



The photobiomodulation (658, 830 and 904nm) on wound healing in histomorphometric analysis

A fotobiomodulação (658, 830 e 904nm) na cicatrização de feridas na análise histomorfométrica

La fotobiomodulación (658, 830 y 904nm) en la cicatrización de heridas en el análisis histomorfométrico

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Abstract

Introduction: Photobiomodulation (PBM) assists in the processes of angiogenesis and cellular mitosis after skin lesion, contributing to tissue repair. Objective: to investigate the effects of photobiomodulation (during the proliferative phase) of 658 nm, 830 nm and 904 nm in the repair of skin lesions in an animal model. Method: 658 nm (G658), 830 nm (G830), 904 nm (G904) PBM, and control group (CG) integrated the research. We submitted the animals to an excisional wound and treatment at different wavelengths for 14 days. On the seventh and 14-1485004059th postoperative days, we calculated the area and percentage of lesion contraction. The animals were sacrificed on the 14-1485004056th postoperative day and cutaneous section of the injured region was collected for histomorphometric evaluation of the cellularity, neovascularization, thickness of the epidermis and volume density of collagen fibers colored with H&E and Picross Sirius respectively. For the statistical analysis, we applied the ANOVA test. Results: the G658 presented higher cellularity than GC ($p = 0.03$). The animals in the G658 group showed a significant increase in the neovascularization in relation to the CG ($p = 0.01$). Type III collagen significantly increased in G904 compared to G830 ($p < 0.0001$) and CG ($p < 0.0001$). The G658 had a

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significant increase in type III collagen fibers compared to G830 ($p < 0.0001$) and GC ($p < 0.0001$). We found no significant difference in the thickness of the epidermis, wound area, and in the percentage wound of contraction between the analyzed groups. Conclusion: PBM was effective to stimulate the tissue repair process, with better results for the 658 nm wavelength.

Keywords: Low-Level Light Therapy. Models, Animal. Wound Healing. Rehabilitation.

Resumo

Introdução: A Fotobiomodulação (FBM) auxilia nos processos de angiogênese e mitose celular após lesão cutânea, contribuindo para reparo do tecido. **Objetivo:** investigar os efeitos da fotobiomodulação (durante a fase proliferativa) com comprimento de onda de 658 nm, 830 nm e 904 nm no reparo de lesões cutâneas em modelo animal. **Método:** FBM 658 nm (G658), 830 nm (G830), 904 nm (G904) e controle (GC) integraram a pesquisa. Os animais foram submetidos a uma ferida excisional e receberam tratamento em diferentes comprimentos de por 14 dias. No 7º e 14º dia pós-operatório, calculou-se a área e a porcentagem de contração da lesão. Os animais foram sacrificados no 14º dia pós-operatório e a secção cutânea da região lesada foi coletada para avaliação histomorfométrica da celularidade, neovascularização, espessura da epiderme e densidade volumétrica das fibras colágenas, corados com H&E e Picross Sirius respectivamente. Para a análise estatística, foi aplicado o teste ANOVA. **Resultados:** o G658 apresentou maior celularidade que GC ($p = 0,03$). Os animais do grupo G658 apresentaram aumento significativo da neovascularização em relação ao GC ($p = 0,01$). Houve aumento significativo do colágeno tipo III no G904 em relação ao G830 ($p < 0,0001$) e GC ($p < 0,0001$). O G658 teve um aumento significativo nas fibras colágenas tipo III em comparação ao G830 ($p < 0,0001$) e GC ($p < 0,0001$). Nenhuma diferença significativa foi encontrada na espessura da epiderme, área da ferida e na porcentagem de contração da ferida entre os grupos analisados. **Conclusão:** a PBM foi efetiva para estimular o processo de reparo tecidual, com melhores resultados para o comprimento de onda de 658 nm.

Palavras-chave: Terapia com Luz de Baixa Intensidade. Modelos Animais. Cicatrização. Reabilitação.

Resumen

Introducción: La fotobiomodulación (FBM) auxilia en los procesos de angiogénesis y mitosis celular después de lesión cutánea, contribuyendo para la reparación. **Objetivo:** investigar los efectos de la fotobiomodulación (durante la fase proliferativa) con longitud de onda de 658 nm, 830 nm y 904 nm en la reparación de lesiones cutáneas en modelo animal. **Método:** grupos de FBM 658 nm (G658), 830 nm (G830), 904 nm (G904) y control (GC) integraron la investigación. Los animales fueron sometidos a una herida excisional y recibieron tratamiento 14 días. En el 7º y 14º día postoperatorio, se calculó el área y el porcentaje de contracción de la lesión. Los animales fueron sacrificados en el 14º día postoperatorio y la sección cutánea de la región lesada fue recolectada para evaluación histomorfométrica de la celularidad, neovascularización, espesor de la epidermis y densidad volumétrica de las fibras colágenas, colorados con H & E y Picross Sirius respectivamente. Para el análisis estadístico, se aplicó la prueba ANOVA. **Resultados:** G658 presentó mayor celularidad que GC ($p = 0,03$). G658 presentaron un aumento significativo de la neovascularización en relación al GC ($p = 0,01$). Se observó un aumento significativo del colágeno tipo III en el G904 con respecto al G830 ($p < 0,0001$) y GC ($p < 0,0001$). El G658 tuvo un aumento significativo en las fibras colágenas tipo III en comparación con el G830 ($p < 0,0001$) y GC ($p < 0,0001$). Ninguna diferencia significativa se encontró en el espesor de la epidermis, área de la herida entre los grupos. **Conclusión:** la PBM fue efectiva para estimular el proceso de reparación del tejido, con mejores resultados para grupo 658 nm.

Palabras clave: Fotobiomodulación. Modelo Animal. Cicatrización. Reabilitación.

Introduction

Cutaneous tissue performs several different functions such as eliminating toxins through the sweat glands, thermoregulation, tissue renewal, and mechanical protection. Altered skin integrity increases the chance of infection, chronic pain, impaired mobility, decreased functions, and may result in the loss of limb and a significant reduction in quality of life [1, 2].

After a tissue injury, the cell repair process begins to restructure and preserve normal tissue function. Wound healing is a complex event that develops in three interrelated phases: inflammatory, proliferative and remodeling. In this process a dynamic interaction occurs between the extracellular matrix, growth factors, and cells [3-6].

Photobiomodulation (PBM) is a form of light therapy that utilizes light sources, including LASERS, LEDs, and broadband light for the relief of pain and the treatment of cutaneous lesions, particularly for its action in the inflammatory and proliferative phases of healing [7-11].

PBM is based on the application of light of low intensity in the tissues. This light is absorbed by specific cellular chromophores, especially the cytochrome C oxidase, located in the mitochondria that generates an increase of ATP by the action of chromophores in the electron transport chain. In addition, oxygen acts as the final electron acceptor in the electron transport chain, being metabolized and promoting the increase of the reactive oxygen species (ROS), which releases nitric oxide (NO) and generates cytosolic responses. With this, transcription factors are activated, leading to the regulation of various stimulatory and protective genes related to cell proliferation, migration, cytokine production, and growth factors [12-15].

PBM also promotes relaxation of vascular endothelial smooth muscle, mediated by NO, that produces vasodilation and increases the availability of oxygen to cells and contributes to accelerated healing of lesions [12, 16].

The PBM with wavelengths of 658 nm [17-19], 830 nm [20-22] and 904 nm [23, 24] are used to improve the cutaneous tissue repair and provides evidence-based support for clinical practice. During the proliferative phase, PBM increases the number of mast cells and promotes angiogenesis when used at short wavelengths [23]. However, few studies use the same methodological parameters to compare the results obtained with these different wavelengths.

Thus, the purpose of this study was to investigate the effects of PBM at wavelengths of 658 nm, 830 nm and 904 nm in the repair of excisional skin lesions in a proliferative phase animal model. With this study, we expect to achieve better results with 658 nm wavelength laser.

Methods

Animals

For this study, we used twenty-four adult male rats (*Rattus norvegicus*, albinus, Wistar, aged 7-8 weeks and weighing 270-280 g) from the main animal house of the Universidade Federal de Alfenas (UNIFAL). The animals were housed in plastic cages (27 × 12 × 17 cm, 4 per cage) at a controlled temperature (24 ± 1° C) under a 12-hour light-dark cycle (dark cycle beginning at 7 pm), and they had free access to food and water. We conducted the experiment in accordance with local guidelines for animal welfare consistent with the National Research Council's "Guide for the Care and Use of Laboratory Animals" (National Academies Press, Washington DC, USA) and after approval by the Committee of Animal Experimentation of the UNIFAL, protocol 602/2014. All possible efforts were made to minimize the number of animals used and their suffering.

Surgical procedure

We anesthetized the animals with an intraperitoneal injection of ketamine chloride (90 mg/kg) and xylazine chloride (10 mg/kg) [1]. The dorsal region was trichotomized and performed asepsis with iodine. We then demarcated the area of the excisional lesion with a dermatographic pen, keeping the anterior border of a quadratic template of 1 cm × 2 cm (2 cm²) at the angle of the scapulae. We made an incision in the demarcated area with a scalpel, and dissected the skin until the exposure of the muscular fascia. The dissection of the skin was performed with Metzembaum scissors, in an areolar anatomical plane at the suprafascial level [1]. At the end of the surgical procedure, the animal was observed until complete recovery of the anesthesia. Each mouse was placed in an isolated cage throughout the experiment. The animals received analgesic Dipirone 160 mg/kg after the surgical procedure in order to avoid stress caused by pain. During the postoperative period, up to the day of euthanasia, we performed a daily clinical evaluation of surgical wounds was performed

to monitor local complications such as dehiscences, infections and bruises.

Experimental protocol

We randomized the animals into four groups (n = 6): PBM groups with 658 nm (G658), 830 nm (G830), 904 nm (G904) wavelengths and the control group (GC). For the application of the PBM we used the HTM Compact® Laser (Amparo, SP, Brazil, License: 80212480005) with a frequency of 60 Hz, power supply, and a maximum current of 54 mA.

Table 1 presents the PBM parameters used with the same energy density standardized for the three groups (4 J/cm²) [24-27].

Table 1 – Energy parameters of laser treatment

Parameters	Groups		
	G658	G830	G904
Density of Power	4 J/cm ²	4 J/cm ²	4 J/cm ²
Peak Power	30 mW	30 mW	50 W
Average Power	0.03 W	0.03 W	0.02 W
Emission Mode	Continuous	Continuous	Pulsed
Spot Size	0.125 cm ²	0.125 cm ²	0.125 cm ²
Irradiation Time	16.6 sec	16.6 sec	25 sec
Pulse Repetition Rate	-	-	5000 Hz
Light Emission Time	-	-	800 ns

The application was given point by point [28], with the probe in perpendicular contact to the edge of the wound (0.5 cm), totaling 12 points (Figure 1). Between the probe and the wound we placed transparent and sterile plastic for protection.

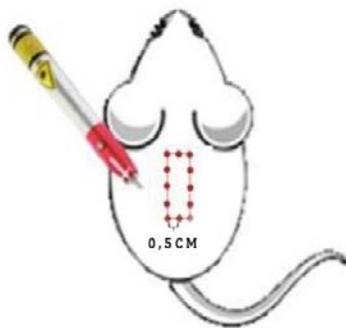


Figure 1 – During the treatment, we attached the laser pen at the edge of the wound, in a punctual way (0.5 cm distance between the application points).

We performed the application for 14 consecutive days. In the control group, the animals received a sham laser application, with the device accoupled but switched off. Before all PMB applications in the intervention group, the Laser Test was performed at the apparatus, which generates a beep and indicates that the laser is being emitted.

Macroscopic analysis

On day zero (day of surgery) and 7-2021876908th and 14-2021876908th postoperative days, we evaluated and measured all wounds from each group. For area and percentage of contraction of lesion calculation, we applied a ruler graduated in millimeters on digitalized images obtained by a digital camera (SX500IS, Nikon, Brazil). We calculated the percentage of wound contraction using the following formula: [initial lesion area (Ao) – area on measurement day (Ai)] / initial lesion area (Ao) × 100 [2].

Histomorphometric evaluation

The animals of each group were sacrificed on the 14-2021876906th postoperative day with a hyperdose of anesthetics xylazine chloride (50 mg/kg) and ketamine chloride (300 mg/kg). We collected a 1 cm × 1 cm cutaneous section of the injured region from all animals for histological observation. We fixed the fragments in 10% formaldehyde, dehydrated in ethanol, diaphanized in xylene and embedded in paraffin. We then sectioned 5 µm thick pieces using a microtome (Reichert-Jung 2045 Multicut, Germany). It was collected one in 20 cuts (four cuts per slide) in order to avoid the evaluation of the same histological area [10]. We stained the sections with hematoxylin-eosin (H&E) and observed them using a light field microscope (Zeiss Scope A1 AXX10, Germany) in order to analyze the volume density of inflammatory cells (cellularity), blood vessel density of the dermis (neovascularization), contact surface of the epidermis/dermis and thickness of the epidermis. Histological images were captured on a digital camera using the image analysis program (AxionVision – AxioCam ICC 3). For the evaluation of the volume density of collagen fibers, the sections were stained with Picrosirius red and analyzed under a polarized light microscope (AxioPhot, Zeiss-Germany) [27] and all the images were captured using the program Toup View For Digital Camera 3.7. We evaluated the collagen

fibers according to their birefringence properties on polarization (red color represents type I collagen; green color corresponds to type III collagen) [10]. In the dermis, we obtained eight photos from each animal using 40x objective lenses (for evaluation of blood vessels and collagen) and 100x (for cellularity). In the epidermis, we took six photos per slide from a 20x objective lens (for epidermal thickness). All digital images were analyzed from ImagePro-Plus 4.5 software (Media Cybernetics, Silver Springs, MD, USA).

Statistical analysis

We applied the Shapiro Wilk test to evaluate the distribution of the data. The variance of the data obtained in all groups was analyzed using the One-Way ANOVA test. When necessary, we compared the

data between groups using the Tukey Post-Hoc test. The statistical analysis of the results was performed by the SPSS program, version 20.0, with level of significance established at 0.05.

Results

At the time of sacrifice, a visual inspection did not detect any inflammatory and/or infectious process around the lesions in all groups investigated.

We found no significant difference in the wound area and in the percentage wound of contraction between the analyzed groups. In the intragroup analyses, all groups (G658, G830, G904 and CG) presented a significant reduction in the wound area between days seven and 14 ($p < 0.01$) and in the percentage wound contraction between days seven and 14 ($p < 0.01$) (Table 2).

Table 2 – Wound area and percentage wound contraction on zero, 7-2021876864th and 14-2021876864th postoperative days

Day	Area (A) / contraction (C)	G658	G830	G940	CG	ANOVA p value
0	A (cm ²)	2.0 ± 0.0 [#]	1.00			
	C (%)	0.0 ± 0.0 [#]	1.00			
7	A (cm ²)	0.47 ± 0.07	0.56 ± 0.19	0.53 ± 0.15	0.49 ± 0.06	0.64
	C (%)	23.90 ± 3.84	28.40 ± 11.7	26.8 ± 10.2	24.50 ± 4.24	0.79
14	A (cm ²)	0.0 ± 0.02	0.08 ± 0.15	0.04 ± 0.09	0.06 ± 0.08	0.51
	C (%)	99.0 ± 1.27	99.3 ± 0.59	95.8 ± 7.97	98.16 ± 0.87	0.47

Note: G658, G830 e G904: the wavelength of 658 nm, 830 nm e 904 nm groups, respectively; CG: control group; A: wound area; C: percentage wound contraction. Data were expressed as mean ± standard deviation and analyzed by one-way analysis of variance (ANOVA) with Tukey post hoc test. [#] $p \leq 0.05$ vs 7-2021876863th and 14-2021876863th postoperative days (intragroup analyses).

The number of mononuclear cells (cellularity) presented a difference between the groups ($F = 0.56$; 95% IC = 33.42 to 38.17; $p = 0.02$), with a significant increase in G658 in relation to CG (95% CI = 0.61 to 16.16; $p = 0.03$). The number of type III collagen presented a difference between the groups ($F = 34.6$; 95% IC = 28.03 to 36.12; $p < 0.0001$). The amount of type III collagen increased in group G904 compared to G830 (95% CI = 5.77 to 19.09; $p < 0.0001$) and CG (95% CI = 7.44 to 20.76; $p < 0.0001$) and when G658 was compared to G830 (95% CI = 12.23 to 25.55; $p < 0.0001$) and CG (95% CI = 13.90 to 27.22; $p < 0.0001$) (Table 3). We found no significant difference between G904 and G658.

In neovascularization we observed a difference between the groups ($F = 3.82$; 95% IC = 2.55 to 3.90; $p = 0.02$). The animals in the G658 group showed

a significant increase in the number of vessels compared to the CG (95% CI = 0.41 to 4.81; $p = 0.01$). The CG presented a significant increase amount of the surface area contact between epidermis and dermis in relation to G658 (95% CI = 6,371.13 to 103,489.51; $p = 0.02$). We found no significant difference in the epidermal thickness between the analyzed groups ($F = 3.83$; 95% IC = 60,147.43 to 89,849.09; $p > 0.05$) (Table 3).

Figure 2 shows the results observed in the histological sections and stained with H&E and Picrosirius red. For irradiated groups, the epithelial proliferation was similar to control group. We observed neutrophils in the subjacent dermis, which also presented inflammatory exudate, lymphocytes and macrophages. The samples from G658 also exhibited a higher content of fibroblast

cells and more new formed capillaries than the other groups (Figure 2, H&E 40x). Irradiated groups showed a larger amount of fibroblasts, which presented conspicuous nuclei, indicating the intense activity of these cells. Irradiated groups

showed a larger amount of thin collagen fibers and were in a parallel alignment with the epithelium surface. Particularly, G904 samples showed better fibers arrangement than other groups (Figure 2, Picrosirius red 40x).

Table 3 – Histological analysis: cellularity, type III collagen, blood vessels, surface area contact epidermis/dermis and epidermal thickness

	G658 M±DP (IC95%)	G830 M±DP (IC95%)	G904 M±DP (IC95%)	CG M±DP (IC95%)	ANOVA <i>p</i> value
Cellularity (n/ 10034.29 μm^2)	41.39±1.46* (38.85 to 42.93)	35.05±5.46 (29.32 to 40.78)	33.72±5.88* (27.55 to 39.9)	33.00±5.09# (27.65 to 38.35)	0.02
Type III collagen (%)	43.55±6.00* (37,25 to 49,85)	24.66±3.94 (20.5 to 28.80)	37.1±3.16 ^ε (33.78 to 40.42)	22.99±2.51 (20.35 to 25.63)	< 0.0001
Blood vessels (%)	4.70±1.80 ^ε (3.57 to 5.84)	3.14±1.06 (2.02 to 4.26)	2,98±1,2 (1,72 to 4,24)	2.09±1.91 (0.08 to 4.09)	0.02
Surface area contact epidermis/dermis (μm^2 / μm^3)	54,986.19±16,760.54 [°] (37,397.07 to 72,575.34)	68,119.9±16,913.55 (50,370.2 to 88,869.59)	66,970.4±24,275.3 (41,495.1 to 92,445.8)	109,916.52±49,554.49 (57,912.27 to 161,920.76)	0.02
Epidermal thickness (μm)	897.94 ± 125.86 (765.85 to 1,030.03)	786.18 ± 145.34 (633.65 to 983.71)	763.58±118.47 (639.24 to 887.41)	863.49±193.62 (660.29 to 1,066.68)	0.37

Note: G658, G830 e G904: the wavelength of 658, 830 e 904 nm groups, respectively; CG: control group. Data were expressed as mean ± standard deviation and 95% confidence interval (CI95%) and analyzed by one-way analysis of variance (ANOVA) with Tukey post hoc test. # $p \leq 0,05$ vs G904 and CG; * $p \leq 0,05$ vs G830 and CG; ^ε $p \leq 0,05$ vs G830 and CG; ^ε $p \leq 0,05$ vs CG; [°] $p \leq 0,05$ vs CG.

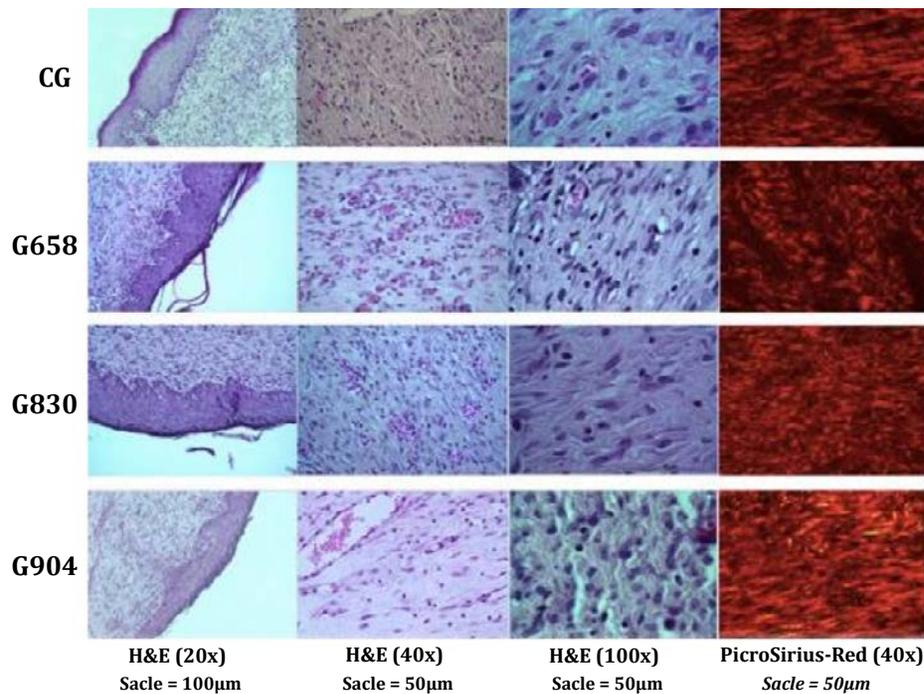


Figure 2 – Histological sections in groups. G658, G830 and G904: LLLT groups 658 nm, 830 nm, and 904 nm, respectively. CG: control group.

Discussion

This study investigated the effects of PBM of 658 nm, 830 nm, and 904 nm on the healing of excisional lesions in an animal model. Using the methodology of histological and lesion contraction analyses, the results indicate that laser irradiation was able to stimulate the tissue repair process, with better results for the 658 nm wavelength.

PBM is currently one of the most widely used modalities in rehabilitation. However, clinical and scientific evidences are often contradictory, a fact that justifies the necessity and relevance of research involving this therapeutic resource [29, 30]. In addition, studies that evaluate therapeutic approaches clarifying the skin repair process are very important, given the impact on morbidity associated with patients' functionality, mortality, and quality of life [31, 32].

In the literature, few studies compare the different wavelengths of laser irradiation in cutaneous repair in same methodology, therefore, our results were considered extremely relevant for describing the parameters involved in PBM application in order to better elucidate and interpret the results of this therapeutic approach.

The PBM stimulation parameters for skin lesion repair still remain unclear in the scientific literature and the values of energy density applied are variable. The low energy density (3 J/cm²) compared to the highest energy (30 J/cm²) in skin lesions of animals showed that both are effective at increasing cell numbers, neovascularization, collagen, and elastic fibers [6]. In this research, we used lower energy of 4 J/cm², obtaining positive results especially in the group that received laser irradiation with wavelength of 658 nm. Chiarotto et al. [20] found similar results using energy density of 4.48 J/cm² in animals submitted to second degree burn, with a greater effectiveness of the laser 670 nm compared to 830 nm. Kerppers et al. [25] reported that small doses of laser fractions at different times are more effective than higher doses in a single application because it sustains mitochondrial activity.

In this work, the laser applications started on the first day after the injury procedure in order to initiate the irradiation in the inflammatory phase of the lesion. The stimulus offered in the inflammatory phase may accelerate the tissue repair process when applied during the inflammatory phase, compared to the onset in the proliferative and remodeling phases [4].

The area and percentage of contraction of the lesion were evaluated on the 7-2021876827th and 14-2021876827th day after the injury and showed no differences between the groups studied. This result may be related to the small lesion area performed in our study. Conversely, Gonçalves et al. [9] when studying the 830 nm laser with energy densities of 30 J/cm² and 90 J/cm² detected a significant and faster repair on days seven and 14 after a cutaneous lesion. In immunodepressed animals, the low intensity laser with wavelength of 810 nm was also effective for a greater percentage of contraction of the lesion area [33]. Oliveira Guirro et al. [34] evaluated the effects of laser irradiation of 670 nm, with energy densities of 4 and 7 J/cm² in the tissue repair process of induced surgical wounds in rats and found positive results in both doses on the seventh day after injury and the animals evaluated on the 14-2021876824th day presented complete closure of the epithelium. Gupta et al. [24] also studied the pulsed 904 nm, evidencing its positive effects for the treatment of wounds in burn-induced rats on the eighth day after injury. However, the diversity of the parameters and methodologies used in their research do not support specific comparisons and show the need for standardization in the use of this resource.

We observed in this research that the cellular proliferation of the 658 nm laser was significantly higher than in the other groups. The infrared lasers of 830 nm and 904 nm showed a cellular proliferation very similar to the group that received simulated treatment. In fact, -2021876821 *in vitro* research shows a greater proliferation of cells with laser in the red spectrum, visible, with radiation of 1 to 3 J/cm², while the laser in the infrared spectrum showed no positive or potential negative effect on proliferation cell [35]. Moreover, according to Brassolatti et al [36], the 660 nm laser is able to stimulate the cellular production of areas with third degree burn injuries, contributing to the tissue regeneration process.

The collagen deposition observed in this study was related to type III collagen. In fact, we analyzed the samples on the 14-2021876820th day after the operative procedure, at which time the collagen type I deposition was not yet observed [37]. Furthermore, a better organization of the collagen fibers implies a better cicatricial process [38]. Gupta et al. [24] analyzed the effect of the 904 nm laser on the collagen deposition after burn in animal model and observed an increase in the amount of collagen seven days after the injury. Tamatsu-Rocha et al. [38] observed that 904 nm laser

radiation promotes a larger and more organized deposition of collagen in healthy or diabetic animals five days after skin lesion. These results support our research, since the animals treated with 904 nm laser had a significant increase of collagen in relation to the laser 830 nm and to the control group. However, we observed the highest deposition of tissue collagen in the group that received 658 nm of radiation, in agreement with the study developed by Trajano et al. [19] which, when applying red spectrum laser in burned animals, showed an increase in type III collagen proliferation, ten and 21 days after the injury.

Another evaluated aspect was the angiogenesis in the injured tissue. Angiogenesis involves an important factor for tissue regeneration after cutaneous injury, being essential for the supply of oxygen and nutrients to tissues [24]. The laser application provides increase in the blood vessels by stimulating angiogenesis, as observed in our study when analyzing vessels after 14 days of injury, with a significantly greater amount for the group that received 658 nm irradiation. The study by Medeiros et al. [26] supported these results since they observed greater angiogenesis in the group that received 660 nm laser on the 14-2021876816th day after the injury compared to the analysis performed in the group that received application on the seventh day after the injury.

The process of skin tissue regeneration involves a greater activity in the formation of granulation tissue and changes in the thickness of the epidermis. Although the results of the epidermis presented greater thickness in the group that received 658 nm of radiation, the results were not significant in the epidermis between the groups that received the different wavelengths of the laser therapy or the control group. Conflicting with these results, studies have noted a greater re-epithelialization of the epidermis after applying laser wavelengths between 808 nm to 830 nm in immunosuppressed [18] and diabetic animals [39]. Moreover, Leite et al. [39] observed positive results of increased epidermal thickness in radical cutaneous flap with 660 nm laser therapy with fluency of 140 J/cm². Different animal models and different wavelength and fluency must explain the different effect observed in this work.

Some limitations were evidenced in this study regarding the low number of the sample, although it is a standard number for animal research. Another important point to emphasize is the lack of study in all phases of the tissue repair process to complement the evidence found here in the proliferative phase.

Finally, the literature limitation on the low intensity and wavelength standard of the PBM techniques prevents a more solid discussion about the results obtained in this study [40].

Conclusion

The PBM promoted increase in the number of cells, increased amount of collagen and blood vessels in the group that received the 658 nm wavelength. The 658 nm PBM was effective to stimulate the tissue repair process.

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