Macro and microscopic analysis of island skin grafts after low-level laser therapy

Análises macro e microscópicas de enxertos cutâneos por semeadura após laserterapia de baixa intensidade

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A B S T R A C T

Objective: To observe the effects of low intensity laser therapy in inflammation, wound healing and epithelialization of island skin grafts. **Methods**: Twenty rats were subjected to this grafting technique and divided subsequently into two equal groups, one treated with laser and the other control. **Results**: there was less inflammation, faster healing, epithelialization and keratinization in the laser-treated animals when compared to the untreated. **Conclusion**: Low intensity laser therapy is helpful to island skin grafting.

Key words: Transplantation, autologous. Skin transplantation. Wound healing. Laser therapy, low-level. Surgery, veterinary.

INTRODUCTION

Skin grafts are alternatives to the closure of extensive lesions where the approximation of the edges is not possible. They become effective when the transplant heals in its new location. In dogs and cats, particularly, they are indicated for the treatment of extensive wounds in which the skin flaps cannot be applied because of the location, type or extent of the lesion[1]. The autografts are more successful, since the graft and the host are immunologically identical[2].

Grafts can be collected with a scalpel blade or a punch biopsy, which, being small and circular, form epithelialized islands in sites with granulation tissue[3].

The natural latex biomembrane is thin, elastic and easy to handle, and has a thin layer of polylysine that increases permeability and microvascular flow[4]. It has also proven biocompatibility and low cost compared with the alternatives found on the market[5-9]. Its particular microarchitecture allows protein and cell adhesion, in particular macrophages involved in repair[5,6,10].

Current research has shown that application of low intensity laser in adequate dosages and exposures and in correct time intervals are decisive factors in the treatment of wounds and accelerate their closure. Adequate laser therapy promotes wound healing by stimulating cell migration, fibroblast proliferation and mitochondrial activity, maintaining viability without causing damage or cellular stress[11].

The therapeutic effects of low intensity laser therapy have been shown in *in vitro* and *in vivo* studies and included regeneration and anti-inflammatory and analgesic effects. Other studies showed gains in local microcirculation[12], lymphatic system[13] and synthesis of collagen by fibroblasts[13,14] and prevention of infections[15-17].

Another study also showed that irradiation of low intensity laser accelerates wound healing because it stimulates the biological activities and differentiation of fibroblasts, causes reduction of the inflammatory process and contributes to the organization of the collagen fibers in the extracellular compartment[18].

Regarding anti-inflammatory action, it was confirmed that the use of laser promotes rapid initiation and resolution of the inflammatory phase and tissue repair, making it more acute and sharp; furthermore, it increases collagen synthesis[19,20]. However, it was not confirmed whether the anti-inflammatory action of the laser, though it accelerates this process, promotes histological quality to the repaired tissue and even activation of keratinocytes[21].

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With the intention of getting better results with respect to the healing process, this study aimed to determine whether application of HeNe low intensity laser accelerates the healing process of island skin grafts.

METHODS

This study was submitted and approved by the Animal Ethics and Welfare Committee – CEBEA of the Faculty of Agriculture and Veterinary Sciences, Paulista State University – UNESP, Jaboticabal - São Paulo, under Protocol 010004-08.

We used 20 male, young adult Wistar rats (Rattus norvegicus), (mean age 20 days), weighing between 200 and 300g. They were randomly divided into two equal groups (n = 10), a control (GC) one, undergoing no treatment and a laser (GL) one, receiving laser applications over the wound. Both groups underwent an operation to create a defect in the skin. Under general anesthesia with Isofluorane by mask, the wound was created with a scalpel and scissors and had dimensions of approximately 4x4cm. The natural latex biomembrane with 1% polylysine (Isoforine - Cristália) was used as biological dressing. The animals also received a bandage strip and adhesive plaster that was replaced after five days. In the immediate postoperative period a single dose of 0.02 ml of enrofloxacin 10% (10% Iflox - IRFA) and 0.02 ml of flunexin meglumine (Flumedin - Jofadel) was administered intramuscularly.

After ten days, in a novel surgical procedure, all animals received grafts, also under inhaled anesthesia. Trichotomy was performed on the left flank, the donor site. Total thickness grafts were harvested with a 5mm-diameter surgical punch and grafted into 4mm-diameter perforations created in the recipient area covered with granulation tissue. The donor sites healed by second intention.

The GL group (n = 10) received low intensity laser irradiation (6J/cm^2/18s) at each grafted island in the immediate postoperative period, 72 hours after and on the seventh day after the procedure. The control group (n = 10) received no irradiation.

On the dates set for applications of low-intensity laser on GL we also changed the dressing in GC. Two animals in each group (GC and GL) were sacrificed in CO_2 chamber in days one, two, four, eight and 14 after the second surgery, and shortly after sacrifice samples were collected covering the grafts and part of the receptor site, being identified and preserved in 10% formalin.

The preserved material was processed in the laboratory, immersed in paraffin and cut according to routine histological methods. The sections were stained with hematoxylin and eosin and Masson Trichrome. The slides were examined and photographed at 100X optical microscope coupled to a camera. The images were transferred to and processed in a computer.

RESULTS

The macroscopic evaluation of the control group showed that one day after the first application of low intensity laser, the wound had a hemorrhagic aspect, particularly around the receptor sites of the grafts (Figure 1 A1). On the second day, there was decreased bleeding, but there was edema and yellowish discharge covering the entire site (Figure 1 B1). On the fourth day, the granulation tissue was paler, the recipient site had smaller area and signs of cicatricial retraction in the sides of the wound, causing the graft to approach the edges (Figure 1 C1). Eight days after surgery, the granulation tissue was markedly reddish, with smaller area and dry aspect on its surface. The receptor sites were already approaching the edges (Fi-

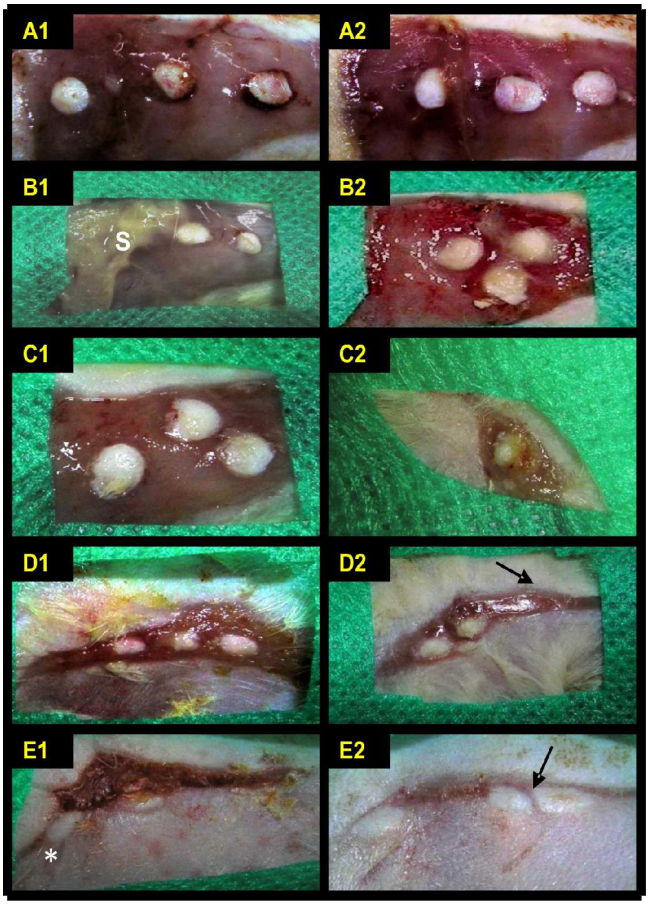

Figure 1 - *Photographic images showing the macroscopic evolution of the wounds. In the images, letters refer to times: A: one day after grafting; B: 2 days; C: 4 days; D: 8 days; and E: 14 days. The numbers indicate the groups: 1: control group; 2 laser group. The symbols embedded in the images mean, respectively: Arrow – re-epithelialized area; (*) - areas of scar retraction.*

gure 1 D1). Finally, at 14 days, a dry crust covered the entire wound. The grafts were practically engaged by intact skin and signs of scar retraction and epithelialization were present at the edges (Figure 1 E1).

The macroscopic evaluation of the laser group showed that on the first day after surgery, the wound had hemorrhagic aspect, although visually less intense than that of the control group (Fig. 1 A2). After two days, the reddish granulation tissue displayed a small petechial bleeding (Figure 1 B2). After three days, the area of the recipient site was smaller, paler and with serous secretion (Figure 1 C2). On the eighth day, epithelialization started in the edges and, although reduced, the wound had no signs of scar contraction (D2 Figure 1). At 14 days, healing was almost complete. The grafts were surrounded by re-epithelialised tissue and by the intact skin adjacent to the wound. Only a small area in the center of the receptor site did not look epithelialized (Figure 1 E2).

In both groups, there was no displacement of the grafts from their receptor site, which is important for success of the technique.

As for microscopic evaluation, staining with hematoxylin-eosin showed that it is possible to visualize the region of the graft and the receptor formed by the granulation tissue, as seen in figure 2 with 100X. On days one and two we observed the presence of inflammatory infiltrate, with more intense aspect in the control group. After four days there was no inflammatory infiltrate in the laser group and in the control group it was already attenuated. On day eight, there was coverage of epithelium only in the region of the graft in the control group, whereas in the laser group the epithelium extended from the region of the graft to the granulation tissue. At 14 days, the control group showed epithelium only over the graft, whilst in the laser group the epithelium covered the granulation tissue and displayed dermal papillae, which confer greater adhesion to the tissue and demonstrate a greater degree of organization.

From the 8th day, in the laser group the transition area between the graft and the recipient site was barely evident, whereas in the control group this condition could only be seen at 14 days.

The Masson Trichrome staining was used to highlight the epithelium and keratin layer on the surface of the graft. Figure 3 depicts the histological cuts at 100X magnification. There is an increase in the proportion of collagen in all times in GL (letters B, D, F, H and J) when compared to GC (letters A, C, E, G and I). In both groups the keratin overlays the graft region. From the eighth day it appears on the recipient site in GL (Figure 3, H). In GL dermal papillae were observed already on the 14th day (Fig. 3, D) and from the fourth day, keratin had covered the granulation tissue (Figure 3 F). In GC the layer of keratin was noted on the adjacent tissue at 14 days, and at this time the epithelium was only in the grafted region (Figure 3, I).

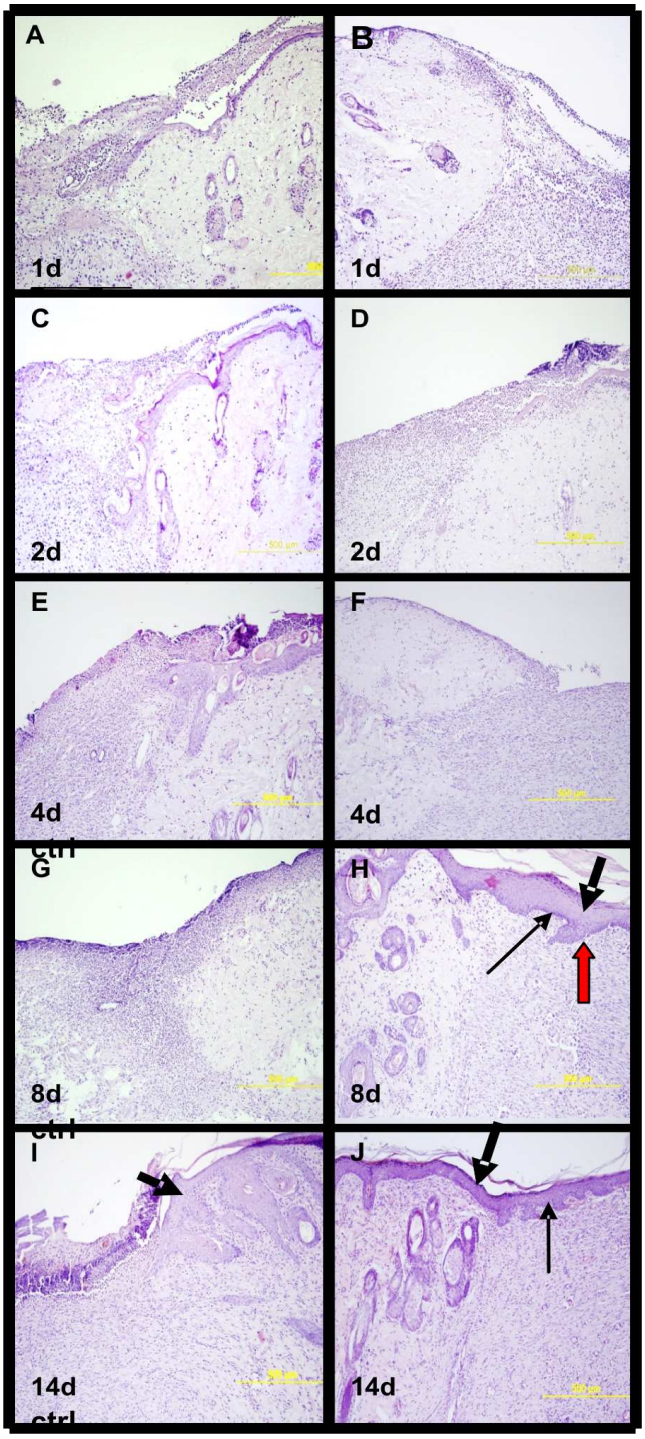

Figure 2 - *Photomicrographs of histological sections of the island grafts in HE staining, 100X magnification, at the different times and groups. The symbols embedded in the images mean: thick arrow: epithelial; thin arrow: dermal papillae; red arrow: epidermal ridge.*

DISCUSSION

The natural latex biomembrane has great potential for tissue repair and formation[9]. The dressing

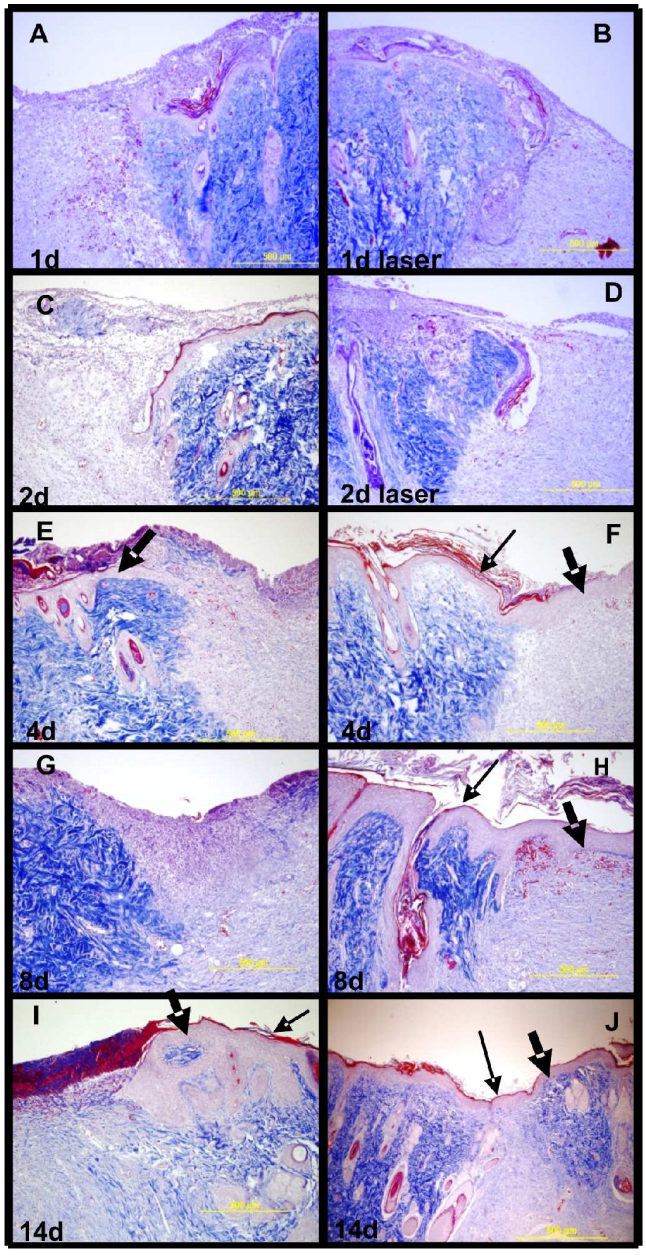

Figure 3 - *Photomicrographs of histological sections of the island grafts in Masson Trichrome staining, 100X magnification, at different times and groups. The symbols embedded in the images mean: thick arrow: epithelium; thin arrows: keratin layer.*

made with this material in the first stage of the study secured rapid granulation of the area of and also a granulation tissue of good quality[9] to be the recipient site for the grafts.

As for the anti-infection action of laser[15,16], the control group showed a yellowish discharge with purulent aspect on the grafted wound, a fact that did not occur in the Laser Group, which may be related to the absence of infection in the individuals treated with low-intensity laser.

Animals from GL group showed no changes in behavior or in ingestion of food and water after the surgical procedures. This could suggest an analgesic action of the laser[12]. However, those from GC did not display changes either, so this parameter should be investigated in a more specific manner, such as with the dosage of endogenous substances, like cortisol, which provides more specific values for this assessment.

Within four days, unlike GL, GC had more reddish wounds, indicating the presence of inflammation. This result was also seen in histological sections stained with hematoxylin and eosin, where the inflammatory infiltrate can be visualized. This type of response characterizes the laser action as an accelerator of the inflammatory process[19] and not as an anti-inflammatory[12,18].

Since healing is a complex process that starts with inflammatory reaction, the statement that best explains laser action in relation to decreased healing time is: the sooner the inflammatory phase ends[19], the sooner the repair phase begins, and the sooner the whole healing process is accomplished. This happened in this study, where in GL group wounds were healed at 14 days, while in GC they were still in the early epithelialization phase. This result was reported in another study in which laser accelerated the first and second phases of the healing process[20].

Both the macro and the microscopic evaluations showed that GL animals had epithelialization of the wound in less time than the GC ones, this being due to increased cell proliferation induced by low-intensity laser[11].

The literature reports the good quality of scar tissue after laser therapy[19], which was confirmed in the analysis of the slides on the 14th day in GL. Unlike GC, dermal papillae were observed therein, which reveals a high degree of tissue organization and thus the quality of repair, since these structures have the function of fixing the epithelial to the granulation tissue.

Histological sections of GL specimens stained with Masson Trichrome demonstrated the presence of keratin on the wounds from the fourth day due to the activating action the low-intensity laser has on keratinocytes[21]. The GC animals only displayed keratin coverage on the recipient site from the 14th day, since they received no irradiation.

In conclusion, the grafts were incorporated and epithelialization commenced on receptor sites more quickly in the group irradiated with laser. Wound healing treated with laser was faster, showing better macro and microscopic appearance in the group treated with low intensity laser when compared to the group that did not receive laser therapy.

R E S U M O

Objetivo: *observar se a laserterapia de baixa intensidade acelera o processo inflamatório, a cicatrização e epitelização de enxertos cutâneos por semeadura.* ***Métodos:*** *vinte ratos foram submetidos a esta técnica de enxertia e divididos em dois grupos iguais, um tratado com laser e outro controle.* ***Resultados:*** *houve menor tempo de reação inflamatória, maior velocidade de cicatrização, epitelização e queratinização nos animais tratados com laser em relação aos não tratados.* ***Conclusão:*** *a laserterapia de baixa intensidade é efetiva no auxílio ao tratamento de enxertos por semeadura.*

Descritores*: Transplante autólogo. Transplante de pele. Cicatrização. Terapia a laser de baixa intensidade. Cirurgia Veterinária.*

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