



Effects of a Low-Cost LED Photobiomodulation Therapy Equipment on the Tissue Repair Process

F. E. D. de Alexandria, N. C. Silva, A. L. M. M. Filho, D. C. L. Ferreira, K. dos S. Silva, L. R. da Silva, L. Assis, N. A. Parizotto, and C. R. Tim

Abstract

Wound is the interruption of tissue continuity, caused by physical, chemical, mechanical trauma or triggered by a clinical condition, which triggers the fronts of organic defense. The body reacts to the installation of the wounds by initiating the healing process, however, these events may fail due comorbidities may be impair the healing response, resulting in a chronic wound. LED, light-emitting diode, have been successfully used as adjunctive therapies for stimulating skin wound healing however, the parameters used in low-cost equipment have not yet been studied yet. This study aims to evaluate the efficiency of a red LED light therapy equipment on wound healing in rats. The project was approved at CEUA/UESPI under protocol 0298/2019. Two experimental groups were delimited, one treated with LED and one control group. The animals were anesthetized and an area of 2 cm² was cut with a skin punch. The treatment was performed for a period of 14 days, in which the regression of the wound was evaluated using the ImageJ program. When comparing the 14-day regression percentage between the LED treated group and the untreated group, it was observed that there was a significant difference in the regression percentage, being $p < 0.05$, in which the group treated with LED presented a higher regression percentage than the untreated. LED photobiomodulation therapy has been widely used demonstrating success in the regeneration of lesions by promoting photobiological effects that stimulate this process. This study showed that the

low-cost red LED light therapy equipment resulted in faster healing process of the wounds, favoring the reepithelization and recovery of skin integrity.

Keywords

Healing • Wounds • Wistar rats • Photobiomodulation • LED

1 Introduction

Wound is the interruption of the continuity of body tissue to a greater or lesser extent, caused by physical, chemical, mechanical trauma or triggered by a clinical condition, which triggers the fronts of organic defense directly interfering in its functions, causing damage to the organism [1, 2]. After the installation of the wounds, the body is expected to react by initiating the complex healing process that involves physiological, biochemical, cellular and molecular events that interact for tissue reconstruction, in order to restore its integrity, however in many cases, these events fail giving rise to wounds that can take years to end the process of tissue reconstruction, and many times this process may not even happen [3].

It is known that the health costs of treating wounds that are difficult to heal in Brazil and in the world are high and have been increasing rapidly, making it difficult to maintain the sustainability of health systems. The constant search for alternatives that reduce the costs of skin lesions, has proven to be an option to balance health expenses. Searching for treatments that can reduce costs, as well as restoring the integrity of patients' skin sees an alternative in the honest process of health systems [4].

The LED (Light Emitting Diode), a light emitting diode, has been studied for decades, whose evidence of wound treatments is based on two main stimulating effects, which is due to the stimulation in the differentiation and proliferation

F. E. D. de Alexandria · N. C. Silva · L. Assis · N. A. Parizotto · C. R. Tim (✉)

Department of Biomedical Engineering, University Brazil, São Paulo, Brazil

e-mail: carla.tim@universidadebrasil.edu.br

F. E. D. de Alexandria · N. C. Silva
Facid, Teresina, Brazil

N. C. Silva · A. L. M. M. Filho · D. C. L. Ferreira · K. dos S. Silva ·
L. R. da Silva
UESPI, Teresina, Brazil

of fibroblasts and collagen synthesis of tissular tissue. It is known that LEDs are generally inexpensive and easy to handle, and can be used together with other wavelengths, forming an applicator tip that covers a larger area of lighting, and can also be associated with other therapeutic resources. This treatment provides professionals in the field to carry out an advanced and non-invasive procedure, which does not promote damage to the skin, without restriction to skin types and can be used at any time and in other pathologies [5, 6].

Although the positive effects of LED interventions on wound healing, the parameters used in low-cost equipment were not studied before. In this context, it was hypothesized that a low-cost red LED light therapy equipment may optimize the healing process and would contribute to mitigate socio-economic spending as well as improving the quality of life of affected individuals. Thus, the aim of the present was evaluated the efficiency of low-cost red LED light therapy on cutaneous wound healing in rats. For this purpose, the macroscopic aspect and wound regression were evaluated.

2 Materials and Methods

In order to development of this work, 14 adult male Wistar rats (*rattus norvegicus albinus*) (over 60 days old), with an average of 250 g body weight, randomly selected from the vivarium of the Health Sciences Center of the State University were used from Piauí. The animals were born and raised on the place, not requiring an acclimatization period. The study was authorized by the ethical committee of animals using (CEUA / UESPI) da State University of Piauí with protocol n° 0312/2019.

During the experimental phase, all animals received standardized rodent food and water ad libitum. They were kept in polypropylene boxes, autoclavable and in a ventilated environment. The temperature was maintained at 25 °C and the humidity controlled. The 12-h light–dark cycle was followed.

After weighing, the rats were anesthetized with an 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, as ethical standards and guidelines for research with animals of Resolution 879 of February 15, 2008, of the Federal Council of Veterinary Medicine [7].

After trichotomy of the dorsal and cervical region, the rats were placed in prone position, asepsis was performed with 70% alcohol, and with a skin punch, an area of 2 cm² was marked on the back of the animals, corresponding to skin area to be removed (Fig. 1). The skin fragments were resected with a steel blade until the muscular fascia was exposed. Hemostasis was performed by digital compression, using sterile gauze.



Fig. 1 Schematic representation of experimental procedure

In the first two postoperative days, paracetamol 200 mg/mL was administered as an analgesic medication at a dose of 1 mL/20 mL of water every 8 h.

The animals were randomly divided into 2 groups containing 7 animals each, being a negative control group, in which the wound on the animals' back was induced and treated with 0.9% saline; and a group treated with LED, in which the wound on the back of the animals was induced and LED was applied, then saline 0.9%

The LED used was Tendlite® medicinal, model 204, red wavelength 660 nm, nominal output power of 160 mW, 60 s. Immediate irradiation 0.9% saline (SF) was applied to the wounds, as well as to the control group.

After the 14-day experimental period, the animals of both groups were humanely killed, with a lethal dose of anesthetic of sodium thiopental at a dose of 100 mg/kg, intraperitoneally.

For the assessment of wound regression, a digital camera was used in basic mode, without flash, without zoom and with a resolution of 8.0 megapixels. To standardize the distance from the camera to the wound, an aluminum support 20 cm apart and perpendicular to the wound was used. A ruler placed next to the animals and next to the wound was used to standardize the lesion area unit in mm². The images were analyzed using the 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, obtaining the following parameters:

- lesion size (mm²)
- residual area (Ar) of the lesion (%)
- A_{day} represents the area measured daily
- A_{initial} is the initial area measured immediately after the puncture.

$$Ar = \frac{A_{\text{day}}}{A_{\text{initial}}} * 100$$

Statistical analyses were performed in the statistical Graph Prism software. The level of significance adopted in the study was $\alpha = 0.05$. The data were processed and analyzed using the Statistical Package for the Social Sciences (SPSS), version 20.0. The normal distribution of all variables was verified by Shapiro–Wilk’s W Test. Comparisons between groups with parametric distribution were performed using the Unpaired t test.

3 Results

In the macroscopic findings obtained from the qualitative analysis of the experimental groups, it was possible to observe the absence of necrosis, odor, presence of fibrosis or liquid in the lesion cavity in both experimental groups. In the control group, there was a slight local inflammation, evidenced between the 5th and 6th day after the lesion was performed, not manifested in the LED group (Fig. 2).

After the end of the 14th day, the morphometric analysis of the lesions of both groups was performed, treated with LED and negative control, by the Image J analytical software, in which the initial wound area, day 0, was measured and the area compared the last day. The program generates a percentage of regression from this comparison.

The obtained value was processed by the parametric test of statistical analysis, the “Unpaired t test”, which showed a significant difference between the two groups tested, the LED treated and the negative control (Fig. 3).

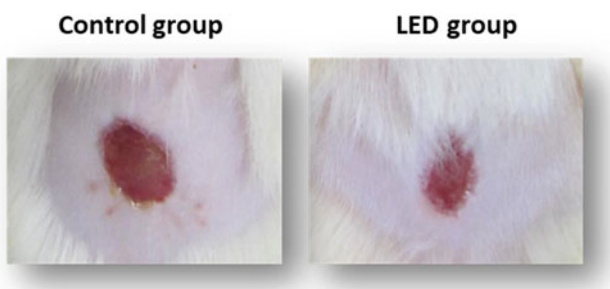


Fig. 2 Macroscopic analysis

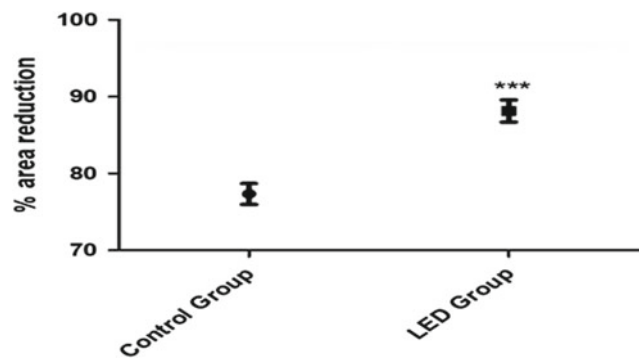


Fig. 3 Record of the percentage of regression of the injured area. *** = $p < 0.05$ when compared to the negative control

4 Discussion

Photobiomodulation has been widely used demonstrating success in the regeneration of injuries, as it promotes photobiological effects that stimulate this process [8, 9].

The use of LED in the healing process is already a reality. There are reports of cases of use of phototherapy that demonstrate the effectiveness of its use in the treatment of wounds. A case study was carried out with a patient from the HC/UEL Clinic Clinic who had ulcers in the lower limbs. The application was made once a week, using LEDs with a wavelength of 628 nm in the ulcer of the left lower limb and the right was used as a control. Evolution was measured by means of photographic record, area measurement and pain measurement. After the eighteen sessions, the results showed changes in the clinical characteristics of the lesion and the healed area was 30% larger compared to the control ulcer. As for pain, the visual analog scale varied from eight to zero in the irradiated limb and from nine to two in the control. It was concluded in this study that LED therapy is a resource of choice in the treatment of venous ulcers, in healing and pain reduction aspects [10].

Another reported case reported a pediatric patient with oral mucositis due to chemotherapy used to treat acute lymphoblastic leukemia, in which the lesions were treated daily with a light-emitting diode (LED). The lesions remitted after 10 days of treatment. It is concluded in the article that the LED was effective in the treatment of mucositis, as it decreased the symptoms of pain and accelerated the tissue repair process [11].

Currently the University of Brasilia (UnB) is developing a project for the use of LED in the treatment of diabetic foot and other types of injuries. Equipment formed by a mobile system of tissue neoformation was developed based on the principles of phototherapy to assist in wound healing. Its light emitting circuit is formed by a control module and a high brightness LED module. Tests were carried out with

nine patients in the Federal District—six, with 11 different ulcers, at the Regional Hospital of Taguatinga (HRT), in 2013, and three at the Regional Hospital of Ceilândia (HRC), in 2016—who used the LED, presenting to the end of the study total healing of ulcers [12].

Research focused on developing strategies to accelerate and improve the healing process is increasingly necessary and valued. Today, one of the biggest drivers of research involving stomatherapy is the search for effectiveness with cost reduction in the area. A study carried out in the wound sector, at UFMG, compared traditional dressings and new technologies, such as the use of new standardized coverings for the treatment and the introduction of photobiomodulation and proved a 5.4% reduction in their costs, with a reduction observed average total cost, up to 60.7% of the initial value of conventional dressings.

Thus, technological advances in the use of new products and procedures in the realization of dressings have been showing benefits to the population with wounds, improving the quality of life of people.

5 Conclusion

The low-cost red LED light therapy equipment was efficient in accelerate healing processes of the wounds, favoring the reepithelization and recovery of skin integrity.

Acknowledgements We would like to acknowledge the contributions of funding agency CAPES for the financial support of this research.

Conflict of Interests The authors declare that they have no conflict of interest.

References

1. Cesaretti IUR (1998) Processo fisiológico de cicatrização da ferida. *Rev Pelle Sana* 2:10–12
2. Blanck M (2009) Curso de Feridas: Anatomia, histologia, fisiologia, imunologia, microbiologia e o processo cicatricial. *Enferm Atual* 9(49):6–12
3. Garcia SJ et al (2018) Protocolo de Tratamento de feridas para o Sistema penitenciário do Estado de São Paulo. Universidade Federal de São Paulo, São Paulo—SP
4. Silva DRA et al (2017) Pressure ulcer dressings in critical patients: a cost analysis. *Rev Esc Enferm USP* 51:1–12
5. Schubert EF (2006) Light emitting diodes, 2nd edn. Cambridge University Press. New York, Cambridge
6. Silva EF, Moraes DEPF, Silva PM (2018) A terapia combinada de LED associada com ácidos no tratamento de acne. *Fisioterapia Brasil* 19(5):63–69
7. Brasil, Conselho Federal de Medicina Veterinária (CFMV) Resolução Nº 879, de 15 de fevereiro de 2008. Manual de Legislação do Sistema CFMV/CRMVs
8. Parizotto NA, Baranauskas V (1998) Structural analysis of collagen fibrils after he-ne laser photostimulated regenerating rat tendon In: Second World Congress Kansas City. *Annals. Kansas City (USA)*, 66–67
9. Martignago CCS et al (2015) Proliferação de células fibroblásticas submetidas a terapia a laser de baixa potência de 904 nm. *Blucher Biochem Proc* 1(2):362–362
10. Siqueira CPCM et al (2009) Efeitos biológicos da luz: aplicação de terapia de baixa potência empregando LEDs (Light Emitting Diode) na cicatrização da úlcera venosa: relato de caso. *Semina: Ciências Biológicas e da Saúde* 30(1):37–46
11. Rimulo AL, Ferreira MC, Abreu MH, Aguirre-Neto JC, Paiva SM (2011) Chemotherapy-induced oral mucositis in a patient with acute lymphoblastic leukaemia. *Eur Arch Paediatr Dent* 12 (2):124–127
12. Caxito C (2017) UnB desenvolve tratamento com LED para cicatrização em diabéticos. *Jornal Brasília Capital, Brasília, Brasil*