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Influence of laser therapy on the dynamic formation of extracellular matrix in standard second degree burns treated with bacterial cellulose membrane

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ABSTRACT

The present study aims to assess the influence of Aluminum-Gallium-Indium-Phosphide laser (AlGaInP laser, λ = 660 nm), whether or not in association with the application of a membrane of bacterial cellulose (Nexfill™), during recovery from induced second-degree burns at the dorsum of Wistar rats. (*Rattus norvegicus, Wistar*). Forty-eight animals have been distributed into four groups: Control (burns remained untreated), Group I (laser-treated), Group II (treated with Nexfill), and Group III (laser + Nexfill™). In addition to a morphological analysis, immunohistochemical analysis has been performed for type I collagen, type III collagen, fibronectin, and laminin. The Fisher's Test was used to assess differences among groups ($p < 0,05$). A larger amount of collagen type III was observed in Control, Group II and Group III when compared with Group I ($p < 0,05$). Group I and Group III have shown a greater collagen deposition when compared with Group II ($p < 0,05$), but the amount of collagen was similar in Group I, Group III, and Control. Group III has shown larger fibronectin amounts in comparison with Group II ($p < 0,05$). As regards laminin, Group I has shown a predominant discontinuity pattern on the basal lamina in comparison with Control, Group II, and Group III ($p < 0,05$). It is concluded that in this current study the laser when used alone (Group I) hasn't influenced collagen deposition neither has it acted on fiber pattern (fibril and/or reticular). Moreover, laser application hasn't accelerated the repair of wounds caused by inflicted second-degree burns.

1. Introduction

Burns are one of the commonest types of trauma facing human beings [1] and, regardless of a worldwide decline in the death rate arising from burns, associated non-fatal sequelae frequently tend to lead to permanent impairments [2]. In such injuries, the clinical treatment and repair process are dependent on the extent and depth of the damage. In many cases, burns can immediately be treated by means of autologous graft – which promptly leads to a permanent and satisfactory wound closure. However, in many situations this type of treatment appears to be impossible or unlikely to succeed, for instance in cases of infected or severely extensive wounds [3,4].

Therefore, temporary wound dressings are deemed necessary in order to maintain the function of the wound, reduce infection, relieve the pain and metabolic stress, in addition to providing blood supply and protection against trauma [3,5,6]. In light of this fact, research into wound dressings has advanced the production of a wide range of synthetic and biological dressings for wound care and management [7]. Options readily available include the bacterial cellulose biomembrane, a biosynthetic polymer that provides optimum conditions for epidermal regeneration owing to its nanomorphological characteristics, protection against infection and ability to hold water; in addition, it enables the transfer of medicines into the wound [8–10].

The influence of laser on wound healing has recently motivated a number of experimental studies - some of which drawing attention to biostimulation and healing properties [11–15]. Laser therapy triggers cellular processes and a response from the vascular system which appear to have a direct impact on tissue repair [16]. As a result, research on the effects of photobiomodulators on burns has become increasingly common [1,17–19] and fairly recently scholarly papers have addressed

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an associated use of laser and occlusive dressings [20,21]. Nonetheless, substantial evidence is still lacking as regards the bacterial biocellulose membrane in association with laser use in the healing of second-degree burns.

Given the above considerations and also taking into account the perceived advantages of bacterial cellulose membrane and laser use, it is arguably valid to undertake research as to assess possible effects of a combined use of both therapies for burn treatment, ensuring faster, more efficient, and less painful healing with better functional and cosmetic results.

2. Materials and Methods

2.1. Sample Selection

A total of forty-eight male, albino, young, healthy rats (*Rattus norvegicus*, *Wistar*) aged two months and weighing 150 g–200 g were randomly selected from the Laboratory Vivarium at Gonçalo Moniz Research Center (FIOCRUZ, Bahia, Brazil). The animals were housed individually at 22 °C under a light/dark cycle established as 12 h light on, with ready access to Labina rat feed and water *ad libitum*. The rats were distributed into four groups of 12 animals as follows: Control, Group I (laser radiation), Group II (Nexfill™ bacterial cellulose membrane), and Group III (laser radiation associated with the application of Nexfill™ membrane).

2.2. Ethical Considerations

In order for this research to be conducted, prior approval was received from the Committee for Experimentation and Animal Use (CEUA) at Gonçalo Moniz Research Center (CPqGM-FIOCRUZ – BA), under the Protocol 008/2007. Adherence to ethical principles was ensured for the performance of experimental use of animals as well as their vivisection for academic-scientific purposes.

2.3. Induction of Standard Second-Degree Burn Injury

The animals were anesthetized with 75 mg/kg Ketamine and 10 mg/kg Xylazine intraperitoneal injection. Next, an area measuring 2 × 2 cm was depilated at the dorsum of the animal so that antisepsis was performed with polyvinylpyrrolidone-iodine solution. Infliction of burn wounds has been performed in accordance with Meyer and Silva's modified technique [22]. To this end, a brass bar with a cube-shaped tip was pre-heated in boiling water for 1 min and subsequently placed on the skin of the animal for 0.5 s, and timed with a digital timer. Once the surgery procedure completed, an oral dose of Sodium Dipyrone was administered to each and every rat according to their weight. The epidermis on the wound area was removed with a scalpel blade no. 15 as to expose the subcutaneous tissue. This was a necessary for the application of the Nexfill™ cellulose membrane on the superficial dermis.

2.4. Laser Radiation Protocol

The animals allocated in Group I and Group III received radiation from a red laser AlGaInP (λ = 660 nm, 40 mW, ø = 4 mm^2, t = 125 s) with a *SAEF* (Spatial Average Energy Fluence) at 20 J/cm^2 split into four points of 5 J/cm^2 each [19,23]. Controls were sham-irradiated. Irradiation was performed immediately after the infliction of burns and every 48 h until one day before the death of the animals, which occurred 24 h, 3 days, 7 days, and 14 days later in a CO_2 chamber. A summary of the laser parameters used on the study is depicted on Table 1.

Table 1
Summary of the laser parameters used on the study.

Parameters	Laser
Wavelength (nm)	660
SAEF (J/cm^2) (per session)	20
Energy density (J/cm^2) (per point)	5 J/cm^2
Power output (mW)	40
Illuminated area (cm^2)	4
Mode	CW
Spot (cm^2)	0.04
Intensity (mW/cm^2)	1.000
Exposure time (per session)	125 s

2.5. Morphological and Immunohistochemical Analysis

Following death, the tissue fragments were removed from the wound area, placed in formalin at 10% and routinely processed for staining with hematoxylin-eosin, picrosirius for fibrosis and orcein analysis of elastic fibers. Histological sections were morphologically diagnosed in a candid fashion by an experienced pathologist by light microscopy (Zeiss Axioskop) and the treated groups were compared to the control group (no treatment). The inflammatory infiltrate was classified as absent, predominantly polymorphonuclear, predominantly mononuclear, or mixed. Inflammatory infiltration and edema (if present), the presence of adipocytes in the dermis, and production of collagen and elastic fibers were semi-quantitatively marked as absent (0), mild (+), moderate (++) and strong (+++).

The deposition of the extracellular matrix was assessed by immunohistochemistry using a polymer system (AdvanceTM, Dako Corporation) and antibodies directed against the following proteins: collagen types I and III, fibronectin, and laminin (Table 2).

In order to undertake immunostaining animals dead at 24 h of the experiment were excluded. Collagen I and III were analyzed in order to calculate the percentage of wound covering, by taking into consideration only the papillary dermis (score 0: 0–10% coverage; score 1: 11–30%; score 2: 31–60% and score 3: > 60%) and the fiber pattern (reticular, fibril, or mixed). The fibers were regarded as reticular when they appeared to be only partially stained, and fibril when stained in its entirety.

For fibronectin protein, the analysis was performed by using the following criteria: presence of protein underlying the epithelial basement membrane and distribution in the papillary dermis in the focal and dispersed patterns; percentage of wound covering (score 1: up to 30%, score 2: 31 to 60%, and score 3: > 60% of coverage) and pattern of the fibers (reticular, fibril, or mixed). For this protein, the animals were also considered for three days.

Analysis of laminin was made according to its location in the subepithelial basement membrane or vascular basement membrane. Criteria for both stainings: percentage for staining ($\leq$50% and > 50%), continuity (continuous and discontinuous), and thickness (thin, thick, or mixed).

2.6. Statistical Analysis

Data were compiled into an Excel™ (Microsoft) Spreadsheet and then transferred to the Graph Pad Prism Version 5.0, Software Inc. (La Jolla, California, USA). For comparison and assessment of statistical differences among the experimental groups, the Fisher's non-parametric test was applied considering the value of $p < 0.05$.

3. Results

In the morphological analysis, acute, dense inflammatory infiltrate was present in the Control Group, in addition to an edema within 24 h. As early as seven days, the specimens showed complete wound healing, with no inflammatory infiltrate and it has been noted an increase in collagen fibers within seven to 14 days. Group I has shown within 24 h dense, acute inflammatory infiltrate, so remaining until the seventh day