser on sutured wound healing in rats[1]

Efeito do laser de baixa potência na cicatrização de ferida cirúrgica em ratos

Valdinaldo Aragão de Melo[I], Denise Cristina Santos dos Anjos[II], Ricardo Albuquerque Júnior[III], Daniel Barreto Melo[IV], Fábio Ursulino Reis Carvalho[V]

[1] Research performed at Postgraduate Program, Department of Medicine, Federal University of Sergipe, Brazil. *Part of Master degree Thesis.*

[I] Full Professor, Department of Experimental Surgical, Federal University of Sergipe, Aracaju-SE, Brazil. *Tutor, conception and design of the study and critical revision.*
[II] Fellow Master degree, Postgraduate Program in Health Sciences, Federal University of Sergipe, Aracaju-SE, Brazil. *Intelectual and scientific contente of the study.*
[III] Full Professor, Department of Pathology of Institute of Technology and Research, Tiradentes University, Aracaju-SE, Brazil. *Histological examinations*.
[IV] Graduate student, Faculty of Physical Education, Federal University of Sergipe, Aracaju-SE, Brazil. *Helped with technical procedures.*
[V] Graduate student, Faculty of Medicine, Federal University of Sergipe, Aracaju-SE, Brazil. *Helped with technical procedures.*

ABSTRACT
Purpose: To evaluate the effect of low-level laser therapy (LLLT) ë904 nm on healing of surgical wounds in rats. **Methods**: Forty male Wistar rats were used, divided into four groups, underwent incision along the lines Alba covering skin, subcutaneous and muscle abdominal, sutured continuously for nylon 5-0. Eight and fifteen days after the surgery process, the repairing area was removed and histological sections were stained with hematoxylin-eosin to assess cellularity inflammatory, Masson´s Trichrome and Picrossirus to quantify the collagen fibers and immunohistochemical technique for counting newly formed vessels. The data were compared statistically using analysis of variance ANOVA, with a "post-hoc Tukey test, p <0.05. **Results**: Low-level laser therapy reduced the intensity of the inflammatory reaction and influenced the dynamic of the immunoinflammatory response by inducing switching of the leukocyte infiltration pattern (neutrophilic to lymphoplasmacytic infiltration). Also stimulate the deposition and enhance the organization of collagen fibers, featuring a delicate collagen type III. Furthermore, it appeared to a significant increase in the average number of newly formed vessels (p = 0.00 and p = 0.02, respectively). **Conclusion**: Low-level laser therapy resulted in modulate of the inflammatory response, enhanced deposition of collagen fibers and increase in the average number of newly formed vessels.
Key words: Wound Healing. Lasers, Semiconductor. Collagen. Macrophages. Rats.

RESUMO
Objetivo: Avaliar o efeito da terapia laser de baixa potência (TLBP) ë904 nm na cicatrização de feridas cirúrgicas em ratos. **Métodos**: Foram utilizados 40 ratos Wistar, machos, divididos em quatro grupos, submetidos à incisão na linha Alba abrangendo pele, subcutâneo e musculatura abdominal, suturados continuamente por fios de nylon 5-0. Oito e quinze dias após o procedimento cirúrgico, a área de reparo foi removida e os cortes histológicos foram corados com hematoxilina-eosina para avaliar celularidade do infiltrado inflamatório, Tricrômico de Masson e Picrosirius para quantificar as fibras de colágeno e técnica imuno-histoquímica para a contagem de vasos neoformados. Os dados foram comparados estatisticamente pela análise de variância ANOVA, com "teste de Tukey post-hoc, p <0,05. **Resultados**: A terapia laser de baixa potência reduziu a intensidade da reação inflamatória e influenciou a dinâmica da resposta imuno por induzir a mudança do padrão de infiltração de leucócitos (neutrófilos a infiltração mononuclear). Como também estimula a deposição e melhora padrão organizacional das fibras colágenas, apresentando um delicado colágeno tipo III. Além disso, verificou-se um aumento significativo no número médio de vasos neoformados (p = 0,00 e p = 0,02, respectivamente). **Conclusão**: A terapia laser de baixa potência modula a resposta inflamatória, com melhor deposição de fibras colágenas e aumento do número médio de vasos neoformados.
Descritores: Cicatrização de Feridas. Lasers Semicondutores. Colágeno. Macrófagos. Ratos.

Introduction

The discontinuity of tissue integrity can be induced either by trauma or by surgical procedures. In this regard, numerous chemicals are released into the injured area causing tissue changes consistent with an acute inflammatory process. This phenomenon is characterized by stimulation, migration and proliferation of stromal cells, synthesis of extracellular matrix, and finally culminating in tissue remodeling[1-3].

Currently, there are methods to alleviate the inflammatory process, providing the improvement of cellular nutrition and favoring the injured tissue repair, particularly the low level laser therapy (LLLT)[2,4]. The LLLT has been clinically used for pain control and wound healing. The effectiveness of this therapy is due to responses induced by the cellular level photobiomodulation promoting acceleration of cellular metabolism, reducing inflammation, increasing local microcirculation and the lymphatic system, leading to the decrease of the interstitial edema and increase of the collagen synthesis[4-7].

This biological activity of LLLT is probably a result of photo-induced acceleration of electron transfer in the respiratory chain and consequently the increase of mitochondrial ATP synthesis. Thus, this electronically excited state of molecules is supposed to promote enhance of cellular metabolism in a several tissues and improve a variety of physiologic and physiopathologic processes, such as wound healing[7].

Due to the extensive controversy observed in the literature concerning the role played by phototherapy on stromal and inflammatory cells involved in the cicatricial repair, the goal of this study is to analyze the effect of a specific protocol of low-energy Laser irradiation on the intensity of the inflammatory response, deposition of collagen fibers and number of newly formed vessels during two different phases of wound healing in rodents.

Methods

The sample consisted of 40 adult male albino Wistar rats (*Rattus norvegicus albinus*), with body weight ranging from 200 ± 20 grams, from the animal colony of the Federal University of Sergipe (UFS), Sao Cristovao, State of Sergipe, Brazil. All experimental procedures were carried out in accordance with the norms of the Brazilian College for Animal Experimentation (COBEA). This study was approved by the Committee of Ethics in Research at, University of Sergipe protocol number 05/08/CEPA/UFS.

The rats were confined in plastic cages (three animals per cage), and supplied with food and water *ad libitum* in a temperature and humidity-controlled environment. Forty rats were subjected to the anesthesia and the surgical procedures as described by Poole Jr *et al.*[8]. The incision length was an inch, demarcated by rule, including skin, subcutaneous tissue and abdominal muscle was subsequently sutured continuously by a nylon thread 5-0 (Med Goldman).

Subsequently, rats were separated into four groups of six animals each, which were randomly assigned to one of the four treatment groups: (G1) untreated group euthanized 8 days after surgical procedures, (G2) photoirradiated group euthanized 8 days after surgical procedures, (G3) untreated group euthanized 15 days after surgical procedures, and (G4) photoirradiated group euthanized 15 days after surgical procedures.

Laser therapy application

Each animal in the treated subgroups received daily transcutaneous GaAlAs laser irradiation (KROMAN, Sao Paulo, Brazil) application during 7 and 14 days at 904nm for 2 min, potency of 45mW; spot area of 0,02cm² and energy density of 3J/cm². Laser array was positioned directly over the animal at a vertical distance of 0.5cm from the edge of the wound. The irradiation technique was by scanning the entire perimeter of the wound, comprising a time of two minutes using a stopwatch digital.

Histological analysis

After the sacrifice of the animals, the wound area was surgically removed, fixed in buffered 10% formalin and paraffin embedded. Subsequently, serial 4ìm sections were obtained and stained in hematoxylin-eosin to assess the inflammatory reaction, Masson´s trichrome and Picrossirus to semiquantify the collagen fibers and immunohistochemical technique for counting newly formed blood vessels. The intensity of the inflammatory response was assessed as following: +1 (inflammatory cells representing less than 10% of the cell population observed within the wound area), +2 (inflammatory cells representing in between 10 and 50% of the cell population observed within the wound area) and +3 (inflammatory cells representing more than 50% of the cell population observed within the wound area). The analysis of the intensity and pattern of the collagenization was performed according to the disposition and appearance of the collagen fibers deposited in the wound site. These fibers were then categorized in +1 (thin, delicate loosely arranged collagen fibers seen throughout the wound area), +2 (thin, delicate loosely arranged collagen fibers are seen in the surface and center of the wound area, but thicker and gross in the deep and margins) and +3 (thick, gross densely arranged collagen fibers seen throughout the wound area). Moreover, the slides also underwent Picrosirius Red technique to examine the collagen fibers, descriptively classified according to its typing, spatial orientation, thickness and size of fibers. When observed under polarized light microscope, the fibers were birefringent, appearing bright against the dark background, indicating the occurrence of type I collagen staining in areas ranging from yellow, through orange dark, reaching the red bright; the description of type III collagen was visualized in the areas of green color bright. The count of newly formed blood vessels was aided by use of the technique of immunohistochemistry (streptoavidin-biotin-peroxidase) using primary antibodies anti-CD34 (Clone Q B End 10, dilution 1:50, Mouse anti-human CD34, lot 512, Novocastra) and analyzed by morphometric techniques in the number of newly formed vessels and endothelial cells. The average number of blood vessels was determined by counting the capillaries across selected in 10 histological fields (400x magnification) of each wound and the

mean was obtained by the ratio of the sum of vessel count and the amount of fields analyzed.

Statistical analysis

The results obtained were subjected to statistical analysis of variance (ANOVA), with extension "post-hoc" Tukey test. The significance level was set at 0.05 or 5%.

Results

As indicated in the Table 1, the intensity of the inflammatory response was severe in all the samples of G1. Besides, the leukocyte infiltrate was predominantly composed of neutrophils, and macrophage, characterizing an acute inflammatory reaction. In general, neutrophils were distributed along the wound surface, particularly close to the fibrinous exudates membrane [Figure 1A]. Lymphocytes were also observed, but they always represented less than 10% of the leukocyte population. The wounds of the irradiated animals of G2 presented severe inflammatory response (+3) in 50% of the samples and moderate in the other half. Lymphocytes were the most frequently observed leukocytic subsets [Figure 1B]. Eosinophils and neutrophils were not observed the site of injury. In 14 days, the intensity of the inflammatory response in G3 and G4 was predominantly moderate [Figure 1C], exhibiting predominance of mononuclear chronic inflammatory cells [Figure 1D]. The data were statistically analyzed and revealed no significant differences between the groups sacrificed at day 8 ($p = 0.08$) and between the groups sacrificed on day 15 ($p = 0.3$).

TABLE 1 - Assessment of the profile of the inflammatory reaction intensity in the control and photoirradiated groups after surgical procedures, sacrificed at the 8th and 15th day

Animals	Groups			
	G1	G2	G3	G4
R1	+3	+2	+2	+2
R2	+3	+3	+2	+2
R3	+3	+2	+2	+2
R4	+3	+2	+2	+2
R5	+3	+3	+2	+2
R6	+3	+2	+2	+2
R7	+3	+3	+2	+2
R8	+3	+2	+2	+2
R9	+3	+3	+2	+2
R10	+3	+3	+2	+2

+ 1 = representing less than 10%; + 2 = representing between 10 and 50%; + 3 = representing more than 50%.

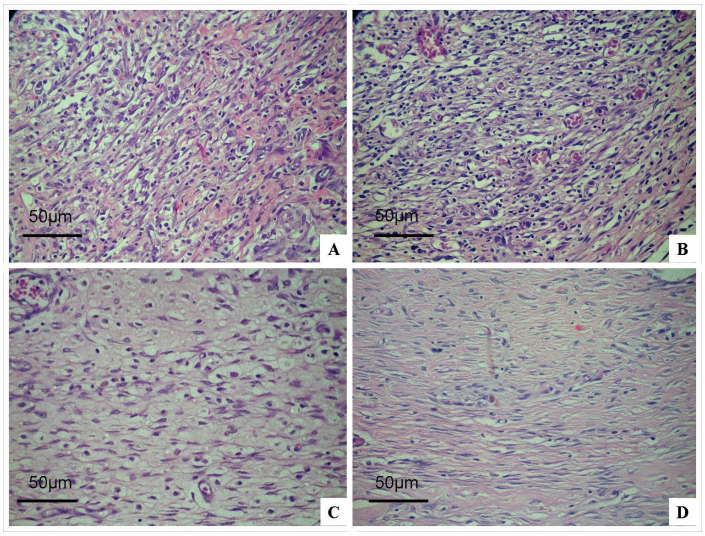

FIGURE 1 - (**A**) Acute inflammatory reaction in the control group G1; (**B**) and subacute in the irradiated group G2, sacrificed at day 8. Reduction of inflammation in both groups killed on day 15, control G3 (**C**) and irradiated G4 (**D**). (H and E, 400x).

As showed in Table 2, fibroplasias were assessed by analyzing the appearance and disposition of collagen fibers stained in blue by Masson's trichrome. In G1, thin collagen fibers arranged in delicate interlaced bundles were observed within a loose connective tissue (pattern +1) in the entire cases studied [Figure 2A]. On the other hand, in G2 showed thick, gross collagen fibers densely arranged in parallel bundles (pattern +3), although in 40% cases the pattern +2 of collagen deposition had persisted [Figure 2B]. In G3, thicker collagen fibers arranged densely in the whole area of the wound (pattern +3) were evidenced in all histological slides [Figure 2C]. In the irradiated group (G4), there was a mixture of collagen fibers, sometimes thicker, with densely arranged fibers, in 70% cases, sometimes presenting thinner fibers close to the the edges of the lesion, but densely compacted gross fibers in the central region in 30% of the samples [Figure 2D]. In general, collagen fibers appeared to be less abundant and thicker in G3 than in G4. However, there was no statistical difference between the groups sacrificed at day 8 ($p = 0.06$) and between the groups sacrificed on day 15 ($p = 0.15$).

TABLE 2 - Morphological assessment of the fibroplasia degree in both experimental and control groups, sacrificed at the 8th and 15th day

Animals	Groups			
	G1-	G2	G3	G4
R1	+1	+3	+3	+2
R2	+1	+1	+3	+2
R3	+1	+2	+3	+3
R4	+2	+3	+3	+3
R5	+3	+3	+3	+3
R6	+1	+2	+3	+3
R7	+3	+3	+3	+3
R8	+1	+3	+3	+3
R9	+1	+2	+3	+3
R10	+2	+2	+3	+2

+1 = thin, delicate, loosely arranged collagen fibers seen throughout the wound area; +2 = thin, delicate, loosely arranged collagen fibers seen in the deeper regions and margins of the wound area, but thicker and gross in the central region; +3 = thick, gross, densely arranged collagen fibers seen throughout the wound area.

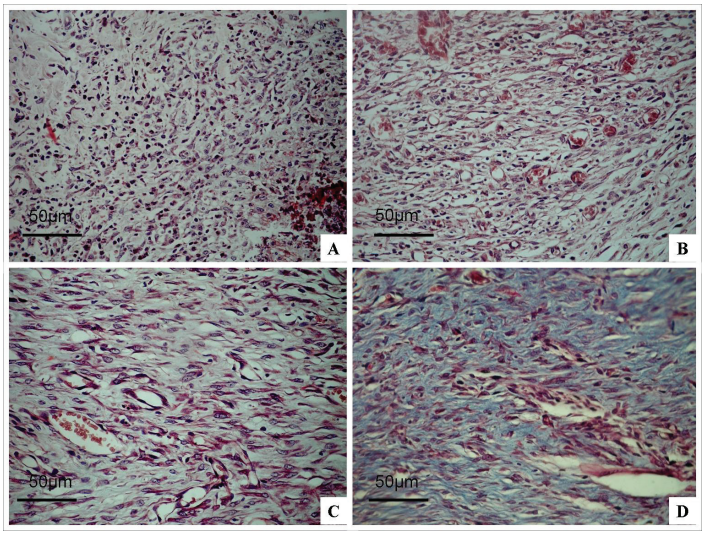

FIGURE 2 - Collagen of animals sacrificed at 8 days; slender collagen, with fibers arranged loosely, +1 score in the control group G1 (**A**), collagen thick with densely arranged fibers, score +3 in the irradiated group G2 (**B**). Score of similar collagen in mice sacrificed at 15 days; collagen thick with thick fibers arranged throughout the wound area, in the control groups G3 (**C**) and irradiated G4 (**D**). (Trichrome, x 400).

The morphological characterization of collagen fibers using the technique of Sirius red [Figure 3] showed that the treated groups (G2 and G4) had a tendency to present predominance of type III collagen, or a mixture of type III and I collagen, disposed in a parallel arrangement, whereas the control groups (G1 and G3) showed clear abundance of type-I collagen, sometimes parallel-arranged sometimes reticularly organized.

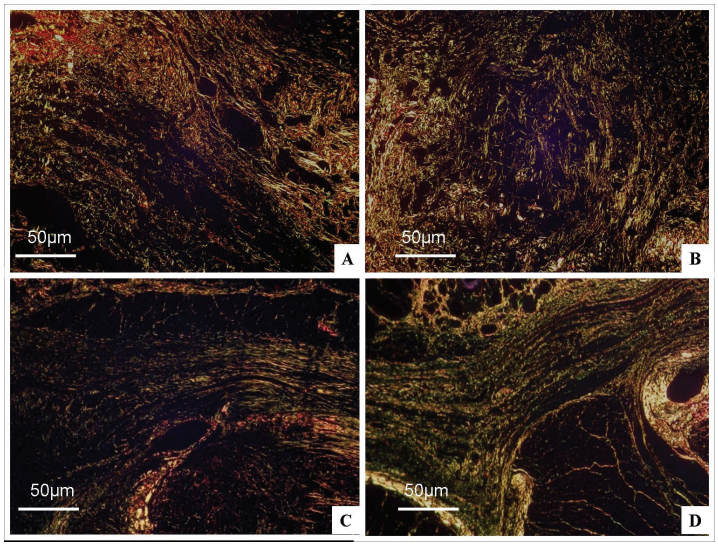

FIGURE 3 - In animals sacrificed at the 8th day, it was observed in the control group (**A**) the predominance of deposition of collagen type I in regions of color ranging from yellow to dark orange, up to bright red. In the irradiated group (**B**) a predominance of collagen type III were seen in areas of bright green color. On the 15th day, it was found in the control group (**C**) A characterization of type I collagen in all specimens, contrasted with the irradiated group (**D**) where the collagen fibers showed a mixture of type III and I (Picrosirius Red , 400 x).

Concerning to the quantitative analysis of the vascularization, it was observed a higher content of newly formed blood vessels in G2 (0.66±0.36) than in G1 (0.16±0.009) (p=0.001) in 8 days, as well as in G4 (0.53±0.16) when compared to G3 (0.37±0.10) (p=0.02) in 15 days [Figures 4 and 5]. Nevertheless, no significant difference was observed between the groups irradiated G2 and G4 (p = 0.30), and between the control group G1 and G3 (p = 0.00).

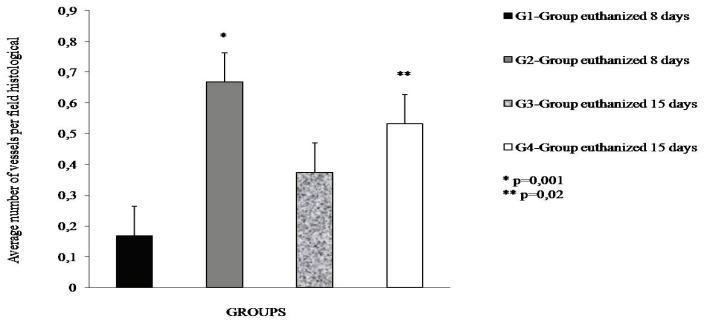

FIGURE 4 - Quantitative analysis of the average number of newly formed vessels by histological field.

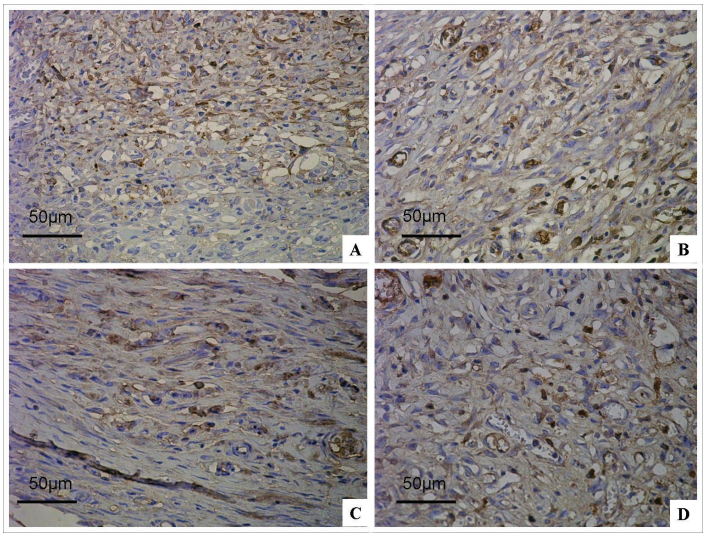

FIGURE 5 - Newly formed blood vessels in the groups sacrificed on the eighth day in the control groups G1 (A) and irradiated G2 (B) Reduction in the number of newly formed vessels in mice sacrificed at 15 days in the control groups G3 (C) and irradiated G4 (D) (Anti-CD34, 400 x).

Discussion

Although the clinical efficacy of the LLLT has been proved through controlled studies[9,10], little is known about the histological alterations that can occur due to its use, particularly regarding modulation of the extracellular matrix elements. Several studies on laser photobiomodulation ensured the improvement of cicatricial repair in wounds with loss of tissue, a particular sort of cicatrization phenomenon known as second-intention wound healing[2,7,11]. However, in this study, the experimental lesions were sutured wounds, resembling the ones carried out after elective surgical procedures, in order to asses the role of LLLT as an alternative therapy to improve the healing process in cases in which the cicatrization might be somehow impaired.

The inflammatory reaction represents the earliest event to take place after tissue injury, whose main function is to eliminate eventual microorganisms and provide wound cleaning. This inflammation is absolutely required to provide wound healing, although its long-term persistence is considered one of the most important reasons of delay in the healing process[12]. During the first days of healing, biological events such as formation of new capillary blood vessels associated with progressive deposition and remodeling of collagen fibers will culminate in a complete cicatricial repair of the injured area[13].

In this study, LLLT reduced the intensity of the inflammatory reaction in both 8 and 15 days after performing the surgical cutaneous wounds. These findings suggest that this protocol of light Laser irradiation is able to downregulate the inflammatory response and favor the acceleration of biological events responsible for the healing process. Similar findings attesting a remarkable anti-inflammatory activity of LLLT in other different protocols have been previously reported[14,15]. Additionally, LLLT apparently influenced the dynamic of the immunoinflammatory response at early stages of wound healing process, once it clearly induced switching of the leukocyte infiltration pattern (neutrophilic to lymphoplasmacytic infiltration) into the damaged tissue of irradiated wounds.

Both modulatory effects of LLLT over the inflammatory response might be a result of an important inhibitory role played by Laser arrays, in a variety of cells, on the synthesis of prostaglandin, a chemical mediator widely supposed to provide chemotactic signals for leukocytes polymorphonuclear neutrophils, as well as on lymphocyte proliferation and maturation[16]. Besides, elevated numbers of polymorphonuclear cells within the first experimental hours, followed by decreased levels on day 3 post-treatment in laser treated wounds have been reported in previous studies, and a close relation between this phenomenon and the stimulation of mast cells degranulation has been pointed[19]. Therefore, LLLT might provide a short term acute inflammatory response in earlier stages of wound healing, accelerating the phagocutic inflammatory phase of the cicatricial repair.

As long as the healing process takes place, the inflammatory phase is gradually substituted by proliferating phase, which is characterized by wide migration of fibroblasts into the wound area[12]. This cell subset is responsible for synthesizing, depositing and remodeling the collagen fibers required to cicatricial repair after tissue injury. In normal tissues, collagen fibers provide strength, integrity and structure; so, when tissues are disrupted following injury, collagen deposition is essential for replacing the lost tissue and restoring anatomic structure and function[17]. In this study, although LLLT appeared to stimulate the deposition of collagen fibers at both evaluated stages of wound healing, these findings were not statistically significant.

Thus, it seems that despite laser arrays is able to upregulate the activity of enzymes responsible for the synthesis of collagen fibers[2,7], this activity, in sutured wounds, seems not to be expressive enough to lead to an overproduction of collagen fibers. These data appears to suggest that the upregulatory effects of LLLT on collagenization, particularly observed in second-intention wound healing[2,9,11], do not enhance the possibility of hypertrophic scars formation in sutured wounds. However, further investigations are required to clarify this mechanism and order to assure the liability of these observations.

The classification of collagen fibers stained with Sirius Red and analyzed using polarized light showed that in the earlier stages of tissue healing, fibers are delicate, showing a loose arrangement and a yellow-green birefringence, featuring a type III collagen. On the other hand, in the later stages of the repair process the fibers exhibit an organizational pattern more compact, thicker bundles of red-orange color, indicating a type I collagen[2,11,19].

In fact, these data disagree with the survey results, where it was noted that the morphological characterization of collagen fibers in the irradiated groups had a predominance of either type III collagen, or the presence of both type III and I collagen in equal proportions, with in parallel orientation, whereas poorly organized type I collagen was dominating in the control groups. It is noteworthy that the predominance of type III collagen, provides a fragile scar on your tension, but on the other hand, excess type I collagen is responsible for a

healing of poor quality, showing hypertrophic appearance. Notwithstanding increased collagen synthesis is considered a good marker of healing evolution, over deposition of these fibers within the wound area may provoke excessive accumulation of scar tissue, resulting in hypertrophic scars or keloids[18]. However, this undesirable effect can be minimized by applying correct dosages of Laser irradiation in order avoid over stimulation of fibroblastic activity.

Since no sign of excessive collagen synthesis was observed, it is possible to suppose that the protocol of photobiomodulation employed in this study was suitable to be applied in sutured wounds. Besides, the better orientation of the collagen bundles, as well as the presence of equal proportions of both type I and III collagen fibers, closely resembling the appearance of the normal tissue, seems to point out an important role played by LLLT in the architectural organization of the collagen during wound healing, as suggested in previous investigations[2].

These data indicate that this protocol was successful in improving wound healing without any apparent deleterious or side effect on scar formation. The exact explanation for these quite beneficial results is not completely understood yet, so that further investigations are still demanded in order to fully elucidate the mechanisms underlying the effects observed in this study.

In this study, LLLT appeared to stimulate the proliferation of new blood vessels, but the pathways of laser-induced endothelial activation are still unclear. It is possible that such pro-vascular effects may reflect an over stimulation of endothelial proliferation by cytokines released by inflammatory cells[16] or result from laser-related direct stimulation of the cell cycle in angioblasts[11,12]. However, the blood vessels content in 8 days was significantly reduced in both irradiated and non-irradiated groups when compared to 15 days. Similar findings were reported in previous investigations[11], so that it is suggested a close relationship between the evolution of the scarring events and progressive reduction of neovascularization during the healing process.

Conclusion

Low-level laser therapy resulted in modulate of the inflammatory response, enhanced deposition of collagen fibers and increase in the average number of newly formed vessels.

References

1. Gillitzer R, Goebeler M. Chemokines in cutaneous wound healing. J Leukoc Biol. 2001;69:513-21.
2. Medrado AP, Soares AP, Santos ET, Reis SR, Andrade ZA. Influence of laser photobiomodulation upon connective tissue remodeling during wound healing. J Photochem Photobiol B. 2008 18;92(3):144-52.
3. Guyton AC, Hall JE. Tratado de Fisiologia Médica. 10ed. Rio de Janeiro: Guanabara Koogan; 2002.
4. Honmura A, Yanase M, Obata J, Haruki E. Analgesic effect of Ga-Al-As diode laser irradiation on hyperalgesia in carrageenin. Lasers Surg Med. 1993;13:463-9.
5. Baxter D. Therapeutic laser: theory and practice. Churchill Livingstone: Edinburgh; 1994.
6. Theodoro LH, Garcia VG, Marcantônio JE. Lasers em implantodontia. Lasers Surg Med. 2002;9:74-8.
7. Ribeiro MA, Albuquerque RL, Ramalho LM, Bonjardim LR, Da Cunha SS. Immunohistochemical assessment of myofibroblastc and lymphoid cells during wound healing in rats submitted to laser photobiomodulation at 660nm. Photomed Laser Surg. 2009;27:49-55.
8. Poole Jr GV, Meredith JW, Kon ND, Martin MB, Kawamoto EH, Myers RT. Suture technique and wound bursting strength. Am Surg. 1984;50:569-72.
9. Harazaki M, Takamashi H, Ito A. Soft laser irradiation induced pain reduction in orthodontic treatment. Bull Tokyo Dent Coll. 1998;39:95-101.
10. Pinheiro ALB, Calvacante ET, Pinheiro ITNR. Low-level laser therapy is na important tool to treat disorders of the maxillofacial region. J Clin Laser Med Surg. 1998;16:223-6.
11. Carvalho PTC, Mazzer N, Reis FA, Belchior ACG, Silva IS. Analysis of the low-power HeNe laser on the healing of skin wounds in diabetic and non-diabetic rats. Acta Cir Bras. 2006;21(3):177-83.
12. Clark RA. Wound repair – overview and general considerations. In: Clark RA. The molecular and cellular biology of wound repair. New York: Plenum Press; 1996. p.3-50.
13. Whelan HT, Smits RL Jr, Buchman EV, Whelan NT, Turner SG, Margolis DA, Cevenini V, Stinson H, Ignatius R, Martin T, Cwiklinski J, Philippi AF, Graf WR, Hodgson B, Gould L, Kane M, Chen G, Caviness J. Effect of NASA light-emitting diode irradiation on wound healing. J Clin Laser Med Surg. 2001;19:305-14.
14. Conlan M, Rapley JW, Cobb CM. Biostimulation of wound healing by low-energy Laser irradiation: a review. J Clin Periodontol. 1996;23:492-6.
15. Freitas AC, Pinheiro AL, Miranda P, Thiers FA, Vieira AL. Assessment of inflammatory effect of 830 Laser light using C-reactive protein levels. Bras Dent J. 2001;12:187-90.
16. Sakurai Y, Tamaguchi M, Abiko Y. Inhibitory effect of low-level Laser irradiation on LPS-stimulated prostaglandin E2 production and cyclooxygenase-2 in human gingival fibroblasts. Eur J Oral Sci. 2000;108:29-34.
17. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. Front Biosci. 2004;9:283-9.
18. Pugliese LS, Medrado AP, Reis SR, Andrade ZA. The influence of lo-level Laser therapy on biomodulation of collagen and elastic fibers. Pesq Odontol Bras. 2003;4:307-13.
19. Pereira MCMC, Pinho CB, Medrado ARP, Andrade ZA, Reis SRA. Influence of 670 nm low-level laser therapy on mast cells and vascular response of cutaneous injuries. J Photochem Photobiol B. 2010;98(3):188-92.
20. Rich L, Whittaker P. Collagen and picrosirius red staining: a polarized light assessment of fibrilar hue and spatial distribution. Braz J Morphol Sci. 2005;22:97-104.

Conflict of interest: none
Financial source: FAPITEC

Correspondence:
Valdinaldo Aragão de Melo
Rua Álvaro Brito, 14/301
49020-400 Aracajú - SE Brasil
Phone: (55 79)9982-2438
vamelo@infonet.com.br

Received: September 21, 2010
Review: November 18, 2010
Accepted: December 20, 2010