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# Analysis of the Cicatricial Acceleration Method (MAC®) in Skin Repair in Wistar *Rattus norvegicus* with Induced Chemical Burns

**Marcus Vinicius de Mello Pinto[1], Sandroval Francisco Torres[2], Aline Ronis Sampaio[1], Karin Yuri Fernandez Iturra[3], Miriam Viviane Baron[4], Patricia Froes[5], Carlos Ruiz da Silva[6], Daiane Paza[7], Maria Elena Silva Alvarez[8], Josefa Jeanette Ugalde[9], Juliano Tibola[2], Esteban Fortuny[10]**

[1]Celulare Institute, Petrópolis, Brazil
[2]State University of Santa Catarina (UDESC), Florianopolis, Brazil
[3]Bienus Clinic, Santiago, Chile
[4]Pontifical Catholic University of Rio Grande do Sul (PUC/RS), Porto Alegre, Brazil
[5]International Research Group (IRG), Natal, Brazil
[6]Academic Training Center (CTA) College, São Paulo, Brazil
[7]Fibrafisio Clinic, Brusque, Brazil
[8]Fisiocare Clinic, Curicó, Chile
[9]Lua Clinic, Coyhaique, Chile
[10]Finis Terrae University, Santiago, Chile
Email: marcuspinto1966@gmail.com

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## Abstract

**Introduction:** The cicatricial acceleration method (MAC®) promotes photobiological effects of an anti-inflammatory and healing nature. Its therapeutic radiation is emitted, producing photobiostimulant effects that result in rapid tissue repair and better tissue quality. The treatment of burns has always been a challenge, which involves both performing surgery and controlling and guiding scar regeneration, avoiding possible morbidities. **Objective:** To evaluate the effects of applying the MAC methodology with an AlGa (aluminum, gallium arsenide) laser on the time and quality of tissue repair in the skin of rats after induced chemical burns. **Method:** 22 adult male rats were subjected to a second-degree chemical burn on the back using 50% trichloroacetic acid. After the burns, the animals were randomly separated into 2 groups: control and experimental. The control group (G1) received placebo laser therapy and the laser group (G2) underwent laser irradiation with an energy density of 100 J/cm$^2$. Histological analysis and macroscopic evaluation were carried out by means of the paper template method. **Results:** Group G1 showed (53%) of the necrosis area and

group G2 showed (11%) necrosis area. **Conclusion:** The cicatricial acceleration method (MAC®) favored the repair of wounds caused by a 2nd-degree chemical burn, optimizing time and improving quality.

## Keywords

Chemical Burn, Healing, Scarring, Cicatricial Acceleration Method (MAC®), Tissue Repair

## 1. Introduction

Laser therapy is an anti-inflammatory and biostimulation device characterized by being monochromatic (it has a well-defined wavelength), coherent (all the photon waves that make up the beam are in phase) and collimated (it propagates as a beam of practically parallel waves) [1] [2].

The word *laser* corresponds to an acronym made up of the first letters of *light amplification by stimulated emission of radiation* [1].

Pinto *et al*. [1] demonstrated that laser therapy using low-power lasers reduces the inflammatory response and is able to increase cell proliferation, collagen deposition and wound contraction in the burn repair process.

According to Karu *et al*. [3], low-intensity laser therapy has shortened the remodeling time and improved the quality of the tissue undergoing neoformation. The basic biological mechanism promoted by this electrophysical resource is the absorption of red and infrared light by chromophores contained in the protein components of the respiratory chain located in the mitochondria, which, in turn, by absorbing the energy, triggering a cascade of biochemical events, resulting in increased enzyme activity, adenosine triphosphate (ATP) production, protein synthesis, cell proliferation, collagen deposition and organization.

Treating burns has always been a challenge, both because of their severity and because of the many complications that usually occur. Curing burns involves not only early skin grafting surgery, but also controlling and guiding cicatricial regeneration, which tends to occur anarchically and with the potential for sequelae and infections [4].

The radiation emitted by low-power lasers is being used to assist in the tissue repair process due to the low energy densities used and the wavelengths capable of penetrating the tissues [1].

The most important defects in the repair of burned skin occur in the early stages, leading to a decrease in cellular elements and alterations in collagen synthesis. Various local and systemic factors interfere with and delay healing and, for this reason, tissue repair has received attention in various studies in search of therapeutic methods that can solve or minimize the flaws in the process [5].

The process of tissue repair has been the subject of study for years, due to the high morbidity related to alterations in the natural course of cicatrization. There is a growing multidisciplinary contribution to this dynamic physiological phenomenon,

which continues to challenge researchers [6].

Wound healing consists of a perfect and coordinated cascade of events that culminate in tissue reconstitution. Collagen is the most abundant protein in biological tissue and is also the main component of the extracellular matrix. It is structured in a dense and dynamic network resulting from its constant deposition and reabsorption [7].

The tissue repair process takes place through 3 phases that are not watertight but interdependent, such as the inflammatory phase (vasoconstriction and vasodilation), the proliferative phase (angiogenesis and the formation of granulation tissue) and remodeling to restructure the resistance and elasticity of the tissue [8].

The synthesis of collagen from fibroblasts, which is responsible for skin resistance and tissue integrity, supports a recent and fragile vascular network and maintains the basis for the formation of granulation tissue [9].

The use of therapy with physical agents has been used to improve tissue repair in burn situations. These include the use of electromagnetic waves, such as lasers.

This photobiological process, which consists of the molecular absorption of light energy, is deposited and transformed into vital energy, thus producing primary effects, such as stimulation of lymphocytes, activation of mast cells and an increase in mitochondrial adenosine triphosphate (ATP) production and general therapeutic effects, which promote actions of an analgesic, anti-inflammatory and healing nature [1] [10].

This resource accelerates the repair process, acting on the sequence of physiological and biochemical events resulting from this process, such as inflammation, collagen synthesis, granulation tissue formation and re-epithelialization [1] [8].

Laser therapy has a healing effect by accelerating the production of adenosine triphosphate (ATP), acting directly on the electron transport chain, providing energy that increases the mitotic speed of cells, stimulating microcirculation and increasing the supply of nutritional elements, triggering optimal conditions in epithelial cells for fast and effective healing. The laser acts on collagen, promoting its deposition and remodeling, increasing the number of collagen cross-bridges and the tensile strength of these fibres, which provides benefits for grafts, vascularization, vasodilation, the lymphatic system and has an antibacterial and immunological effect [1] [11] [12].

The aim of this study was to analyze the effects of low-intensity laser application on the time and quality of tissue repair in the skin of rats after chemical burns.

## 2. Cicatricial Acceleration Method (MAC®)

The cicatricial acceleration method (MAC) is a proposal for therapeutic intervention based on the premise of evaluation, identification and interpretation so that treatment can be carried out effectively. In order to carry out the MAC methodology, it is necessary for the assessment to be based on anamnesis, detailed physical examination and complementary examinations and imaging, while the treatment should be based on the principle of doping by photodynamic therapy with an association of photopharmaceuticals and cell markers specific to each case [1]. Clinical

sovereignty guides the development of the physiotherapeutic diagnosis and, consequently, the short, medium and long-term objectives of the treatment plan, making it more effective and precise in physical-functional rehabilitation and improving the patient's quality of life.

MAC uses lasers and LEDs as treatment tools for photodynamic therapy, using cell markers, photosensitive substances, photopharmaceuticals and photobiomodulation, more precisely immunophotobiomodulation, to modulate cell biological processes.

The method uses doping in two treatment modalities as Monodoses and Doses. Therefore, it is necessary to assess the type of tissue, taking into account its density, redox states, severity and depth of tissue damage, and type of microorganism that may be colonizing the tissue.

Monodoses are underdoses. Time folds over time are used, fractionating the time at each wavelength. This doubling time can be increased or decreased depending on the time of the comorbidities (chronic or acute), the characteristics of the lesion, the aim of the treatment and the clinical assessment. Dosimetry is increased or decreased depending on each case.

## 3. Method

An experimental and prospective study was carried out using 22 rats (*Rattus norvegicus*) of the Wistar strain supplied by the Faculty of Medicine Bioterium (UNEC). This study was approved by the Animal Experimentation Ethics Committee of the UNEC Faculty of Medicine under protocol No. 001-2012.

The animals were handled carefully in the morning by the same researcher and accompanied by a veterinarian. The animals were adapted to the proposed environment, prioritizing their well-being [5].

The animals were anaesthetized with a combination of Ketamine 50 mg/Kg and Xylazine 10 mg/Kg intraperitoneally prior to the burn, bidigital trichotomy was performed, followed by delineation of the standardized area of 6 cm × 3 cm, with a total area of 18 $cm^2$, in the dorsal region, 1 cm below the $4^{th}$ thoracic vertebra laterally to the left.

Once the area had been marked, the 50% ATA acid was applied with a volume of 0.2 ml, using the uninterrupted technique of 3 passes over the 18 $cm^2$ area on each animal. A special wooden swab measuring 3 cm in length and 4 mm in diameter was used to make the burn in this experiment.

This technique was carried out by applying trichloroacetic acid topically to the back, applying strong, uniform pressure in accordance with the model cited by Santos *et al*. [13], and work coordinated by *Dr. Marcus Vinicius de Mello Pinto*.

### 3.1. Experimental Groups

Twenty-two male Wistar rats weighing between 250 g and 350 g were used. The animals were kept in a controlled environment in the animal house. The temperature was monitored at around 26˚C, humidity at approximately 60% and the

light-dark cycle was 12 hours each. Nuvilab (Nuvital) feed and filtered water were offered *ad libitum*. Figure 1 shows groups G1 and G2 after burns and their photobiological response to the MAC methodology.

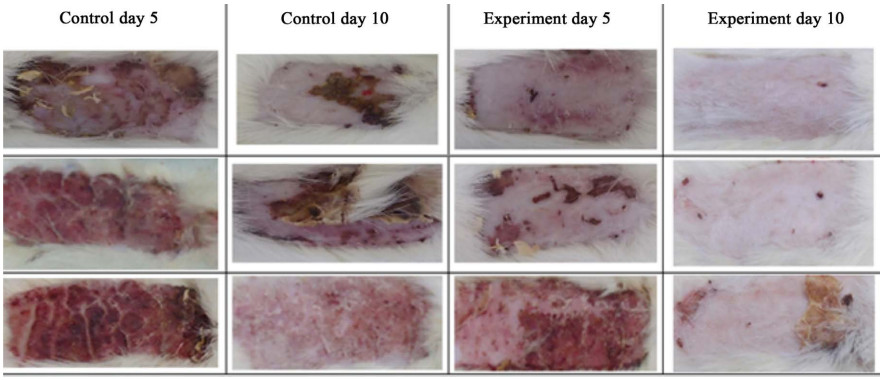


Figure 1. Demonstration of control group (G1) and laser group (G2) on the 5th and 10th day post-burn showing the tissue repair process after MAC within the time folds.

The rats were randomly allocated to 2 groups (n = 11): the control group (G1) and the laser group (G2). The rats remained in individual polypropylene cages lined with sawdust in order to avoid any injuries resulting from fights between them. The therapies were carried out in the morning (11 am to 15 am).

The proposed treatment started 2 hours after the injury and was applied daily for 10 consecutive days. Group G1 received the placebo protocol with the laser switched off. For group G2, the laser was applied using the HTM® Fluence Maxx device (HTM, São Paulo, Brazil), with the following characteristics: visible laser (AlGa) in the 660 nm range, continuous mode, power of 100 mW, dose of 100 J/cm$^2$ and energy of 0.1 J per application point, for 8 seconds at each point inside the burn. The application was carried out using the point technique in direct contact with the wound, where the probe was positioned with light pressure at an angle of 90˚. The 2.0 cm interval between points was respected, totaling 3 points on the burn bed per animal.

After 24 hours of the last therapy, the animals were sacrificed using a $CO_2$ chamber after being anesthetized. The moment after the sacrifice, a biopsy of the skin tissue was taken 3 cm from the base of the skull for histological study, including the wound under repair (Figure 2).

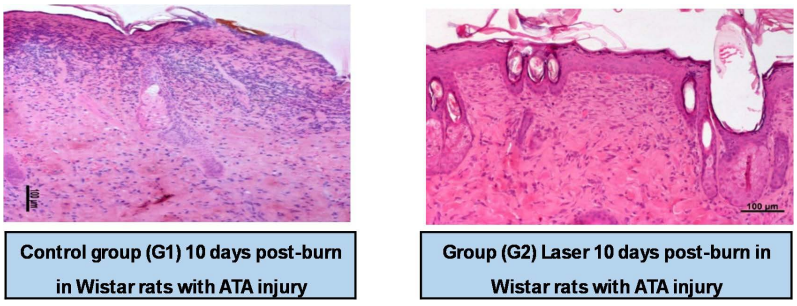


Figure 2. Demonstration of control group (G1) and laser group (G2) 10 days post-burn in Wistar rats with ATA injury.

### 3.2. Evaluation Method

To calculate the percentages of necrosis area, we used the paper template method described by Sasaki *et al*. [14] on the 5th and 10th postoperative days. The method consists of drawing the boundary between viable tissue (soft, pink, warm skin with hair) and necrotic tissue (rigid, darkened, cold, hairless skin with a crust).

After obtaining the mold of the burned area through the decal, it was cut out on transparent tracing paper, weighed on a precision scale (error of ±0.0001 g) and then the following formula already in the literature was used:

$$\text{Percentage of Necrosis Area} = \frac{\text{Weight of Paper Template of Necrosis Area}}{\text{Weight of Paper Template of Total Area of Necrosis}} \times 100$$

### 3.3. Histological Analysis

The collected material was embedded in historresin and after histological sections were made, the material was stained with hematoxylin and eosin (HE). The tissue was analyzed by a blind examiner using an optical microscope with a magnification of 100× Nikon® (Nikon, Tokyo, Japan). Using all the criteria applied in the histological analysis.

Its aim was to illustrate cell proliferation, the synthesis of elements that make up the extracellular matrix (collagen, elastin and reticular fibres), fibroblastic proliferation and the existence of neovascularization.

### 3.4. Statistical Analysis

The GraphPad Prisma version 5.0 statistical program was used for data analysis. The ANOVA test for repeated measures was carried out, followed by the Newman-Keulspost-test. **Figure 3** shows the percentage of the lesions in the fur of Wistar rats submitted to the surgical technique of chemical burns by ATA.

## 4. Results

In order to verify whether or not there were statistically significant differences between the two weight measurements on the fifth- and tenth-day post-burn, a repeated measures ANOVA test was applied, followed by a Newman-Keuls post-test in the Control (G1) and Laser (G2) groups.

According to the results shown, statistically significant differences were found between the percentages of necrosis, the highest values were obtained with Group 1 (53%) on the tenth day, *i.e*. Group G2 (11%) showed the lowest percentage of necrotic area.

The results of the histological analysis of the slides from the groups (G1), the HE cut in a solution of continuity of the epidermis, showed involvement of the underlying skin tissue, with a mixed inflammatory infiltrate with a predominance of polymorphonuclear cells, with hyalinized collagen and preservation of dermal appendages, mainly represented by hair follicles.

The area of lesion described here extends to the deep dermis and subcutaneous tissue up to the muscular layer. In the areas corresponding to the adipose and

muscular tissue, there is an intense infiltrate, also with a predominance of mononuclear cells (macrophages and lymphocytes). It is worth noting that in the deeper areas, there are numerous, sparsely distributed, intact mast cells.

The group (G2) treated with laser for 10 days showed regeneration of the epidermis, in the papillary dermis, granulation tissue was observed with a scarcity of newly formed vessels, with increased cellularity, composed of numerous fibroblasts, some with typical mitosis figures and those arranged to form bundles of packed cells, resembling a dense modeled tissue.

A large amount of collagen is also deposited between the cells. The dermal appendages, represented by hair follicles and sebaceous glands, are histologically preserved.

It was observed that the appearance of the wound of the G1 (control) animal—devitalized and necrotic tissue—10 days after the injury and the appearance of the wound of the G2 (laser) animal—epithelialized tissue—10 days after the injury. It was also observed a demonstration of group G1 (control) and group G2 (laser) on the 5$^{th}$ and 10$^{th}$ day post-burn showing the repair process.

## 5. Discussion

The investigation into the time and quality of tissue repair when subjected to a chemical burn by trichloroacetic acid with the application of a low-power AlGa (arsenide, gallium) laser, identified that the group subjected to laser radiation (G2) on day 10 had an 11% necrotic area, compared to the placebo group (G1) which had 53% (macroscopic view).

In the histological analysis, group G2 showed epidermal regeneration, papillary dermis and granulation tissue with neoformed vessels, increased cellularity, numerous fibroblasts and a large amount of collagen between the cells. No significant inflammatory process was observed. Group G1, on the other hand, showed impairment of the underlying tissue, a mixed inflammatory infiltrate with hyalinized collagen and numerous intact mast cells.

Using a burn model in Wistar rats, it was observed that when applied, the AlGa laser (660 nm and 100 mW power) promoted significant improvement compared to the control group.

Peplow *et al*. [15], suggest that the use of red wavelength in a series of low-dose parameters results in significant benefits in wound healing in animal models and pathological processes in humans, as observed in our study.

Studies differ on the positive and negative effects of visible light irradiation on cell metabolism. Pinto *et al*. [1] observed an increase in cell viability after irradiation with a red wavelength laser. Reddy *et al*. [16] stated that the red laser caused inhibition of cell activity at doses of 10 to 16 J/cm$^2$. This disagrees with the author Rigau [17], who used the HeNe laser for 8 days at a dose of 2 to 4 J/cm$^2$ and an output power of approximately 4 mW and states that it is the most widely accepted laser for treating skin burns.

Al-Watban *et al*. [18], observed that the effects of the laser depended on the

dose and frequency of treatment, with more than 5 days a week having a negative effect on healing. In contrast to these results, our analysis shows improved regeneration over a period of 10 consecutive days, highlighting that there was a significant improvement from the 5th day onwards.

Lagan *et al*. [6], reported that there was no benefit in the treatment of surgical wounds after the application of the AlGa laser at a fixed dose of 9 J/cm$^{2.}$ This statement is justified by Pinto *et al*. [1] and Bai *et al*. [19], that the negative effect occurs due to the hypothesis of cell fatigue, changes in signaling or metabolic pathways in the cell. The results of de Carvalho *et al*. [5], also corroborate the idea that low doses (2 J/cm$^2$) are stimulating, while higher doses (16 J/cm$^2$) can have the opposite effect. Thus, the use of 2 J/cm$^2$ promoted repair in less time and better quality on the 10th day after the burn, showing a statistically significant difference ($p < 0.001$), as shown in **Figure 3**.

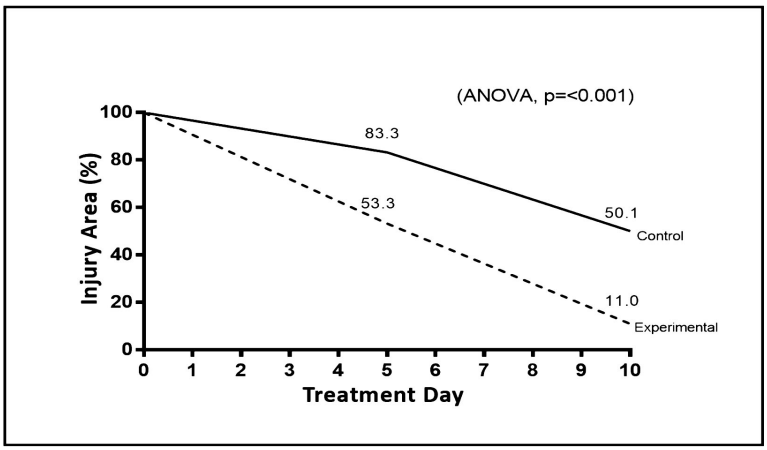


**Figure 3**. Results of the percentage of hair damage in Wistar rats submitted to the ATA chemical burn surgical technique for the MAC methodology.

When the lesions were analyzed macroscopically, it was observed that the animals that received laser irradiation showed no signs of infection or marked inflammation, as confirmed by Pinto *et al*. [1] and de Carvalho *et al*. [5].

In a microscopic view, it was possible to analyze that the inflammatory infiltrate was lower in the group (G2) that received the laser, unlike the placebo group (G1), which, even on the 10th postoperative day, still had predominantly chronic inflammation.

Walker *et al*. [20], admit that doses of 2 J/cm$^2$ had positive effects on neoformation and angiogenesis. In agreement with the authors, Pinto *et al*. [1], Garcia *et al*. [10] and Rigau [17] states that several mechanisms are involved in the action of the laser, such as: fibroblast proliferation, stimulation of the production of basic fibroblast growth factor (BFGF), differentiation of fibroblasts into myofibroblasts, which are responsible for wound contraction and other cells of the immune system responsible for secreting cytokines.

Clinically, a better quality of skin could be observed after the chemical burn in

terms of elasticity, compared to the control group. At the end of the tenth day, all the burns had been repaired in the group that received the laser and microscopically, there was a histological difference in the collagen fibres.

The results obtained were observed through an experimental procedure on animals and cannot be considered in humans, thus limiting the study. Unlike some studies that suggest the use of high doses of laser for skin healing processes, we suggest the use of doses (100 J/cm$^2$), distributed within the mathematical curves of the MAC methodology, which can be confirmed because high doses most often generate an immunophotobiological effect.

Clinically analyzing the application of the laser in humans, taking into account the use of the appropriate dose-power suggested, significant results are obtained in the regenerative, neovascular and collagenesis and elastogenesis processes, obtaining significant results when the time factor of the dosimetric criteria of the MAC methodology is proposed.

The analysis of cellular structures that are activated and/or inhibited by this resource has helped to elucidate how to promote a quality and rapid regenerative process. The therapeutic effects have been attributed to the interaction between the external energy stimulus and the biological tissue (biostimulation), promoting an increase in cellular activity during the repair process [1] [13] [19].

According to Karu *et al*. [3], low-intensity laser therapy has reduced remodeling time and improved the quality of the tissue undergoing neoformation. The basic biological mechanism promoted by this electrophysical resource is the absorption of red and infrared light by chromophores contained in the protein components of the respiratory chain located in the mitochondria, which in turn absorbs the energy verified in the MAC methodology, the absorptive effect of lasers and LEDs triggers a cascade of biochemical events, resulting in an increase in enzymatic activity and the production of adenosine when the dosimetric factor is distributed in the space-time curve seen in this methodology [1].

## 6. Conclusion

In view of the method used, it was concluded that the methodology of scar acceleration (MAC®) favored the repair of lesions caused by a 2$^{nd}$-degree chemical burn, optimizing time and presenting better tissue quality, with the advantage of adapting to cell markers and photofarms to optimize treatment time and symptomatology.

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## Conflicts of Interest

Dr. Marcus Vinicius de Mello Pinto is the creator of MAC®. The other authors have no relevant conflicts to disclose.

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