LED Photobiomodulation Therapy Associated with Heterologous Fibrin Biopolymer Optimizes Wound Repair in Rats

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Abstract

This study aimed to evaluate the effect of LED photobiomodulation therapy associated or not with heterologous fibrin biopolymer to repair skin wounds in rats. Full-thickness skin wounds were induced on the back of 84 Wistar rats and they were randomly divided into 4 groups: control group (CG), LED group (LED), heterologous fibrin biopolymer group (HFB), and LED + heterologous fibrin biopolymer group (LED + HFB). The animals were euthanized at 7, 14, and 21 days after surgery. In the macroscopic findings, it was observed in the control group, mild local inflammation, evidenced between the 5th and 6th day after the injury, not manifested in the other groups. The analysis of the wound reduction index shown that the treated groups presented greater wound reduction in the three periods evaluated. The results of the degree of inflammation showed that the LED and LED + HFB groups showed a predominance of chronic inflammation, while the control group showed a predominance of diffuse acute inflammation. The quantity of type I collagen it was bigger after 7 days in the LED and LED + HFB groups, and at 14 and 21 days this type of collagen appears more intensely in the group that used only HFB. The traction force at 7 days of the experiment, the treated groups showed better performance, while at 14 and 21 days the group that used only the biopolymer obtained greater strength in the suture region. Results have indicated that treatments using HFB and LED, either isolated or associated, stimulated the wound repair process in rats. HFB promote collagen maturation.

Introduction

The skin is a complex organ that covers the entire surface of the body. It provides protection as a physical barrier between the body and the environment. Any situation that interrupts the integrity of the skin, whether by accidents, trauma, disease, or surgery is defined as a skin wound (Smaniotto et al. 2012; Nguyen and Soulika 2019). When a skin lesion occurs, the body initiates a series of complex events that involve physiological, biochemical, cellular, and molecular events that interact for tissue reconstitution, with the intention of restoring its integrity. However, in many cases, these events fail in their objectives, giving rise to a difficult-to-repair wound, which can take years to complete the tissue reconstitution process, and often this process may not happen (Gonzalez et al. 2016).

Although individuals affected with wounds receive the usual treatment, chronic wounds cannot adequately complete the repair process. Thus, in addition to burdening the patient, difficult-to-repair wounds have a great economic impact, as it is known the high costs of healthcare on their treatment, in Brazil and worldwide. The constant search for alternative treatments that can restore the integrity of the patients' skin and also reduce costs is seen as an alternative in this costly process (Araújo et al. 2017).

From this perspective, several strategies have been studied, with the aim of accelerating skin repair. Among them, the use of heterologous fibrin biopolymer (HFB) has been highlighted, an adhesive, sealant and hemostatic agent that has the ability to promote tissue repair. HFB consists of the addition of a fibrinogen-rich cryoprecipitate, to thrombin-like enzyme, simultaneously. The union of these two components leads to the formation of a stable fibrin clot (Ferreira et al. 2017). It is known that fibrin in the

tissue repair process has the function of producing a matrix to adhere to other cells, such as endothelial cells and fibroblasts, which lead to angiogenesis, and tissue remodeling. Fibroblasts then re-modulate the fibrin structure and synthesize collagen, giving shape and stability to capillaries in vitro, thus favoring the tissue repair process (Gassling et al. 2009).

Another resource that can be an ally to the tissue repair process is the use of photobiomodulation therapy, through the use of light-emitting diode, also known by the acronym LED (Light Emitting Diode). LED has been studied for decades, whose evidence of wound treatments is based on the stimulation of cell differentiation, fibroblast proliferation, and collagen synthesis in the tissue. Another advantage for its use is that LEDs are generally cheap, easy to handle, and can be used along with an applicator tip, the clusters, which cover a larger treatment area (Chaves et al. 2014). Although the literature demonstrates some encouraging data on the potential effects of LED and HFB on tissue repair, research focused on developing strategies to accelerate and improve the repair process is increasingly needed and valued. Thus, technological advances in the use of new products and procedures in performing dressings have shown benefits to the population with wounds, improving people's quality of life (Nourian et al. 2019). Given the above, this study sought to evaluate the effect of LED photobiomodulation therapy associated or not with heterologous fibrin biopolymer the use in the repair of skin wounds in rats.

Material And Methods

SURGICAL PROCEDURE

For the development of this work, 84 adults male Wistar rats (*Rattus norvegicus albinus*) were used (over 60 days old), with an average of 250 g of body mass, randomly selected. During the experimental phase, all animals received standardized rodent chow and water ad libitum. They were kept in self-washing polypropylene boxes, and in a ventilated environment, housing 7 animals in each box. The temperature was kept at 25°C and the humidity controlled with a 12-hour light-dark cycle. It is noteworthy that during the experiment no fights, mutilation actions, or cannibalism between the animals were detected.

The animals, after weighing, were anesthetized with intramuscular injection (IM) of ketamine hydrochloride, at a dose of 60-80 mg/kg, and chlorpromazine hydrochloride, at a dose of 1.6-2.0 mg/kg. After trichotomy of the dorsal region, the rats were placed in ventral decubitus, and asepsis with 70% alcohol was done to perform two wounds, one longitudinal and the other circular, in each animal. For this purpose, a 3-cm linear lesion was demarcated on the animals' backs below the scapulae and then the edges were approximated with 3 suture stitches. This injury was intended to assess the quality of repair through the tensile strength test. Later, in the same surgical procedure, the second lesion was performed with a dermatological punch, an area of 2 cm in diameter from all skin layers was removed. Hemostasis was performed by digital compression, using sterilized gauze.

In the first three post-operative days, acetaminophen 200 mg/ml every 8 hours was administered as analgesic medication. The animals were monitored daily by the researcher. Cleaning of the boxes,

maintenance of food, and change of the water were performed regularly by a qualified professional. Animals were randomly divided into 4 groups containing 21 animals each.

Control group (CG): the wound on the animals' back was induced and treated with 0.9% saline solution.

LED Group (LED): the wound was induced on the animals' back and LED was applied immediately after the surgical procedure and every 72 hours.

Heterologous fibrin biopolymer group (HFB): the wound was induced on the animals' back and the HFB was applied immediately after the surgical procedure and every 72 hours.

LED group associated with heterologous fibrin biopolymer (LED + HFB): the wound on the back of the animals was induced by irradiating the LED and then applying the heterologous fibrin biopolymer. Likewise, this procedure was repeated every 72h.

Subsequently, the animals were subdivided into three experimental periods: 7, 14 and 21 days.

TREATMENT

Tendlite® medical LED equipment, model 204, red wavelength 660 nm was used in the parameters described in table 1.

In the animals in the LED and LED + HFB group, after wound induction, hemostasis was performed and then LED irradiation was carried out punctually with 1 cm of distance from the lesion, standardized by means of a support, being applied to each animal the same LED parameters.

The application of heterologous fibrin biopolymer was applied topically to lesions in the HFB and LED + HFB groups. 2g of heterologous fibrin biopolymer was applied to the injured area and spread with the aid of a spatula, so that the entire area was covered.

Both treatments took place immediately after injury induction and the procedures were repeated every 72 hours in all animals, according to the groups to which they belonged. Still, it is noteworthy that in the group in which the combination of therapies occurred, irradiation with the LED always took place first and then the application of the HFB was performed.

The animals in the experimental groups were anesthetized, according to the protocol described above, and euthanized with an overdose of thiopental sodium at a dose of 100 mg/kg, intraperitoneally, after the 7th, 14th and 21st postoperative days, according to the group that they belong.

MACROSCOPIC ANALYSIS

The macroscopic analysis of the wound was performed daily, where the region of the lesion was investigated for the following occurrences: necrosis, local inflammation, odor, presence of fibrosis, or fluid in the cavity.

MORPHOMETRIC ANALYSIS OF THE WOUND REPROCESS INDEX

Morphometric analysis of the wound reprocess index was performed using photographic records using a 14-megapixel Canon PowerShot SX30 IS digital camera on day 0 and on the day of euthanasia of the animal (7, 14 and 21 days). A digital camera in basic mode, no flash, no zoom was used. To standardize the distance between the camera and the wound, an aluminum support was used at 20 cm and perpendicular to the wound. A ruler placed beside the animals and close to the wound was used to standardize the unit of area of the lesions in mm. The images were analyzed by ImageJ 1.45 software (Research Services Branch, National Institutes of Health – NIH (Bethesda, Maryland, USA)) The residual area of the lesion was calculated based on the images, using the following formula:

$$A_r = \frac{A_{day}}{A_{initial}} \times 100$$

Subtitle: residual area (A_r) of the lesion (%); A_{day} represents the area measured daily; $A_{initial}$ is the initial area measured immediately after wound induction.

SAMPLE PREPARATION

After the macroscopic analysis, the collection of skin samples was performed along the entire extent of the wounds, with a margin of 1 cm of intact skin.

The circular wound samples were destined for histological analysis. For this, they were fixed in a 10% buffered formalin solution for 24 hours, and sent for conventional histological processing, consisting of dehydration with alcohol, diaphanization in xylene, and embedding in paraffin.

The blocks were cut on a rotating microtome, with sections 5 micrometers thick, and stained with Hematoxylin and Eosin (HE) and Picrosirius Red. All slides were coded, so that the examiner did not know which group they belonged to.

The longitudinal wound samples were destined to the scar's tensile strength test, they were removed and irrigated with 0.9% saline solution, and soon after they were submitted to the traction force test.

DESCRIPTIVE HISTOLOGICAL ANALYSIS

Samples stained with H.E were used to assess histopathological changes in the skin. Thus, a descriptive analysis was performed evaluating the qualitatively reepithelialization, granulation tissue, fibroblastic proliferation, blood vessels, inflammatory infiltrate, and tissue organization. The specimens were examined using an Olympus CX31, model YS100 trinocular optical microscope.

HISTOMORPHOMETRIC ANALYSIS

The histomorphometric evaluation of the lesion was performed by a semi-quantitative analysis using a score described by Solmaz et al. (2016), considering values from 0 to 4 points, as written in table 2.

INFLAMMATION GRADE

The grade of inflammation was measured with the phases of resolution of the inflammatory process: grade 1, acute inflammation (pyogenic membrane formation); grade 2, predominance of diffuse acute inflammation (predominance of granulation tissue); grade 3, predominance of chronic inflammation (fibroblasts beginning to proliferate); grade 4, resolution and repair (reduction or disappearance of chronic inflammation, although occasional round cells may persist).

COLLAGEN ANALYSIS

After microtomy, the histological sections were stained with picosirius red - F3BA. To identify mature (type I) and immature (type III) collagens, the histological sections were analyzed using an optical microscope, at 400x magnification, with a polarized light source. Images were captured by a camera, transmitted to a color monitor, frozen, and digitized using plates. Finally, image analysis was performed using Image Pro-Plus version 4.5 for Windows (Cibernética Media, São Paulo, SP).

In the RGB system (Red, Blue, Green) values were considered for red, yellow, and orange tones (type I collagen), and for green tones (type III collagen). All slides were evaluated under the same adjustment conditions, within the parameters required by the aforementioned application. In the histological sections, four microscopic fields were acquired over the lesion area, where measurements of the different areas were obtained. In each of them, the software calculated the percentage of the area occupied by the fibers that contained collagen types I and III in relation to the total area examined.

From the measurement of the percentage of collagen fibers, the collagen maturation index (CMaI) was calculated, as described by Simões et al. (2020), being obtained through the percentage ratio of collagen type I by the percentage of collagen type III, where values greater than 1 indicate that the percentage of type I collagen is greater than the percentage of type III collagen.

IMMUNOHISTOCHEMISTRY

After deparaffinization and rehydration of the slides, endogenous peroxidase was blocked using a 30% solution of hydrogen peroxide. The following procedure consisted of the samples being incubated with CD34 monoclonal primary antibody (ab81289, abcam, UK), 1:200 dilution ratio, for 32 minutes. The secondary antibody was exposed to 1:50 dilution for 30 minutes before use. The obtained samples were stained with Diaminobenzidine (DAB) for 30 minutes and then stained with Hematoxylin. After the staining protocol was performed, 10 fields of each slide section were evaluated at 100X magnification

through light microscopy (Axiolab Zeiss). Slides were scored on a scale of 1 to 4 (1 = absent, 2 = weak, 3 = moderate and 4 = strong target staining) for immunohistochemical analysis.

TRACTION FORCE ANALYSIS

The evaluation of the traction force followed the procedure described by Trubian et al. (2007). For the test, a universal mechanical testing machine with manually adjustable pressure grips and electronic data acquisition system (dynamometer) was used. The ends of the skin fragment, which contained the suture in its middle portion, were fixed to the barrettes, with manual adjustment. The traction was carried out at a constant speed, using a 10 kg load, with a sensitivity of 50 g, until the specimen was completely broken. The traction force was constantly measured using a computer attached to the cleats system, considering the maximum traction force (FMT) in kgf as a variable.

STATISTICAL ANALYSIS

The results are described in mean and standard deviation values, and presented in tables and graphs. Data normality was tested with the KS test and Homogeneity with the Levene test. For comparisons between groups, One-Way ANOVA with Tukey Post-Hoc was used. For the statistical difference, a significance value of 5% was adopted. Statistical analysis was performed using the GraphPad Prism program.

Results

MACROSCOPIC ANALYSIS

In the macroscopic findings obtained in the qualitative analysis of the experimental groups, it was possible to observe the absence of necrosis, odor, presence of fibrosis, or fluid in the lesion cavity in all experimental groups. In the control group, there was mild local inflammation, evidenced between the 5th and 6th day after the injury, not manifested in the other groups, as shown in Fig. 1.

MORPHOMETRIC ANALYSIS OF THE WOUND REPAIR INDEX

The analysis of the wound repair index (Fig. 2) demonstrates that the control group had a statistically lower percentage of reduction in the injured area compared to the other groups after 7 days. Also in this period, it can be observed that the LED group associated with HFB showed a higher rate of repair, statistically significant, compared to the group that used HFB alone. After 14 days, the HFB and LED + HFB groups showed greater wound reduction when compared to the control and LED groups. There was no statistical difference between the CG and LED groups. In the last experimental period, that is, 21 days, it was observed that the three treated groups had a higher repair rate compared to the control group. When the treated groups were compared, it was noted that the LED + HFB group showed greater wound reduction compared to the group that was treated only with LED.

DESCRIPTIVE HISTOLOGICAL ANALYSIS

Representative histological images of the wounds are shown in Fig. 3. The histopathological analysis revealed that in all experimental groups, 7 days after the operation, the wounds were covered by a fibrin-neutrophilic scab of variable thickness. Re-epithelialization was more evident only in the peripheral areas of the wounds of the treated groups. In the wound bed of the treated groups, granulation tissue, fibroblasts, numerous blood capillaries, and a predominance of chronic inflammation were observed, when compared to the control group. When comparing the treatments, it was observed that the LED+HFB group demonstrated slightly more organized granulation tissue.

After fourteen days of evolution, the fibrin-neutrophilic scab was still present in all groups, especially in the control group. All treated groups showed a better repair process than the control group. Re-epithelialization was a little more advanced, and granulation tissue was more organized in the HFB and LED+HFB groups compared to the control and LED groups.

With twenty-one days of evolution, it can be observed that in the Control Group, re-epithelialization was not yet complete, noting the injured central area still covered by a scab. In the superficial dermis, the extracellular matrix was less dense, containing numerous blood vessels and inflammatory cells. In the other groups, the wounds had an almost completely neoformed epidermis. The treated groups showed fibroblasts arranged in bundles and a marked reduction in inflammatory activity, which was reduced to a discrete focus of macrophage infiltrates. In the HFB group, the dermis exhibited a denser extracellular matrix.

HISTOMORPHOMETRIC ANALYSIS

The results of the histopathological evaluation (Fig. 4) showed a statistical difference in the tissue repair phases between the experimental groups. For the re-epithelialization criterion, after 7 days it was observed that the control group had a statistically lower score when compared to the LED and LED groups associated with HFB. After 14 days, it can be seen that the control group had a statistically lower score compared to the HFB and LED + HFB groups. Also, in this same period, it was observed that the LED + HFB group showed a statistically higher difference compared to the group that received LED alone. In the last period evaluated, 21 days, it was found that only the group that received the association of treatments (LED + HFB) showed a statistically greater difference when compared to the control group.

For the histomorphometric analysis of fibroblasts, it was observed that all treated groups showed a statistically greater difference compared to the control group after 7 and 21 days. After 14 days, only the HFB and LED + HFB groups showed a statistically greater difference than the control group.

The histomorphometric analysis of collagen showed that all treated groups had statistically higher scores when compared to the control group in the three periods evaluated.

Blood vessel evaluation showed that 7 and 14 days the control group had decreased blood vessels compared to treatments, then at 21 days the control group showed increased blood vessels compared to treatments.

INFLAMMATION GRADE

Fig. 5 demonstrates the grade of inflammation. It can be observed in the 7-day period that the LED and LED + HFB groups presented a predominance of chronic inflammation, represented by a score of 3, while in the control group the predominance of diffuse acute inflammation was still noted (score 2). Likewise, in the 14 and 21-day periods, the treated groups showed a score of 4 that indicates resolution and repair (reduction or disappearance of chronic inflammation, although occasional round cells persist) while the control group still had a predominance of chronic inflammation (score 3).

IMMUNOHISTOCHEMISTRY

Qualitative immunohistochemical analysis demonstrated immunostaining for CD34 predominantly in the cytoplasm of blood vessel endothelial cells of all experimental groups. The semiquantitative immunohistochemical analysis showed that after 7 and 14 days all treated groups had higher CD34 immunoexpression compared to CG. After 21 days CG showed higher immunostaining compared to all treated groups.

COLLAGEN ANALYSIS

Fig. 6 demonstrates the quantification of collagen I and III, and the collagen maturation index of the experimental groups.

In the quantification of type I collagen, it can be observed in a period of 7 days that the LED and LED+HFB groups presented a higher quantity of type I collagen compared to the control group. When comparing the treatments, the HFB group showed a higher statistically significant difference, when compared to the LED group. Also, LED+HFB had a higher amount of collagen I when compared to the other treated groups. Likewise, within 21 days, the treated groups had a greater amount of collagen I compared to the control group. The HFB group showed a higher statistically difference in collagen I compared to the LED and LED+HFB groups.

For collagen III, it can be observed, in the three experimental periods, that in the control group, there was a higher quantification of collagen III. Comparing the treatments after 7 days, the LED group showed a higher amount of collagen III compared to the HFB and LED+HFB groups, as well as the HFB group also showed a higher amount of collagen III compared to the LED+HFB group. After 14 days, the LED group also had a higher amount of collagen III compared to the HFB and LED+HFB groups. Furthermore, the LED+HFB group showed a statistically significant difference compared to the HFB group. After 21 days, the LED and LED+HFB groups had a higher amount of collagen III compared to the HFB group.

The analysis of the Collagen Maturation Index, showed lower statistical differences when compared to the control group with the groups treated in the 3 experimental periods. Within 7 days, the LED+HFB group showed a higher statistical difference when compared to the other groups. After 7 and 14 days, the HFB group showed a statistically significant difference when compared to the LED and LED+HFB groups.

TRACTION FORCE ANALYSIS

Fig. 7 shows the results of tensile strength resistance tests. It is possible to notice that after 7 days it can be seen that the traction force was statistically significant in the treated groups compared to the control group. At 14 days, we can see that the LED and HFB groups showed greater traction force compared to the control group. Also, the HFB group had better performance compared to the LED+HFB group. At 21 days, all treated groups showed a higher statistical difference compared to the control group, as well as the HFB group showed a better result of traction force compared to the LED and LED+HFB groups.

Discussion

Despite technological and scientific advances in healthcare, chronic or difficult-to-repair skin wounds, such as diabetic wounds, burns, or pressure ulcers, still impose an economic burden on the affected patient and the health system due to the high cost of wound treatment, such as clinical consultations, dressing changes, nursing care, hospital admissions, and surgical procedures (Werdin et al. 2008; Crovetti et al. 2004). It is estimated that the number of patients with chronic wounds will continue to increase due to the growing population aging, and the high prevalence of chronic diseases and comorbidities such as diabetes mellitus, obesity, venous hypertension, and peripheral vascular disease, which increases the cost of wound care (Werdin et al. 2008; Simka et al. 2003; Boyce and Warden 2002). In addition, optimal wound care often requires changes in practice, including the implementation of advanced methods and technologies.

This work investigated the effect of LED photobiomodulation therapy associated or not with the use of heterologous fibrin biopolymer in the repair of skin wounds in rats. According to the macroscopic findings and wound repair index, the treatments used in isolation, LED and HFB, demonstrated advanced skin repair when compared to the control group. In addition to the positive results observed with the use of LED and HFB in the macroscopic evaluation, this study also demonstrated microscopically the variables fibroblasts, collagen, and blood vessels showed a statistically significant difference greater compared to the control group. However, in the variable epithelialization, there was only a significant difference after 7 days for the LED group and 14 days for the HFB group. As well, it can be observed in microscopic analysis that both treatments used in isolation modulated the inflammatory process, as they were able to promote the reduction or disappearance of chronic inflammation, previously compared to the control group. These findings are in agreement with Dall Agnol et al. (2009) who showed that the diameter of the skin wound in rats was significantly reduced in the group that received photobiomodulation therapy. As well, Paraguassu et al. (2014) demonstrated a significant increase in skin wound re-epithelialization after irradiation in an experimental model of excisional skin wound in rats. The use of fibrin biopolymer has

also shown a positive effect on the repair process. Abbade et al. (2021) demonstrated that HFB proved to be safe and non-immunogenic, with good preliminary efficacy for the treatment of chronic venous wounds (Abbade et al. 2020, 2021).

It is believed that such results occur because photobiomodulation therapy is able to promote biochemical, bioelectrical, and bioenergetic changes, accelerate tissue metabolism, promote modulation of the inflammatory response, increase cell proliferation as well as the angiogenesis, amount of granulation tissue and collagen deposition, stimulating the repair process (Stein et al. 2005; Soleimani et al. 2021; Tim et al. 2016). In addition, the fibrin biopolymer activity mimics the coagulation cascade, in which thrombin-like enzyme converts fibrinogen to fibrin, leading to the formation of a stable fibrin clot that, reinforced by the activation of factor XIII, stimulates fibroblast proliferation, and participates in collagen synthesis (Kasai et al. 1893; Valbonesi 2006; Orsi et al. 2017). This study suggests that the increase in the variable fibroblasts observed microscopically in the LED and HFB groups were able to promote an increase in collagen deposition.

Collagen, the main component of the extracellular matrix, constitutes a family of proteins, with more than twenty-nine types of collagen identified, and types I, II, and III are predominant in connective tissues, with type I collagen being the most important component involved in tissue repair (Rehfeld et al. 2017). In the present study, collagen I and III depositions were investigated by picrosirius red analysis, which allowed the recognition of its maturation index. This staining is used to investigate shades of yellow or red which is indicative of collagen type I, whereas type III shows fine fibers, weakly birefringent, and in shades of green (Junqueira et al. 1982). Collagen analysis showed that LED and HFB increased type I collagen deposition and decreased type III collagen. This result suggests that the treatments accelerated tissue repair, since it is known that as the process of granulation tissue repair and maturation evolves type III collagen, which is the first to be synthesized, is gradually replaced by type I collagen. The increase in type I collagen accumulation leads to the formation of dense fibrous fibers, thus increasing the traction force of the extracellular matrix at the scar site. Scar maturation occurs simultaneously with the continuous elevation of type I collagen synthesis and the gradual degradation of type III collagen (Martinez-Hernandez and Amenta 1990; Michelle et al. 2001). Our results demonstrated that LED and HFB promoted collagen maturation as well as increased traction force at the new-formed tissue site.

When comparing the LED and HFB treatments, it is seen that both were able to stimulate and accelerate the tissue repair process. However, when we evaluated tissue maturation, we could observe that the HFB treatment proved to be more efficient in stimulating collagen organization and consequently increase the traction force at the injury site, especially after 14 days.

In addition, it is known that fibrin plays a central role in the repair process, as it acts as a scaffold that joins the wound margins, allowing and facilitating the movement, adherence, migration, proliferation, and differentiation of cells, such as fibroblasts and endothelial cells. Furthermore, fibrin cross-links fibronectin through factor XIII (fibrin stabilizing factor), reinforcing adhesion to fibroblasts that initiate collagen deposition, particularly type I in the structural architecture of the fibrin network along with the tissue of

newly formed granulation (Barros et al. 2009). Frauz et al. (2019) demonstrated that the use of heterologous fibrin biopolymer acted as a scaffold, since the groups that used HFB had higher birefringence values, showing that fibrin is slowly replaced by connective tissue. Similarly, Tuan et al. (1996) showed that fibroblasts grown in fibrin gel synthesized collagen. Recently authors demonstrated excellent performance of HFB as a drug delivery system (Pinto et al. 2021; Creste et al. 2020; Venante et al. 2021; Buchaim et al. 2019).

Surprisingly, the LED+HFB group, which combined the treatments, showed the same potential to promote tissue repair as when the treatments were used alone, that is, photobiomodulation therapy did not potentiate the effects of HFB, especially after 14 and 21 days. Likewise, Dutra Júnior et al. (2012) also did not observe an extra effect with the association of HFB with photobiomodulation therapy to stimulate tendon repair. On the other hand, the association of HFB with photobiomodulation in other tissues, such as to stimulate morphofunctional repair of the facial nerve (Raposo et al. 2017) and during the reconstruction of lesions in long bones, demonstrated the great potential of the association of treatments to aid in the process of peripheral nerve repair, and also in bone reconstruction (de Oliveira Gonçalves et al. 2016; de Oliveira et al. 2020). However, it is difficult to compare the results, because previous studies used different experimental periods and/or dosimetric parameters of the TFBM compared to those used in the present study.

This controversy regarding the association of photobiomodulation therapy and heterologous fibrin biopolymer may be related to the energy used and the frequency of application used in this study, as it is known that these parameters play an important role in the biological response, and the existence of a dose-response curve is well known (Hamblin 2016; Zein et al. 2018). Therefore, the present study suggests that the number of applications of LED + HFB promoted a dependent effect, that is, it may have caused an energy overload, especially after 14 and 21 days.

Conclusion

The present study suggests that the use of LED photobiomodulation therapy and heterologous fibrin biopolymer, used alone or in association, were effective to promote tissue repair in induced wounds in rats. However, the use of heterologous fibrin biopolymer was more effective to promote collagen maturation.

Declarations

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Author contributions - NCS made substantial contributions to the conception or design of the work and drafted the work; FEDA, LA and BB: revised it critically for important intellectual content; ALMMF: made substantial contributions to the analysis and interpretation of data; RSFJ, NAP and JFS: revised it critically for important intellectual content and approved the version to be published; CRT: made substantial contributions to the conception, drafted the work and approved the version to be published.

Ethics approval: This study was approved by the Institutional Medical Ethics Commission (protocol nº 0312/2019).

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References

- 1 Smaniotto PHS, Ferreira MC, Isaac C, Galli R (2012) Systematization of dressings for clinical treatment of wounds. *Rev. Bras. Cir. Plást.* <https://doi.org/10.1590/S1983-51752012000400026>
- 2 Nguyen AV, Soulika AM (2019) The Dynamics of the Skin's Immune System. *Int J Mol Sci.* <https://doi:10.3390/ijms20081811>
- 3 Gonzalez ACO, Costa TF, Andrade ZA, ARAP Medrado (2016) Wound healing - A literature review. *An. Bras. Dermatol.* 91 (5). <https://doi.org/10.1590/abd1806-4841.20164741>
- 4 Araújo DRS, Bezerra SMG, Costa JP et al (2017) Pressure ulcer dressings in critical patients: a cost analysis. *Rev Esc Enferm USP.* <http://dx.doi.org/10.1590/S1980-220X2016014803231>
- 5 Ferreira RS, de Barros LC, Abbade LPF, et al (2017) Heterologous fibrin sealant derived from snake venom: From bench to bedside - an overview. *J Venom Anim Toxins Incl Trop Dis* 23:1–12.
- 6 Gassling VL, Açı Y, Springer IN, Hubert N, Wiltfang J (2009) Platelet-rich plasma and platelet-rich fibrin in human cell culture. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 108(1):48-55. <https://doi:10.1016/j.tripleo.2009.02.007>
- 7 Chaves MEA, Araújo Angélica RA, Piancastelli CC, Pinotti M (2014) Effects of low-power light therapy on wound healing: LASER x LED *An. bras. dermatol.* <https://doi.org/10.1590/abd1806-4841.20142519>.
- 8 Nourian AD, Mirahmadi Babaheydari F, Chehelgerdi M et al (2019) Skin tissue engineering: wound healing based on stem-cell-based therapeutic strategies. *Stem Cell Res Ther* 10, 111. <https://doi.org/10.1186/s13287-019-1212-2>

- 9 Solmaz H, Dervisoglu S, Gulsoy M, Ulgen Y. (2016) Laser biostimulation of wound healing: bioimpedance measurements support histology. *Lasers Med Sci.* 31(8):1547-1554. <https://doi:10.1007/s10103-016-2013-9>
- 10 Simões TMS, Fernandes Neto JA, de Oliveira TKB, Nonaka CFW, Catão MHCV (2020) Photobiomodulation of red and green lights in the repair process of third-degree skin burns. *Lasers Med Sci.* 35(1):51-61. <https://doi:10.1007/s10103-019-02776-7>
- 11 Trubian, PS, Branco, AB, Campos, A., Ferreira, M., Trubian, PS, & Matias, JE (2007) Effect of octreotide administered subcutaneously on the healing of gastric sutures in rats. *Arq Bras Cir Dig.* 20 (3):172-178. <https://doi: 10.1590 / S0102-67202007000300010>
- 13 Croveti G, Martinelli G, Issi M, et al (2004) Platelet gel for healing cutaneous chronic wounds. *Transfus Apher Sci.* 30(2):145-151. <https://doi:10.1016/j.transci.2004.01.004>
- 14 Simka M, Majewski E. (2003) The social and economic burden of venous leg ulcers: focus on the role of micronized purified flavonoid fraction adjuvant therapy. *Am J Clin Dermatol.* 4(8):573-581. <https://doi:10.2165/00128071-200304080-00007>.
- 15 Boyce ST, Warden GD (2002) Principles and practices for treatment of cutaneous wounds with cultured skin substitutes. *Am J Surg.* 183(4):445-456. [https://doi:10.1016/s0002-9610\(02\)00813-9](https://doi:10.1016/s0002-9610(02)00813-9)
- 16 Dall Agnol MA, Nicolau RA, de Lima CJ, Munin E (2009) Comparative analysis of coherent light action (laser) versus non-coherent light (light-emitting diode) for tissue repair in diabetic rats. *Lasers Med Sci* 24:909-916. <https://doi:10.1007/s10103-009-0648-5>
- 18 Abbade LPF, Barraviera SRCS, Silveiras MRC, et al (2021) Treatment of chronic venous ulcers with heterologous fibrin sealant: A phase I/II clinical trial. *Front Immunol.* 12:627541. <https://doi:10.3389/fimmu.2021.627541>
- 19 Abbade LPF, Ferreira RS Jr, Dos Santos LD, Barraviera B (2020) Chronic venous ulcers: a review on treatment with fibrin sealant and prognostic advances using proteomic strategies. *J Venom Anim Toxins Incl Trop Dis.* 22;26:e20190101. doi: 10.1590/1678-9199-JVATITD-2019-0101.
- 22 Tim CR, Bossini PS, Kido HW, et al (2016) Effects of low level laser therapy on inflammatory and angiogenic gene expression during the process of bone healing: A microarray analysis. *J Photochem Photobiol B.* 154:8-15. <https://doi:10.1016/j.jphotobiol.2015.10.028>
- 23 Kasai S, Kunimoto T, Nitta K (1893) Cross-linking of fibrin by activated factor XIII stimulates attachment, morphological changes and proliferation of fibroblasts. *Biomed.* 4:155-60.
- 33 Pinto CG, Leite APS, Sartori AA, Tibúrcio FC, Barraviera B, Ferreira Jr RS, Filadelpho AL, Carvalho SC, Matheus SMM (2021). Heterologous fibrin biopolymer associated to a single suture stitch enables the

return of neuromuscular junction to its mature pattern after peripheral nerve injury. *Injury*, 52(1):731-7 Doi: 10.1016/j.injury.2020.10.070

34 Creste CFZ, Orsi PR, Landim-Alvarenga FC, et al (2020) Highly effective fibrin biopolymer scaffold for stem cells upgrading bone regeneration. *Materials* 13, 2747.

35 Venante HS, Chappuis-Chocano AP, Marcillo-Toala OO, et al (2021). Fibrin Biopolymer Incorporated with Antimicrobial Agents: A Proposal for Coating Denture Bases. *Materials* 4;1618. Doi 10.3390/ma14071618

36 Buchaim DV, Cassaro CV, Shindo JVTC et al (2019). Unique heterologous fibrin biopolymer with hemostatic, adhesive, sealant, scaffold and drug delivery properties: A systematic review. *J. Venom. Anim. Toxins Incl. Trop. Dis.* 25, e20190038.

37 de Freitas Dutra Júnior, Enéas et al (2021) Treatment of partial injury of the calcaneus tendon with heterologous fibrin biopolymer and/or photobiomodulation in rats. *Lasers in Medical Science*. <https://doi.org/10.1007/s10103-021-03341-x>.

38 Rosso MPO, Rosa Júnior GM, Buchaim DV, et al (2017) Stimulation of morphofunctional repair of the facial nerve with photobiomodulation, using the end-to-side technique or a new heterologous fibrin sealant. *J Photochem Photobiol B.* 175:20-28. <https://doi:10.1016/j.jphotobiol.2017.08.023>

42 Zein R, Selting W, Hamblin MR (2018) Review of light parameters and photobiomodulation efficacy: dive into complexity. *J Biomed Opt.* 23(12):1-17. <https://doi:10.1117/1.JBO.23.12.120901>

Tables

Table 1: LED photobiomodulation therapy application parameters.

WAVE-LENGTH	660 nm
OUTPUT POWER	0,166 W
TIME OF IRRADIATION	60 seconds
ENERGY	9,96 J
BEAM AREA	2 cm ²
NUMBER OF POINTS	1
FREQUENCY OF APPLICATION	Every 72 hours, for 7, 14 or 21 days.

Table 2: Scale for semi-quantitative analysis of histopathological evaluation (SOLMAZ, et al., 2016)

Scale	Epithelialization	Fibroblasts	Collagen	Blood vessels
0	Absent	Absent	Absent	Absent
1	Thickness of cut edges	Light around the tissue	Minimal granulation tissue	Light around the tissue
2	Cell migration	Light granulation tissue	Light granulation tissue	Light granulation tissue
3	Incision connection	Moderate granulation tissue	Moderate granulation tissue	Moderate granulation tissue
4	Keratinization	Intense granulation tissue	Intense granulation tissue	Intense granulation tissue

Figures

Figure 1

Macroscopic analysis of the wound of the experimental groups. CG= control group; LED = Light Emitting Diode; HFB = Heterologous fibrin biopolymer; LED + HFB = Light Emitting Diode associated with Heterologous Fibrin Biopolymer.

Figure 2

Wound repair index of experimental groups. CG= control group; LED = Light Emitting Diode; HFB = Heterologous fibrin biopolymer; LED + HFB = Light Emitting Diode associated with Heterologous Fibrin Biopolymer.

Figure 3

Representative photomicrographs of the histological analysis. CG= control group; LED = Light Emitting Diode; HFB = Heterologous fibrin biopolymer; LED + HFB = Light Emitting Diode associated with Heterologous Fibrin Biopolymer. Color = H.E. Magnification: 20x.

Figure 4

Histomorphometric analysis. CG= control group; LED = Light Emitting Diode; HFB = Heterologous fibrin biopolymer; LED + HFB = Light Emitting Diode associated with Heterologous Fibrin Biopolymer.

Figure 5

Histopathological analysis for evaluation of inflammatory infiltrate. CG= control group; LED = Light Emitting Diode; HFB = Heterologous fibrin biopolymer; LED + HFB = Light Emitting Diode associated with Heterologous Fibrin Biopolymer.

Figure 6

Quantification of collagen and maturation index. CG= control group; LED = Light Emitting Diode; HFB = Heterologous fibrin biopolymer; LED + HFB = Light Emitting Diode associated with Heterologous Fibrin Biopolymer.

Figure 7

Tensile strength endurance test. CG= control group; LED = Light Emitting Diode; HFB = Heterologous fibrin biopolymer; LED + HFB = Light Emitting Diode associated with Heterologous Fibrin Biopolymer.