



## Comparative study of Low-level laser therapy and microcurrent on the healing of skin burns in rats

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**ABSTRACT.** This study investigated and compared the effects of low-level laser therapy (LLLT) and microcurrent in the burn healing process in Wistar rats. We conducted a randomized controlled study with 30 rats divided into 3 groups (n = 10); control group (CG), laser group (LG) and microcurrent group (MG). After thermal damage, 10 applications of 660 nm diode laser were performed in GL and 10 applications of 60 Hz microcurrent (160  $\mu$ A) in MG. The semi-quantitative histological analysis was done using scores (0–3), in sections stained by hematoxylin and eosin and Masson's trichrome. The results indicated a significant improvement in the fibroblasts proliferation, collagen fibers deposition, neoangiogenesis, and cutaneous appendages regeneration in MG and LG. When microcurrent and LLLT were compared, no difference was detected, except the regeneration and formation of new cutaneous appendages, observed in MG. Despite the similar effects, GM showed faster tissue repair with the formation of skin appendages.

**Keywords:** low-level laser therapy, electric stimulation therapy, wound healing.

## Estudo comparativo da laserterapia de baixa intensidade e microcorrente no reparo tecidual de queimaduras em ratos

**RESUMO.** Este trabalho teve por objetivo investigar e comparar os efeitos da laserterapia de baixa intensidade (LTBI) e microcorrente no processo de reparo de queimadura em ratos Wistar. Foi realizado um estudo randomizado controlado com 30 ratos divididos em 3 grupos (n = 10); grupo controle (GC), grupo laser (GL) e grupo microcorrente (GM). Após lesão térmica, 10 aplicações de laser InGaAlP de 660 nm foram submetidas ao GL e 10 aplicações de microcorrente de 60 Hz (160  $\mu$ A) ao GM. Foi realizada análise semi-quantificativa dos dados histológicos através de escores (0-3), em cortes corados por hematoxilina e eosina e tricrômico de Masson. Os resultados indicaram que houve melhora significativa na proliferação de fibroblastos, deposição de fibras colágenas, neoangiogênese e regeneração de apêndices cutâneos no GM e GL. Quando a microcorrente e a LTBI foram comparados, não houve diferença, exceto na regeneração e formação de apêndices cutâneos, observada no GM. O laser e a microcorrente produziram melhora no reparo tecidual de queimaduras em ratos. Embora possua efeitos semelhantes, o GM apresentou reparação tecidual mais rápida com o aparecimento de apêndices cutâneos.

**Palavras-chave:** terapia a laser de baixa intensidade, terapia por estimulação elétrica, cicatrização.

### Introduction

Thermal injuries are one of the main causes of morbidity and mortality, given skin tissue destruction and risk of infection, with significant implications for health such as: extended hospitalization, high cost of medication, multiple surgeries and prolonged rehabilitation (KHORASANI et al., 2008). This type of injury can also cause functional sequelae and deficits, thereby contributing to the emergence of social and psychological alterations. The burn wound healing process and recovery of functional alterations

remains a challenge for modern medicine. Skin tissue repair is a complex process that involves a sequence of physiological and biochemical events such as inflammation, collagen synthesis, granulation tissue formation, epithelization and tissue remodeling (MAIYA et al., 2005).

Photo stimulation by low-level laser therapy (LLLT) has proven to be effective in promoting analgesia and speeding up the repair process in soft tissue injuries (REDDY et al., 2001). In tissue repair this resource can facilitate burn wound healing, reducing remodeling time and improving the quality

of the repaired tissue (MAIYA et al., 2005; REDDY et al., 2001). When damaged tissue cells absorb laser energy, they trigger a host of biochemical events, resulting in increased enzymatic activity, greater production of adenosine triphosphate (ATP), increased proteinaceous synthesis, cell proliferation and deposition and organization of collagen (RIBEIRO et al., 2004; VIEIRA et al., 2006).

Other authors (LEE et al., 2010; SANTOS et al., 2004; CHENG et al., 1982) report that electric stimulation with microcurrents can speed up ATP synthesis, stimulate transmembrane amino acid transport at the lesion site, reestablish bioelectricity in the tissue, promote a reduction of the inflammatory process, a decrease in pain and an acceleration of the remodeling process. This method aims at normalizing current flow, which is interrupted when tissue damage occurs.

Therefore, both physical modalities can be used for treating burn injuries. However, it has not been established which resource can produce the fastest and most efficient results, or which can emphasize the inflammatory process, neoangiogenesis, collagen production and fibroblast activity (REDDY et al., 1998; WOODRUFF et al., 2004).

The aim of the study was to compare the effect of visible LLLT and microcurrent therapy on burn wound healing in Wistar rats using histological analysis.

## Material and methods

The study was approved by the Research Ethics Committee of Potiguar University (number 062/2008).

We used 30 randomly chosen Wistar rats, weighing approximately 250 to 300 grams. The rats were obtained from the vivarium of the aforementioned institution. After weighing the animals, we divided them randomly into 3 groups ( $n = 10$ ): control group (CG), laser group (LG) and microcurrent group (MG). All the groups had been subjected to the same environment, with the same day/night biological cycle 10 to 12 hours of light, controlled temperature ( $\sim 23^{\circ}\text{C}$ ) and illumination, air condition-controlled humidity and minimal noise exposure. They were confined in sawdust-lined individual cages, where they were given Purina<sup>®</sup> ration and water *ad libitum*. For all the groups the experimental procedures were applied in the following sequence: (1) intramuscular injection in the quadriceps muscle of 50 mg kg<sup>-1</sup> doses of anesthetic tiletamine-zolazepam (Zoletil<sup>®</sup>) according to body weight; (2) skin incision on the back of the

animal; (3) burn induction (MEYERHOLZ et al., 2009) using a 6 x 3 cm aluminum plate heated with boiling water at 100°C, pressed against the skin on the back of the animal 4cm from the base of the skull, for 10 seconds. The aluminum plate was soaked in a beaker of boiling water for 10 minutes. The proposed treatments were started immediately after the injury intervention and were applied daily for 10 days. The CG carefully received the same experimental protocol manipulations, except for treatment exposure.

The LG were exposed daily to a low-level laser AlGaInP, (Photon Laser III device DMC) wavelength of 660 nm, continuous power level of 30 mW, dose of 10 J cm<sup>-2</sup> and energy of 0.27 J per point for 9 seconds inside the burn. In the adjacent region (wound edges) was used, a dose of 12 J cm<sup>-2</sup> and energy of 0.33 J per point for 11 seconds. The application was done by direct contact with the wound and a spot laser positioned through a plastic mold to ensure the proper distance between the points. A distance of 1.5 cm was maintained between the points, totaling 6 points and total energy per session of the 1.62 J inside the burn and 14 points and total energy of 4.62 J in the adjacent region, for a total of 20 points per animal. For the MG, the microcurrents were applied using the Physiotonus Microcurrent Stimulator (Bioset<sup>®</sup>), through two adhesive electrodes (Valutrode<sup>®</sup> 3.2 cm<sup>2</sup> diameter) placed around the lesion with a continuous square wave, intensity of 160  $\mu\text{A}$  and frequency of 60 Hz for 15 minutes. Both therapies were applied in the afternoon (14 to 17h) and all equipments were previously calibrated. The LLLT protocol was designed by (IORDANOU et al., 2009). The method of microcurrent application was described by (DEMIR et al., 2004).

All animals were sacrificed in a sealed box filled with CO<sub>2</sub>. Immediately after sacrifice, biopsy of the healing tissue was carried out for histological study, including part of the adjacent skin at the edge of the wound and the healing tissue. The unburned skin of the control rats were used as baseline study. Samples were fixed in formalin, embedded in paraffin blocks, and sagittal 5  $\mu\text{m}$ -thick sections were cut from all regions of the samples. Sagittal sections were stained with hematoxylin-eosin and Masson's trichrome. Tissue analysis was performed by a blinded researcher using Nikon<sup>®</sup> light microscope (Nikon, Tokyo, Japan) at a magnification of 400X. The histological analysis of the healing skin was performed in sections stained by hematoxylin and eosin to assess re-epithelialization, acute inflammation, neo-angiogenesis, cutaneous appendages regeneration and fibroblasts proliferation. The Masson's trichrome stain was used to evidence the

presence and intensity of the collagen fibers deposition. The criteria used for the analysis are shown in Table 1. The results of histological analysis were semi-quantified into scores on a scale ranging from 0 to 3 (MEIRELES et al., 2008; IORDANOU et al., 2009).

**Table 1.** Criteria used for semi-quantitative under light microscopy analysis. Adapted from Meireles et al. (2008) and Iordanou et al. (2009).

Criterion	Score			
	Null = 0	Mild = 1	Moderate = 2	Intense = 3
Re-epithelialization	Absent	Present: Covering <50% of the wound	Present: Covering >50% of the wound	Present: Covering 100% with regular or irregular thickness
Acute inflammation	Absent	Mild: <25% neutrophils seen in the cells in the field	Moderate: <25% - 50% neutrophils seen in the cells in the field	Intense: >50% neutrophils seen in the cells in the field
Fibroblasts	Absent	Mild: <25% young and less differentiated fibroblasts among other cell types	Moderate: <25%-50% young and less differentiated fibroblasts among other cell types	Intense: >50% young and less differentiated fibroblasts among other cell types
Neoangiogenesis	Absent	Mild: Less than that seen in the healthy adjacent tissue	Moderate: Amount similar to that seen in the healthy adjacent tissue	Intense: More than that seen in the healthy adjacent tissue
Cutaneous appendages	Absent	Mild: <25% seen in all the section	Moderate: >25% - 50% in all the section	Intense: > 50% in all the section
Collagen fibers	Absent	Mild: Masson's trichrome staining less intense than that observed in the healthy adjacent tissue	Moderate: Masson's trichrome staining similar to that observed in the healthy adjacent tissue	Intense: Masson's trichrome staining more intense than that observed in the healthy adjacent tissue

Data were analyzed using GraphPad Prism 5. Summary statistics were used to calculate measures and standard deviations. Bartlett's test was used to test homogeneity between the groups. The Kruskal-Wallis test (Non-parametric ANOVA) was used to compare the data from the semi-quantitative analysis, in addition to Dunn's Multiple Comparison Post-Test used to identify any differences between the groups. A value of  $p < 0.05$  was considered for statistically significant results.

## Results

The animals did not suffer any complications during the treatment period. All groups showed moderate acute inflammatory reactions.

Control subjects showed low signals of fibroblastic activity, collagen fibers, neoangiogenesis and cutaneous appendages. However, in the LG and

MG the amount of granulation, collagen deposition tissue and neoangiogenesis was moderate. We observed moderate presence of cutaneous appendages only in the MG.

There were no significant differences between the groups in epithelial regeneration ( $p = 0.3873$ ) and acute inflammatory process ( $p = 0.8765$ ). Significant difference was observed between the treated and control groups, with an increase in production of fibroblasts ( $p = 0.0005$ ), collagen ( $p = 0.0110$ ), neoangiogenesis ( $p = 0.0099$ ) and cutaneous appendages ( $p = 0.0008$ ) (Table 2).

When MG and LG were compared using Dunn's Multiple Comparison Test, both therapies showed improvement in the repair of burns, with no difference between them, except the regeneration and the formation of new cutaneous appendages ( $p < 0.01$ ), observed in microcurrent therapy (Table 3).

**Table 2.** Evaluation of the histological variables after treatment between LG, MG and CG.

Histological Analysis	Microcurrent	LLT	Control	p value
	mean/SD	mean/SD	mean/SD	
Re-epithelialization	1.9 ± 0.9	1.7 ± 0.9	1.6 ± 0.4	0.3873
Acute Inflammation	2 ± 0.8	2.1 ± 0.8	1.9 ± 0.9	0.8765
Fibroblasts	2.5 ± 0.5	2 ± 0.6	1.2 ± 0.4	0.0005*
Collagen fibers	2.4 ± 0.5	2.5 ± 0.5	1.5 ± 0.8	0.0110*
Neoangiogenesis	1.8 ± 0.7	1.7 ± 0.6	1 ± 0	0.0099*
Cutaneous appendages	1.9 ± 0.8	1 ± 0	1 ± 0	0.0008*

\*Statistically significant difference between the groups ( $p < 0.05$ ) using the Kruskal-Wallis test.

**Table 3.** Intergroup comparison of the effect of treatment on histological variables.

Histological Analysis	p- value		
	MG x LG	MG x CG	LG x CG
Number of fibroblasts	>0.05	<0.001*	<0.05*
Number of collagen fibers	>0.05	<0.05*	<0.05*
Neoangiogenesis	>0.05	<0.05*	<0.05*
Cutaneous appendages	<0.01*	<0.01*	>0.05

Laser group (LG), microcurrent group (MG) and control group (CG). \*Statistically significant difference ( $p < 0.05$ ) between the groups using Dunn's Multiple Comparison Test.

## Discussion

In the present study, it was observed a significant difference between treated and control groups. Our findings also show that there was a statistically significant improvement in production of fibroblasts, collagen and neoangiogenesis, indicating that LLLT and microcurrent had a positive effect on wound healing. Only MG demonstrated new cutaneous appendages, particularly hair follicle, final process of epithelial repair.

Researches comparing LLLT and microcurrent techniques in burns using histological analysis is scarce. Those that evaluate one of the techniques present reliable evidence, albeit insufficient to draw conclusions about the contribution of LLLT and

microcurrents in wound healing (SANTOS et al., 2004; STADLER et al., 2001; AL-WATBAN et al., 2003; CULLUM et al., 2001).

Byrnes et al. (2004) and Clark et al. (1985) report that during the first days of tissue repair, events are aimed at preventing blood loss (hemostasis) and forming a fibrin network, in order to build a matrix for the following processes, in which platelets adhere to the collagen in the perivascular space. This contact activates the platelets, releasing platelet factors that accelerate the migration and proliferation of the main cell types involved in the healing process: the fibroblasts. In our study, histological analyses of neoformation tissue, obtained on the tenth day post-lesion, showed an increase in number of fibroblasts and collagen fibers in the two treated groups. These data suggest that the action of LLLT and microcurrents accelerates the granulation process and the formation of fibroblasts. The MG showed epidermis in an advanced stage of remodeling and the significant presence of cutaneous appendages, such as hair follicles, consequently with more advanced tissue regeneration than the LG. The lack of total regeneration is possibly due to treatment duration (10 days), requiring longer remodeling to achieve the total healing of the injured tissue (MEYERHOLZ et al., 2009).

Ribeiro et al. (2004) in a study conducted with 20 rats, using LLLT HE-NE (632.8 nm) with 10 mW of output power, observed an acceleration in epidermis formation, increased epithelial layer thickness, neovascularization, collagen fiber reorganization and improvement in burn healing, findings similar to our study even using different parameters.

In a study with microcurrents with a current of 50  $\mu$ A, applied to injuries induced in rats by the use of acid peeling, Santos et al. (2004) verified an increase in collagen fiber and fibroblast production with epithelial tissue regeneration and fibrosis formation. Several studies (KLOTH, 2005; MERTZ et al., 1993; STEFANOVSKA et al., 1993) *in vitro*, animal experiments, and clinical trials using electrical stimulation in tissue repair show similar results, but none made a comparison between microcurrent therapy and LLLT.

It has been shown that electrical stimulation increases the healing capacity of pressure ulcers in a significant number of individuals with this type of injury, demonstrating the beneficial effects during the proliferation, inflammatory and maturation phase. Microcurrent therapy, a modality that uses a subsensory current with power output between 1 and 999  $\mu$ A, has been successful in increasing the

healing of soft tissues and fractures (DEMIR et al., 2004; LEE et al., 2007). This type of current induces electron flow in the skin and in subcutaneous tissue. It also appears that the transportation of  $\text{Na}^+$  into the cell, through the cell membrane, maintains skin battery thereby contributing to wound healing (BALAKATOUNIS; ANGOULES, 2008). In electroacupuncture, a technique that uses the stimulation of acupuncture needles with a low frequency microcurrent, the suppression of myostatin expression occurs, leading to a proliferative activation of satellite cells and skeletal muscle repair (TAKAOKA et al., 2007). Cells have a complex bioelectrical system that is sensitive to variations and changes in electric fields. Dermal lesions cause electrical changes in the cell, hindering the healing process. It was demonstrated that the electric fields control the direction and rate of epithelial cells that migrate into the wound. Metabolic, immunological and physiological alterations have been found in different cell cultures after application of the electric current (BALAKATOUNIS; ANGOULES, 2008; LI et al., 2002).

Electric fields stimulate growth factor secretion (ZHAO et al., 2003) and stimulate adenosine triphosphate production (CHENG et al., 1982). Another study found that microcurrents stimulate dermal fibroblasts and U937 cells to secrete transforming growth factor- $\beta$ 1, an important regulator of cell-mediated inflammation and tissue regeneration. In addition, microcurrents promote an increase in collagen concentration around the wound and higher intracellular calcium levels, generating an increase in adenosine triphosphate and protein synthesis, thus promoting cell repair and proliferation (CHENG et al., 1982). While the microcurrent stimulates ionic membrane channels, several models have suggested that mechanisms involved in laser biophotomodulation stimulate mitochondria to promote an increase in adenosine triphosphate (IORDANOU et al., 2009; ZHAO et al., 2003).

Demir et al. (2004) compared microcurrent of 300  $\mu$ A for 30 minutes a day and LLLT with gallium arsenide (GaAs), with a wave length of 904 nm, energy density of 1 J  $\text{cm}^{-2}$  and average power output of 6 mW for 10 minutes daily over a 10-day period. The authors observed that microcurrent and LLLT were efficient in the inflammatory phase compared to the control groups; however, microcurrents were even more efficient than LLLT, obtaining more significant results, corroborating our study. The authors concluded that microcurrent and LLLT treatments have beneficial

effects during inflammatory proliferation and maturation phases of wound healing. In our study we did not observe a reduced inflammatory phase; however, a favorable effect was found in the proliferative and maturation phases. The parameters that we used may have influenced the inflammatory process, contributing to the wound repair, but not specific for attenuating the inflammatory process (WOODRUFF et al., 2004).

Iordanou et al. (2009) evaluated the effect of LLLT on the wound healing process in rats, using parameters with power density of  $40 \text{ mW cm}^{-2}$ , energy of  $2.4 \text{ J}$  and a dose of  $16.8 \text{ J cm}^{-2}$ , and found a beneficial effect on wound healing, leading to rapid epithelization and better healing. The author observed an increase in reepithelization on the 15<sup>th</sup> day of treatment; however, no improvement was observed in the inflammatory process or in the number of fibroblasts and collagens fibers. In a study carried out by Bayat et al. (2010) on the effect of pulsed laser on skin healing, there was a significant increase in fibroblasts and blood vessels and a reduction in macrophages. Both treatments can be successfully used against ulcers and chronic wounds, in combination with conventional therapies, such as daily care, drugs and wound debridement.

It seems that different laser parameters may influence organic responses in models of tissue lesions in rats (WOODRUFF et al., 2004; STADLER et al., 2001). More studies are needed to define the appropriate amount of power, energy and dose in laser therapy as well as amperage and duration of the microcurrent, in order to optimize and improve wound healing. The clinical implications of this study may also be considered. Healing of skin burns is a challenge and a delicate healthcare issue for the physiotherapist. The rehabilitation programs occasionally have to dedicate much time on wound care. Healing is sometimes delayed, and the wound by burn may not respond to standard treatment.

It seems clear that LLLT and microcurrent electrotherapy result in more adequate tissue repair by increasing local cell metabolism, forming new cells (mitosis) and promoting greater synthesis of substances responsible for the regeneration of injured tissue (WOODRUFF et al., 2004; ZHAO et al., 2003; TODD et al., 2001).

## Conclusion

In the present study, it was observed that LLLT (AlGaInP 660 nm) and microcurrent (160  $\mu\text{A}$ ) have accelerated the healing in burned tissue in Wistar

rats. However, the MG seemed to exhibit faster tissue repair compared to the LG, evidenced by the presence of cutaneous appendages. Further studies with a randomized clinical assay are needed to compare the two therapies and determine if the physical effects of each therapy will produce an even more satisfactory healing process.

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Received on September 2, 2011.

Accepted on October 10, 2011.

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