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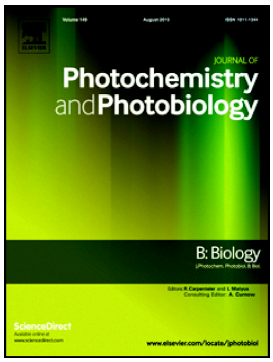

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Photobiomodulation mechanisms in the kinetics of the wound healing process in rats

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Abstract

Objectives:
The healing process of cutaneous lesions is considered a complex event divided into distinct and overlapping phases, which responds satisfactorily to photobiomodulation (PBM). PBM is indicated as a therapeutic resource capable of assisting tissue repair. The present study aimed to analyze the kinetics of cutaneous wounds healing process after application of the GaAlAs laser for treating wounds on the dorsum of rats.

Materials and Methods:
This study was approved by the Animal Ethics Committee of UFSCar. The animals were divided into 2 groups (n = 10); control group (CG) used 0.9% saline solution and the laser group (LG) used GaAlAs, 670nm continuous pulse, 30mW power, 14.28J /cm^2 energy density, irradiating 1 point per wound for 30s, totaling 15 consecutive days of treatment. Samples were collected on the 4th, 11th and 16th days for histological analysis of HE, Picrosirius-Red, immunohistochemistry (Collagen1, TNF-α, VEGF). Statistical analyzes used the one-way ANOVA test for intra and inter group evaluations, and the Tukey post-test. Level of significance was set at $p < 0.05$.

Results:
The histopathological analysis (HE) showed a statistically significant difference for lower values of inflammatory infiltrate in LG versus CG on the 16th day; and for the increase of collagen in the 11th and 16th days of treatment. There was a statistically significant difference in the increase of VEGF on the 11th day for LG; decrease of TNF-α on the 4th and 11th day for LG, and increase of collagen type 1 on the 4th and 16th days for LG. The birefringence analysis of the percentage of collagen fibers presented on the 11th day of treatment revealed a greater quantity and significant statistical difference. Collagen fibers showed improved organization and arrangement on the 11th day for LG.

Conclusion:
Our results show that PBM is effective in helping the kinetics of the cutaneous wound healing process in rats and promotes the necessary stimuli for the satisfactory evolution of healing process, ultimately leading to structurally desirable tissue.

KEYWORDS: Collagen, GaAlAs Laser, Photobiomodulation, Wound Repair

Introduction

Cutaneous wounds are defined as interruptions of cutaneous mucosal tissue that promote considerable changes in their anatomical structure and/or physiological function. Depending on the severity of the disease, they progress to considerable morbidity and mortality rates [1-3]. There are currently no reliable estimates of the prevalence and incidence of chronic wounds, since this term encompasses different types of cutaneous lesions, with several classifications and categories [4,5].

The healing process of cutaneous lesions is considered a complex event divided into distinct and overlapping phases, called inflammation, proliferation and remodeling [2,6,7]. The inflammatory phase, which spans from the beginning of the lesion to approximately 4 days after, is characterized by the recruitment of inflammatory neutrophils and cytokines, particularly TNF-α, responsible for stimulating keratinocytes, macrophages and fibroblasts, as well as acting on the expression of growth factors that contribute to angiogenesis and collagen synthesis [8]. The proliferative phase lasts from 5 to 14 days [9,10] and includes vascular endothelial reestablishment by angiogenesis, as well as extracellular matrix formation, and epithelialization [2,11,12]. The remodeling phase begins after 21 days after the injury and can persist for months depending on the extent and depth of the wound. Its main characteristic is the remodeling of collagen, in which the fibers become thick, resistant and organized, and the covalent chemical bonds are directly responsible for their maturation [8,9].

The wound healing process may often be less satisfactory due to excessive inflammation, extensive/continuous trauma, and infections. Thus, the literature presents many therapies that attempt to accelerate healing, as well as ensure a better quality of the healed tissue. Among these, photobiomodulation (PBM) has been successfully used to regenerate several cutaneous lesions [13-16].

PBM consists of the therapeutic use of coherent, collimated, monochromatic and polarized light, absorbed by endogenous chromophores (cytochrome C), triggering non-thermal and non-cytotoxic biological reactions, through photochemical and photophysical events, ultimately leading to physiological alterations. The use of low density energy and wavelength in this therapy facilitates the penetration of the beams into the cells and tissues, with biomodulator effects. One of the biomodulator effects created by this therapy is the light's ability of photobiological interaction with the

injured tissues, stimulating specific events such as inflammation modulation [17, 14], endothelial cells proliferation due to growth factors like VEGF, and fibroblasts proliferation, which increases the synthesis of collagen [18-23]. These are considered key events for a good evolution of the healing process.

Fig 1: Illustration of the PBM mechanism of action (Adapted from Avci, 2013).

In addition, the efficiency of PBM in cellular mechanisms, whether by proliferative and energetic pathways, transduction of electrical signals, biochemical or immune activity, are directly dependent on the parameters employed, such as electromagnetic wavelength, dose, light beam area, specificity tissue, time and type of injury [24,25]. Therefore, it is important to highlight that the choice of a suitable protocol to treat cutaneous lesions induced by surgical instrumentation is still a challenge, since the literature compares different parameters in different types and sizes of lesions, which makes it difficult to understand the mechanisms involved in the kinetics of the process evolution in its entirety.

Therefore, this work aimed to evaluate the action of PBM on the kinetics of the healing process of cutaneous lesions induced on the dorsum of rats, and better explore the photobiological mechanisms triggered by phototherapy in this type of lesion.

Materials and Methods

Experimental Animals

Twenty adult, male Wistar rats (*Rattus norvegicus albinus*), weighting 250-300 g, were kept at the Animal Hospital of the Physiotherapy Department of UFSCar, for 16 days. The animals were individually allocated in appropriate standard polyethylene cages, under controlled environmental conditions (19-23 °C and 12/12h light/dark cycles), with free access to adequate food and water. This study was submitted and approved by the Ethics Committee on the Use of Animals of UFSCar, n° 2-007 / 2014. The animals were randomly divided into two groups (n = 10):

- Control Group (CG): Wounds and PBM simulation;
- Laser Group (LG): Wounds and treatment with PBM;

Surgical Procedure

The animals were weighed and anesthetized by Ketamine (40 mg/kg, Agener, SP, Brazil) and Xylazine (15 mg/kg, Dopaser, SP, Brazil) prior wounding. The animals were then placed in the ventral decubitus for the digital trichotomy of the dorsal region, and three dermatological punch lesions of 10mm diameter were performed with a circular blade, including all 3 cutaneous layers, equidistant 1cm from each other, on the back of each animal [26] (Figure 2).

Samples were collected on the 4th, 11th and 16th day of the treatment, with random selection of wounds at the end of each period.

Fig 2: Illustration of the three wounds performed by surgical procedure using 10mm dermatological *punch*.

Photobiomodulation Treatment

PBM was performed with a red laser (LASERPULSE, IBRAMED, Brazil), wavelength of 670nm, output power of 30mW, energy density of 14.28 J/cm^2 and beam cross-section of 0.063cm^2. The equipment was calibrated prior to the beginning of the experiment at the Institute of Physics of the School of Engineering of São Carlos of the University of São Paulo (EESC-USP) by a qualified technician. The application started one hour after wounding, and was carried out with daily applications performed in a single point positioned perpendicularly to the back of the animal, continuously, with the beam of light covering the entire area of the wound. The total number of applications was 3, 10 and 15 according to the evaluated experimental times. The LG received PBM uninterruptedly until the time of sample collection at each proposed experimental time (4th, 11th and 16th days). At the time of treatment, the animals were immobilized by a cotton blanket that served as aid both for the application of the therapy and to minimize the animal's stress. The CG received simulated PBM application. The detailed parameters of PBM are expressed in table 1.

Table 1: Detailed parameters used for treatment with PBM.

Parameters	Values
Power (mW)	30
Irradiance (W/cm^2)	0.47
Wavelength (nm)	670
Mode of Action	Continuous
Beam transverse area (cm^2)	0.063
Energy Density (J/cm^2)	14.28
Time (s)	30
No. of irradiation points	1
Energy (J)	0.9

Sample collection

Tissue samples were collected using dermatological *punch* with total area of 10mm, on the 4th, 11th and 16th day of treatment. The sample collected in each experimental period was randomly selected in order to avoid bias.

Euthanasia

The animals were euthanized, by decapitation with guillotine, on the 16th day after the surgical wound.

Fig 3: Illustration of the time line involving surgical procedures, treatments and material collection for analysis.

Histopathological Analysis (HE)

Immediately after sample collection for analysis, the tissue was cut longitudinally (with reference to the craniocaudal axis of the animal, covering both the center and the initial margin of the wound and part of the healthy tissue) with scalpel, fixed in buffered formalin 10% for 24 hours, washed in running water for 24 hours, kept in 70% alcohol and processed for inclusion in paraffin. For the preparation of the slides the tissue samples were sectioned in 5µm thickness.

We obtained three sections of each sample, which were subsequently stained with hematoxylin and eosin (HE, Merck) and analyzed. The HE evaluation was performed using a light microscope (Zeiss Axioshop, Carl Zeiss, with 20X objective). Tissue re-epithelization and collagen expression were evaluated by the semi quantitative analysis, considering the values of 0-4, described in table 2 [27]. The expression of the inflammatory infiltrate was analyzed by the semi quantitative evaluation using scores according to [28], considering the values of 0-4 described in table 3. All analyzes were performed by three evaluators, blinded to the experimental groups.

Table 2: HE classification scale for semi-quantitative analysis of the tissue epithelization and collagen in slides stained with hematoxylin and eosin (HE, Merck).
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Scale	Epithelization	Collagen Expression
0	Absent	Absent-GT
1	Thickness of cut edges	Minimal-GT
2	Migration of the cells	Mild-GT
3	Bridging of the excision	Moderate-GT
4	Keratinization	Marked-GT

GT- granulation tissue

Table 3: Histopathological classification scale for semi-quantitative analysis of the inflammatory infiltrate in slides stained with hematoxylin and eosin (HE, Merck).

Histopathological classification scale for evaluation of Inflammatory Infiltrate	
1	Acute inflammation (pyogenic membrane is formed)
2	Predominance of diffuse acute inflammation (predominance of granulation tissue)
3	Predominance of chronic inflammation (fibroblasts beginning to proliferate)
4	Resolution and healing (decrease or absence of chronic inflammation, with occasional round cells)

Immunohistochemical Analysis

The samples were inserted into silanized slides for better adhesion of the studied biological material and then maintained for 24h at 37 °C. After dewaxing and hydration, the histological sections were stained with a hydrophobic pen and then washed twice in a buffer solution enriched with Tween for 3 min. Sections were then immersed in hydrogen peroxide for 10 minutes, washed twice in phosphate buffered saline (PBS) twice for 3 minutes and finally immersed in endogenous peroxidase for 30 min. The slides were then incubated with the primary antibodies as follows. Vascular endothelial growth factor (VEGF): polyclonal primary anti-VEGF antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at a concentration of 1:400; tumor necrosis factor (TNF-α): primary anti-TNF-α antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) at a concentration of 1:400, and polyclonal anti-collagen type I Cruz Biotechnology Inc., Santa Cruz, CA, USA) at a concentration of 1:200. Both were incubated for 2 h and washed twice in PBS. The slides were subsequently incubated

with a secondary antibody (anti-rabbit IgG) (Vector Laboratories, Burlingame, CA) at a concentration of 1:200 in PBS for 30 min.

Immunoblots of VEGF, TNF-α and type I collagen were quantitatively assessed by Image J 1.37a Software. The morphometric analysis used the total pixels percentage of the marked area in each image using the Threshold color (software ImageJ) [29].

Birefringence Analysis

The histological sections stained by the Picrosirius-Red method were analyzed in a polarized light microscope (Leica, with a 20x objective) to evaluate the organization of collagen fibers.

Quantitative analysis used the ImageJ 1.37a software to evaluate the percentage of orange-reddish coloration. The thicker and strongly birefringent collagen fibers correspond to collagen type I.

We captured three images per cut at a magnification of 20x (1st, 2nd and 3rd quadrant) of the three cutaneous layers. Color Deconvolution ImageJ software was used to evaluate the percentage of red color (collagen) in the image area. This software recognizes the colors of the image and decomposes them into three basic colors: blue (collagen), red and purple. The morphometric analysis, referring to purple color, was measured as the percentage of the total pixels in each image using *Threshold color* (ImageJ software) [29].

Statistical analysis

The results were expressed as average ± standard deviation. The results analysis was performed with the *Software* Graph PadPrism 5.0. We performed the Saphiro Wilk test to assess the data normality. Intergroup comparisons were performed with ANOVA *one-way*. For multiple comparisons we used the Tukey post-hoc test with significance level of $p<0.05$.

Results

Histopathological Analysis (HE)

The HE analysis showed differences in the tissue repair process phases during the course of treatment between the experimental groups.

HE analysis for the expression of type I collagen revealed significant statistical difference ($p \leq 0.01$) between the CG and LG in 2 moments throughout the treatment (11th and 16th day). The LG showed a mild increase on the 11th day of treatment and a

moderate increase on the 16th day of treatment compared to CG, characterizing a more advanced stage in the tissue repair process (Figure 4).

The results showed a statistically significant difference ($p \leq 0.05$) for the amount of inflammatory infiltrate, which was lower for LG versus CG on the 16th day of treatment (Figure 4).

Fig 4: Quantitative evaluation of the HE analysis of the expression values of type I collagen and inflammatory infiltrate for the CG (control group), and LG (Laser group) on the 4th, 11th and 16th day after the lesion, with significance level of $p < 0.05$.

The semi-quantitative analysis of the wound reepithelialization values evidenced predominance of incision connection and keratinization for LG on the 16th day of treatment, in agreement with the findings of the descriptive analysis by Solmaz (2016), in which the comparison between the groups showed that LG is in a more advanced stage of tissue repair (Figure 5).

Fig 5: Photomicrographs representing the experimental groups related to inflammatory infiltrate, collagen expression and wound reepithelialization. A: CG-4 (control group on 4th day); B: CG-11 (control group on 11th day); C: CG-16 (control group on 16th day); D: LG-4 (laser group on 4th day); E: LG-11 (laser group on the 11th day); and F: LG-16 (laser group on 16th day); (n = 8). The white arrow, # and * indicate the expression of type I collagen, reepithelialization of the wound and the presence of inflammatory infiltrate, respectively (100x).

Immunohistochemical Analysis

VEGF factor immunoexpression

VEGF factor analysis was evaluated by the expression of brownish color, observing that on the 11th day the LG showed a statistically significant difference ($p \leq 0.05$) with higher immunolabeling when compared to the CG (Figure 6).

TNF-α factor Immunoexpression

The results obtained from the immunoexpression of TNF-α showed a decrease in LG values during the treatment period, with a statistically significant difference ($p \leq 0.05$) between CG and LG on the 4th and 11th day (Figure 6).

Type I Collagen Immunoexpression

Regarding the immunoexpression of type I collagen, we observed a statistically significant difference ($p \leq 0.05$), showing an increase in the 4th and 16th day of treatment for the LG (Figure 6).

Fig 6: Quantitative analysis of the immunoexpression of VEGF, TNF-a and type I Collagen for the CG (control group), and LG (Laser group) on the 4th, 11th and 16th day after the lesion, with significance level of $p < 0.05$.

Birefringence Analysis

The analysis of the percentage of the collagen fibers presented a greater amount and a statistically significant difference ($p \leq 0.05$) for LG versus CG on the 11th day of the treatment (Figure 7).

Fig 7: Quantitative birefringence analysis for the CG (control group), and LG (Laser group) at the 4th, 11th and 16th day after treatment, with significance level of $p < 0.05$.

We observed that on the 11th day of treatment there was a better organization and arrangement in the LG compared to the CG, and on the 16th day the fiber organization intensifies even more, indicating a tissue repair process (Figure 8).

Fig 8: Photomicrographs representative of the experimental groups regarding the birefringence of the collagen fibers of the wound. A: CG-4 (control group on 4th day); B: CG-11 (control group on 11th day); C: CG-16 (control group on 16th day); D: LG-4 (laser group on 4th day); E: LG-11 (laser group on the 11th day); and F: LG-16 (laser group on 16th day); (n = 8). The white arrows indicate the collagen fiber (100x).

Discussion

The present study shows that the use of PBM at the wavelength 670nm, 30W and energy density of 14.28 J/cm^2 provides positive stimulus for the evolution kinetics of the healing process on cutaneous wounds. Although the literature shows several evidences about the effects promoted by PBM, controversies about the standardization of the best protocol to be used in surgical skin lesions are still unclear. We observed the comparison of such protocols in different wounds with different degrees of impairment and severity [19,20,22,30-32].

Recent studies investigating the effects of PBM on cutaneous lesions emphasize that laser light is able to accelerate tissue repair, modifying the cellular environment that cause modulation of inflammation, improving angiogenesis, increasing collagen

synthesis, and reepithelialization [13,16,24,33-36]. The HE results found in our study reveal that the laser light has a direct action in the modulation of inflammation, which is demonstrated in the first days of treatment, when we observed a significant reduction of the levels of inflammatory infiltrate with decreased expression of TNF-α when compared to the control. In addition, it is noteworthy that the presence of TNF-α in late time of the healing process indicates a possible collagen degradation mechanism, which would affect the final result of the repair, and thus, the reduction and/or modulation of their performance is beneficial and should be considered.

Still on the influence of PBM in the inflammatory phase, our findings corroborate with other studies that also identified the modulation of inflammation after treatment with PBM in cutaneous tissues, suggesting that this resource can anticipate the resolution of this phase from its first applications, benefiting the subsequent phases [37-39].

Angiogenesis, in turn, is a critical and complex event, coordinated by specific growth factors associated with extracellular matrix components, and dependent on the formation of granulation tissue and the microvascular environment. VEGF is the predominant growth factor with specific biological activity that deliberates the events of the cellular cascade responsible for vascular reestablishment. Studies have investigated the action of PBM on the induction of VEGF expression in various conditions and lesions [13,14,24,32,34]. Brassolatti et al. (2016) using a laser (660 nm, 100 mW and 25 J/cm^2) observed both the greater presence of new vessels in the layer of the new dermis and the relative increase in VEGF factor expression. Renno et al. (2011), using a 660nm, 100mW laser, but with lower creep (0.5J/cm^2) also reported that their results were favorable in the early immunoexpression of VEGF factor, with consequent improvement in angiogenesis.

In turn, Colombo et al. (2013), investigated the process of angiogenesis in cutaneous wounds induced on the back of laser- treated rats (660nm, 16mW, 10J/cm^2), and concluded that laser light contributes positively by increasing angiogenesis. Differently, Szymanska et al. (2013), conducted an in vitro study, where they evaluated the effects of PBM on endothelial proliferation and expression of VEGF factor, and also concluded that laser light stimulates endothelial proliferation, with consequent decrease in VEGF, thus suggesting the role of VEGF in the microvascular reestablishment of the lesion environment. Our findings corroborate previous studies, showing higher percentage of VEGF expression in LG compared to CG. In addition, it is important to

note that this fact was evidenced on the eleventh day of treatment, when the laser light was able to stimulate the release of growth factors, particularly the VEGF, in the period comprising the transition between the inflammatory and proliferative phases. These results corroborate with the findings of Fiorio, et al. (2017), which observed that PBM stimulated further release of VEGF at the seventh day of treatment.

Interestingly, angiogenesis in addition to restoring the oxygen and nutrients level to the newly formed tissue through a high metabolic demand, directly favors the protein synthesis, since it is interconnected to the processes of cell proliferation and migration, which includes the presence of fibroblasts, responsible for synthesize or collagen.

Of the factors evaluated in the proliferative phase, we emphasize that the synthesis of collagen, key protein for the restoration and elasticity of the new dermis, is indispensable for understanding the evolution kinetics of the healing process. Therefore, it is known that the synthesis quality is directly related to both the functional and aesthetic results presented by the new tissue. Therefore, the observation of imbalances becomes essential, since any intercurrences, such as excessive formations of both extracellular matrix and disorganized fibers, may evolve into significant tissue adherence frames that prevent the proper physiological functioning of the site [42]. Therefore, the evaluation of not only the amount of fibers present in the site but also their quality in the environment of the forming dermis is reinforced.

Gonçalves et al., 2013 report that the maturation of collagen and its rearrangement are crucial steps that directly affect the mechanical resistance of the new tissue. It occurs with the remodeling and gradual replacement of Type III preformed collagen for type I collagen, generating an increase in the molecular interactions between the newly formed fibers. Meirelles et al. (2008) report that this maturation can be observed with 21 days of treatment with fluency of 20 J/cm^2. Brassolatti et al. 2016, reported a difference in collagen synthesis at the tenth day of treatment, evidencing an early synthesis with improvement in the structural organization of the fibers, when the wound was treated with laser light.

In view of the presented results, it is pertinent to emphasize that the PBM is effective in assisting the kinetics of the healing process of cutaneous wounds in rats. All the biological events evaluated demonstrate an important interconnection that suggests that the benefits of PBM range from the modulation of inflammation to the reestablishment of the new tissue, contributing effectively to primordial events such as

cell proliferation and differentiation, with consequent increase and structural quality of collagen.

Conclusion

The use of PBM with 670 nm laser promoted the necessary stimuli for the satisfactory evolution of the wound healing process, and led to a structurally adequate tissue at the end of the treatment.

Bibliographic references

1. Eming SA1, Krieg T, Davidson JM (2007) Inflammation in wound repair: molecular and cellular mechanisms. J Invest Dermatol 127:514–525 https://www.ncbi.nlm.nih.gov/pubmed/17299434
2. Reinke JM1 & Sorg H (2012) Wound Repair and Regeneration. Eur Surg Res 49:35–43 https://www.ncbi.nlm.nih.gov/pubmed/22797712
3. Buchanan PJ1, Kung TA, Cederna PS (2014) Evidence-Based Medicine: Wound Closure. Plastic and Reconstructive Surgery 134(6):1391-1404 https://www.ncbi.nlm.nih.gov/pubmed/25415102
4. Graves N1, Zheng H (2014) The prevalence and incidence of chronic wounds: a literature review. Journal of the Australian Wound Management Association 22(1):4-19 https://search.informit.com.au/documentSummary;dn=272162994803134;res=IELHEA
5. Jarbrink K1, Ni G, Sönnergren H, Schmidtchen A, Pang C, Bajpai R, Car J (2016) Prevalence and incidence of chronic wounds and related complications: a protocol for a systematic review. Syst Rev.8;5(1):152 https://www.ncbi.nlm.nih.gov/pubmed/27609108
6. Martin P1 (1997) Wound healing – Aiming for perfect skin regeneration. Science 276(5309):75-81 https://www.ncbi.nlm.nih.gov/pubmed/9082989
7. Li J1, Chen J, Kirsner R (2007) Pathophysiology of acute wound healing. Clinics in Dermatology 25:9–18 https://www.ncbi.nlm.nih.gov/pubmed/17276196
8. Childs DR1, Murthy AS (2017) Overview of Wound Healing and Management. Surg Clin North Am 97(1):189-207 https://www.ncbi.nlm.nih.gov/pubmed/27894427
9. Enoch S1, Leaper DJ (2005) Basic science of wound Healing. Surgery 23(2):37-42 http://www.sciencedirect.com/science/article/pii/S0263931906700679
10. Muzzarelli RAA, 2009 Chitins and chitosans for the repair of wounded skin, nerve, cartilage and bone. Carbohydrate Polymers 76:167–182 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2852840/
11. Broughton G1, Janis JE, Attinger CE (2006) Wound Healing: An Overvew. Plastic and Reconstructive Surgery117(7 Suppl):1e-S-32e-Shttps://www.ncbi.nlm.nih.gov/pubmed/168017500

12. Chow O1 & Barbul A (2014) Immunonutrition: Role in Wound Healing and Tissue Regeneration. Advances in wound care, 3(1): 46-53 https://www.ncbi.nlm.nih.gov/pubmed/24761344
13. Ezzati A1, Bayat M, Khoshvaghti A (2010) Low-level laser therapy with a pulsed infrared laser accelerates second-degree burrn healing in rat: a clinical and microbiologic study. Photomedinine and Laser Surgery 28(5):603-611 https://www.ncbi.nlm.nih.gov/pubmed/20860542
14. Guirro ECO1, Montebelo MIL, et al (2010) Effect of Laser (670 nm) on Healing of Wounds Covered with Occlusive Dressing: A Histologic and Biomechanical Analysis. Photomedicine and Laser Surgery 28(5):629–634 http://www.producao.usp.br/handle/BDPI/15250
15. Hussein AJ1, Alfars AA, Falih MAJ, Hassan A-NA (2011) Effects of a low level laser on the acceleration of wound healing in rabbits. North Am J Med Sci 3: 193-197 https://www.ncbi.nlm.nih.gov/pubmed/22540090
16. Brassolatti P1, Bossini PS, Oliveira MCD, Kido HW, Tim CR, Almeida-Lopes L, De Avó LRS, Moreira FMA, Parizotto NA (2016) Comparative effects of two different doses of low-level laser therapy on wound healing third-degree burns in rats. Microscopy Research and Technique 79:313-320 https://www.ncbi.nlm.nih.gov/pubmed/26853699
17. Bayat A1, Arscott G, Ollier WE, McGrouther DA, Ferguson MW (2005) Keloid disease: clinical relevance of sinLGe versus multiple site scars. Br J Plast Surg, 58(1):28-37 https://www.ncbi.nlm.nih.gov/pubmed/15629164
18. Kubota J (2004) Defocused diode laser therapy (830 nm) in the treatment of unresponsive skin ulcers: a preliminary trial. J Cosmet Laser Ther 6:96–102 https://www.ncbi.nlm.nih.gov/pubmed/15204000
19. Gal P1, Vidinsky B, Toporcer T, Mokry M, Mozes S, Longauer F, Sabo J (2006) Histological Assessment of the Effect of Laser Irradiation on Skin Wound Healing in Rats. Photomedicine and Laser Surgery 24(4):480-488 https://www.ncbi.nlm.nih.gov/pubmed/16942428
20. Silva JP1, Silva MA, Almeida APF, Junior IL, Matos AP (2010) Laser Therapy in the Tissue Repair Process: A Literature Review. Photomedicine and Laser Surgery 28(1):17-21 https://www.ncbi.nlm.nih.gov/pubmed/19764898

21. Aust MC1 et al (2011) Percutaneous collagen induction-regeneration in place of cicatrisation? Journal of Plastic, Reconstructive et Aesthetic Surgery 64:97-107 https://www.ncbi.nlm.nih.gov/pubmed/20413357
22. Melo VA1, Anjos DC, Albuquerque Júnior R, Melo DB, Carvalho FU (2011) Effect of low level laser on sutured wound healing in rats. Acta Cir Bras 26(2):129-34 https://www.ncbi.nlm.nih.gov/pubmed/21445476
23. Fortuna T1, Gonzales AC, Sá MF, Andrade ZA, Reis SRA, Medrado ARAP (2017) Effect of 670 nm laser photobiomodulation on vascular density and fibroplasia in late stages of tissue repair. https://www.ncbi.nlm.nih.gov/m/pubmed/29239111/#fft
24. Bossini, PS1, Fangel R, Habenschus RM, Renno AC, Benze B, Antonio J, et al. (2009). Low-level laser therapy (670 nm) on viability of random skin flap in rats. Lasers in Medical Science;24(2):209-13 https://www.ncbi.nlm.nih.gov/pubmed/18351431
25. Silveira PCL1, Silva LA, Freitas TP, Latini A, Pinho R (2011) Effects of low-power laser irradiation (LPLI) at different wavelengths and doses on oxidative stress and fibrogenesis parameters in an animal model of wound healing. Lasers Med Sci 26:125-131 https://www.ncbi.nlm.nih.gov/pubmed/20865435
26. Moreira CF1, Cassini-Vieira P, Silva MF, Barcelos LS (2015) Skin Wound Healing Model - Excisional Wounding and Assessment of Lesion Area. , 5(22):1-4 https://bio-protocol.org/e1661
27. Solmaz H1, DervisoLGu S, Gulsoy M, Ulgen Y (2016) Laser biostimulation of wound healing: bioimpedance measurements support histology. Laser Med Sci 31(8):1547-1554 https://www.ncbi.nlm.nih.gov/pubmed/27371448
28. Camacho-Alonso F1, López-Jounet P (2007) Clinical-pathological study of the healing of wounds provoked on the dorso-lingual mucosa in 186 albino rats. Otolaryngology–Head and Neck Surgery 136:119-124 https://www.ncbi.nlm.nih.gov/pubmed/17210346
29. Caetano GF1, Frade MAC, Andrade TAM, Leite MN, Bueno CZ, Moraes AM, Ribeiro-Paes JT, (2014) Chitosan-alginate membranes accelerate wound healing. J Biomed Mater Res B Appl Biomater. 2015 Jul;103(5):1013-22 https://www.ncbi.nlm.nih.gov/pubmed/25220821
30. Mendez TMTV1, Pinheiro ALB, Pacheco MTT, Nascimento PM, Ramalho LMP (2004) Dose and Wavelength of Laser Light Have Influence on the Repair of

Cutaneous Wounds. Journal of Clinical LaserMedicine & Surgery 22(1):19-25 https://www.ncbi.nlm.nih.gov/pubmed/15117483

31. Marques ACF1 et al (2016) Photobiomodulation therapy on collagen type I and III, vascular endothelial growth factor, and metalloproteinase in experimentally induced tendinopathy in aged rats. Laser Med Sci 31(9):1915-1923 https://www.ncbi.nlm.nih.gov/pubmed/27624782
32. Fiorio FB1, Albertini R, Pinto Leal-Junior EC, Camillo de Carvalho PT (2014) Effect of low-level laser therapy on types I and III collagen and inflammatory cells in rats with induced third-degree burns. Lasers in Medical Science;29(1):313-19 https://www.ncbi.nlm.nih.gov/pubmed/23677436
33. Karu TI1, Pyatibrat LV, Kalendo GS (2004) Photobiological modulation of cell attachment via cytochrome c oxidase. Photochem Photobiol Sci 3:211-216 https://www.ncbi.nlm.nih.gov/pubmed/14872239
34. Renno ACM1, Iwama AM, Shima P, Fernandes KR, Carvalho JG, De Oliveira P (2011). Effect of low-level laser therapy (660 nm) on the healing of second-degree skin burns in rats. Journal of Cosmetic and Laser Therapy;13(5):237-42 https://www.ncbi.nlm.nih.gov/pubmed/21774661
35. Avci P1, Gupta A, Sadasivam M, Vecchio D, Pam Z, Pam N, Hamblin MR (2013) Low Lewel Laser (Light) Therapy (LLLT) in Skin: Stimulating, Healing, Restoring. Semin Cutan Med Surg 32:41-52 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4126803/
36. Hamblin MR1 (2017) Mechanisms and applications of the anti-inflammatory effects of photobiomodulation. AIMS Biophys 4(3):337–361 https://www.ncbi.nlm.nih.gov/pubmed/28748217
37. Chen X (2010) Role of TNF–α in Wound Repair in Human Vocal Fold Fibroblasts. Laryngoscope 120(9): 1819–1825 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2965638/
38. Mesquita-Ferrari RA1 et al (2011) Effects of low-level laser therapy on expression of TNF-α and TGF-β in skeletal muscle during the repair process. Laser Med Sci 26(3):335-40 https://www.ncbi.nlm.nih.gov/pubmed/21053039
39. Chu YH1, Chen SY, Hsieh YL, Teng YH, Cheng Y (2017) Low-level laser therapy prevents endothelial cells from TNF-α/cycloheximide-induced apoptosis. Laser Med Sci i https://doi.org/10.1007/s10103-017-2364-x https://www.ncbi.nlm.nih.gov/pubmed/29098460

40. Colombo F1, Neto AdAPV, Sousa APCd, Marchionni AMT, Pinheiro ALB, Reis SRDA (2013). Effect of Low-Level Laser Therapy (660 nm) on Angiogenesis in Wound Healing: A Immunohistochemical Study in a Rodent Model. Brazilian Dental Journal;24(4):308-12 https://www.ncbi.nlm.nih.gov/pubmed/24173246

41. Szymanska J1, Goralczyk K, Klawe JJ, Lukowicz M, Michalska M, Goralczyk B, Zalewski P et al (2013) Phototherapy with low-level laser influences the proliferation of endothelial cells and vascular endothelial growth factor and transforming growth factor-beta secretion. J Physiol Pharmacol 64(3):387–391 https://www.ncbi.nlm.nih.gov/pubmed/23959736

42. Pessolato AGT1, Martins DdS, Ambrosio CE, Furlanetto Mancanares CA, de Carvalho AF. 2011. Propolis and amnion reepithelialise second-degree burns in rats. Burns 37: 1192-201 https://www.ncbi.nlm.nih.gov/pubmed/21741176

43. Goncalves RV, Novaes RD, Cupertino MdC, Moraes B, Viana Leite JP, Gouveia Peluzio MdC1, et al. (2013). Time-dependent effects of low-level laser therapy on the morphology and oxidative response in the skin wound healing in rats. Lasers in Medical Science;28(2):383-90 https://www.ncbi.nlm.nih.gov/pubmed/22354548

44. Meireles CG1, Santos JN, Chagas PO, Moura AP, Pinheiro AL (2008) Effectiveness of laser photobiomodulation at 660 or 780 nanometers on the repair of third-degree burns in diabetic rats. Photomed Laser Surg, 26(1):47-54 https://www.ncbi.nlm.nih.gov/pubmed/18248161

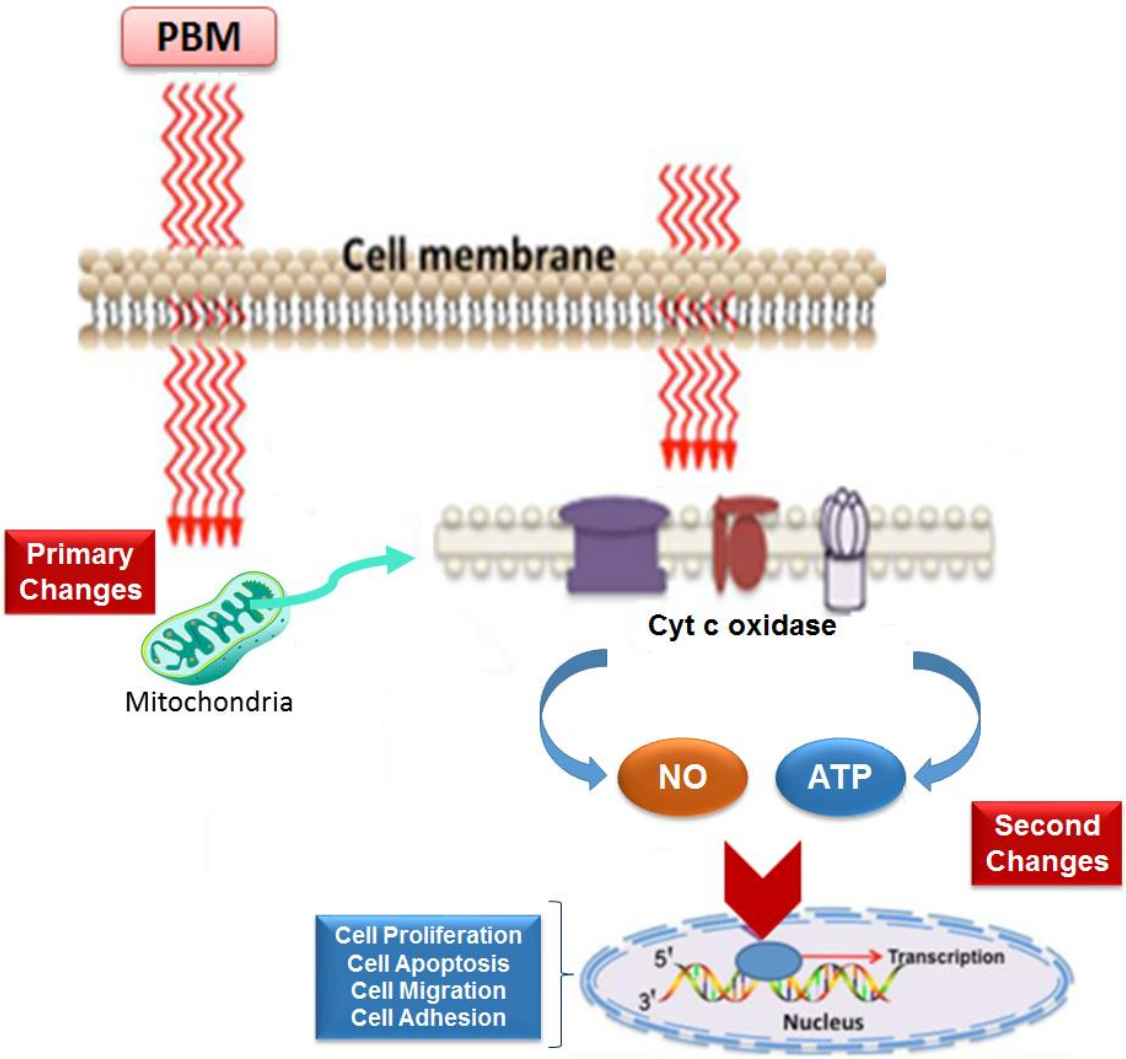
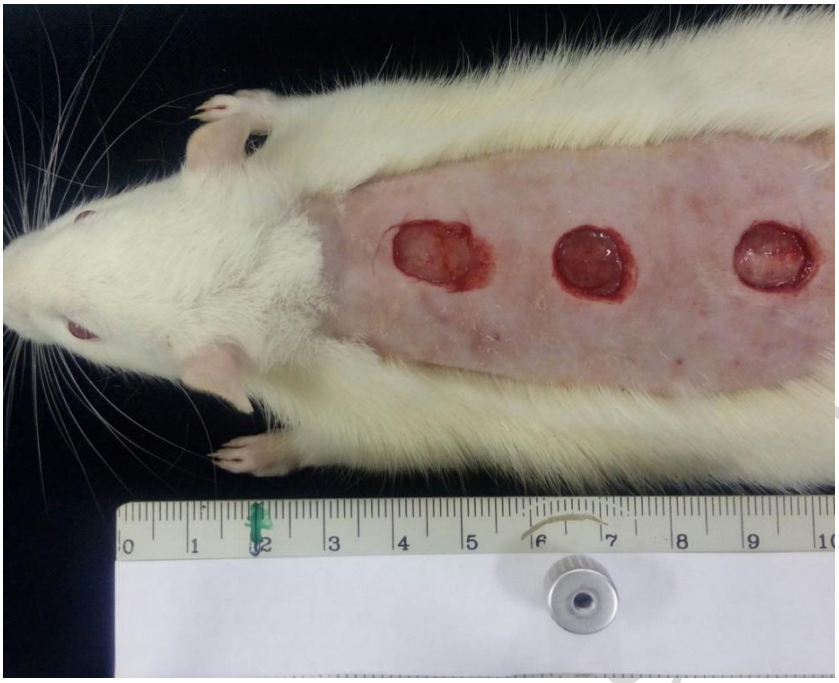


Figure 1



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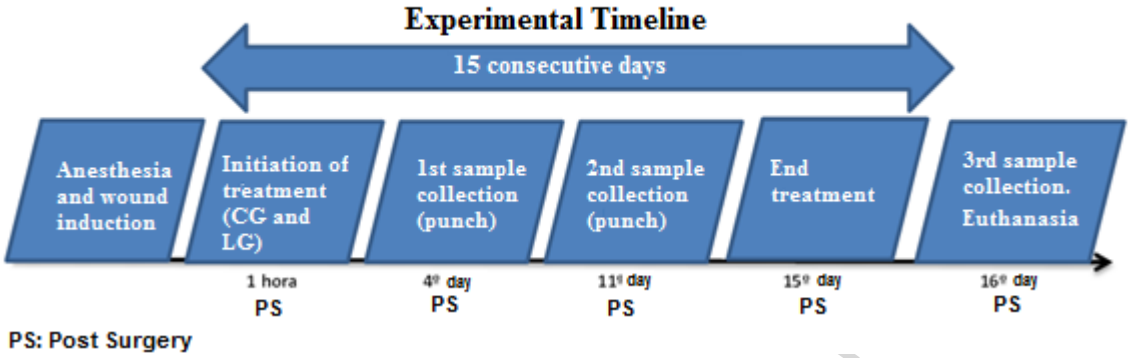

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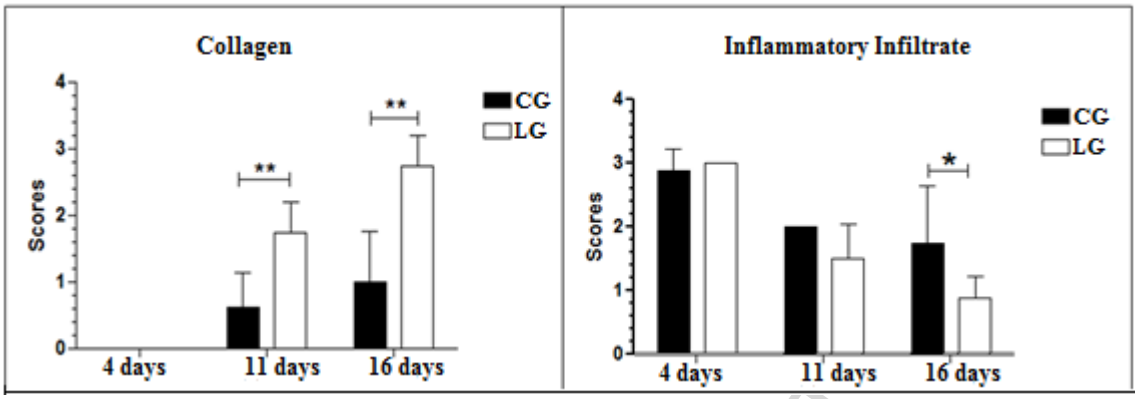
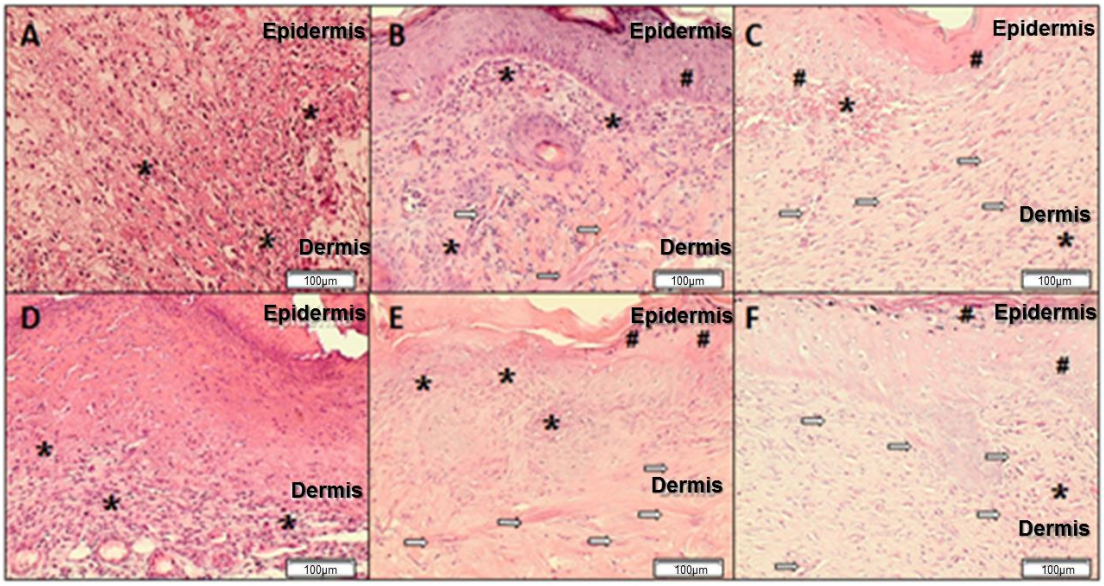


Figure 4



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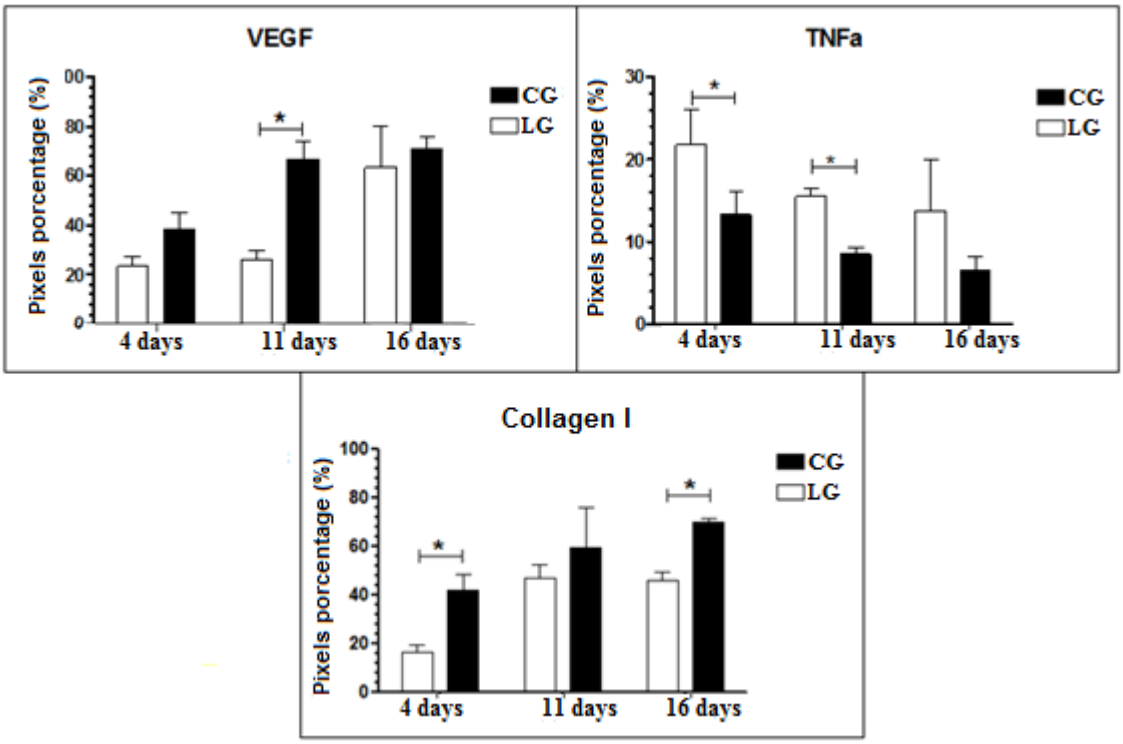

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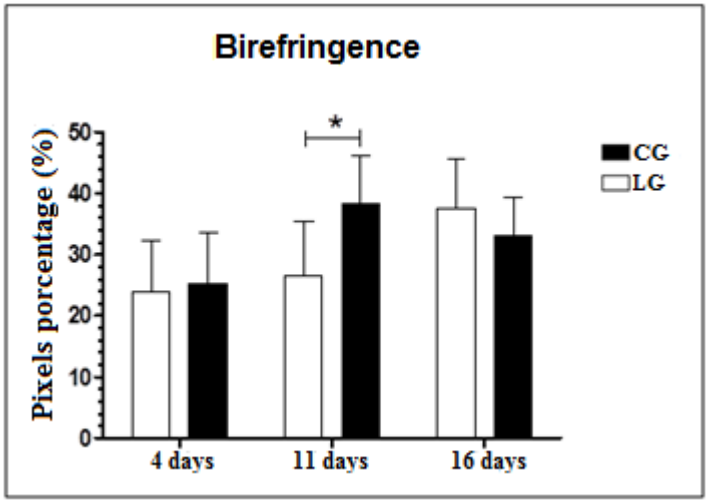

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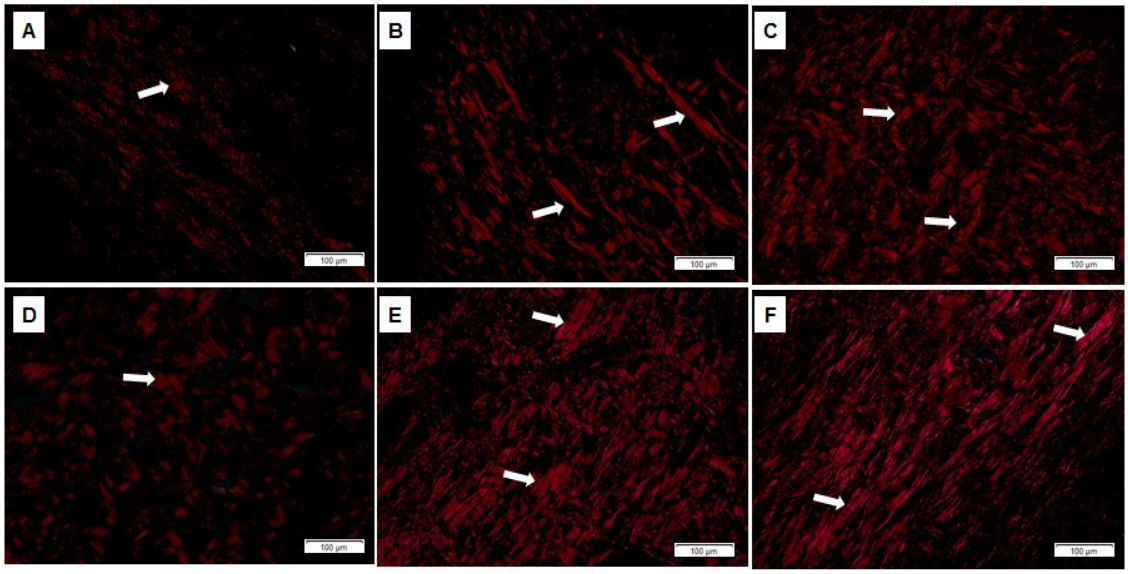

Figure 8

HIGHLIGTS

- Photobiomodulation 670nm is able to accelerate the process of wound healing
- Photobiomodulation resolve the inflammatory process by modulating cytokines
- Photobiomodulation is able to accelerate the maturation of type I collagen
- Scientific rigor is needed to define protocols in optimize therapeutic action