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Biomodulative Effects of Polarized Light on the Healing of Cutaneous Wounds on Nourished and Undernourished Wistar Rats

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ABSTRACT

Objective: **This study aimed to evaluate, by light microcopy, the differences in healing process of cutaneous wounds on nourished or undernourished rats following illumination by polarized light (λ400–2000 nm) with 20 or 40 J/cm². *Background Data:* There are some reports in the literature on different effects of polarized light on wound healing. Amongst the factors that interfere with wound healing one is the nutritional status of the subject. *Methods:* Thirty nourished or undernourished Wistar rats had one standardized surgical wound created on the dorsum and were divided into six groups: group 1, control (standard diet); group 2, control (Northeastern Brazilian Basic Diet [DBR]); group 3, standard diet + polarized light (20 J/cm²); group 4, standard diet + polarized light (40 J/cm²); group 5, DBR + polarized light (20 J/cm²); group 6, DBR + polarized light (40 J/cm²). The first application of treatment was carried out immediately after wounding and repeated every 24 h during 7 days. The animals were sacrificed, and specimens were taken and routinely processed to wax, cut, and stain with hematoxylin and eosin (H&E) and Sirius Red. These were then analyzed under light microscopy. The analysis included re-epitheliialization, inflammatory infiltrate, and fibroblastic proliferation. Sirius Red–stained slides were used to perform descriptive analysis of collagen. *Results:* The analysis of the results showed better results in these groups illuminated with 20 J/cm². *Conclusion:* It is concluded that nutritional status influenced the progression of the healing process as well as the quality of the healed tissue, and that the use of polarized light resulted in a positive biomodulatory effect.**

INTRODUCTION

Awareness of the mechanisms involved in wound healing is very important for the understanding of the possible effects of therapeutic methods that may help the process. The healing of skin wounds requires the participation of several cell lineages, and each one of these cells may behave differently based on the therapeutic used.

Systemic conditions such as undernourishment have been shown to negatively influence the healing process at all stages. A previous report showed that protein deficiency delays the contraction of the wound and modifies the morphologic substrate necessary for repair; introduction of a balanced diet restores the natural responses of the organism.

Nowadays there is a tendency to use therapeutic agents that positively impact repair of a wound. One of the therapies recommended is the use of a light source, which has been used as a therapeutic agent since ancient times. However, the use of different light sources and protocols has pointed out the need for assessment of efficacy. Polarized light uses wavelengths

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similar to those produced by sunlight. These light sources are not considered a "cure"; rather, they trigger or regulate biological processes, thus aiding in healing. [2]

A wound is the interruption (anatomical or functional) of the continuity of tissue which is followed by damage or cellular death. Wound healing occurs, due to a competitive mechanism between the syntheses and lyses of collagen. Any factor that increases the lyses or reduces the syntheses of collagen may result in changes in the healing process.[3]

Factors that affect healing may be divided into systemic or local. Both of these kinds of factors influence the inflammatory response.[4] Nutritional deficiencies have a great effect on wound healing, changing tissue regeneration, the inflammatory reaction, and immunologic function, interfering at any point of the healing process.[5] It has been shown that delay of healing may occur in subjects with a deficiency of any one of the essential nutrients.[6,7] However, this delay is reversed by the introduction of a diet with appropriate levels of nutrients.[1]

The present work aimed to evaluate, histologically, the differences in healing of cutaneous wounds in nourished or undernourished rats fed with the Northeastern Brazilian Basic Diet (DBR) following illumination with polarized light (λ400–2000 nm) using doses of 20 or 40 J/cm².

METHODS

Thirty male and female Wistar rats, average of 21-days-old, were divided into two groups: in Group I, the animals were fed with standard pelted laboratory diet (Labina®; Purina Nutrimentos, São Paulo, Brazil); in Group II, animals were fed the Northeastern Brazilian basic diet (DBR; Department of Nutrition, Federal University of Pernambuco (UFPE), Recife, Brazil) during 30 days in order to induce undernourishment. The procedures for making the DBR were carried out at the Laboratory of Experimental Nutrition, Department of Nutrition, UFPE (*Phaseolus vulgaris* = 37.1g%; salted meat and dried meat = 13.9g%; *Iponaea potatoes* = 32.0%; *Manihot esculenta* = 67.4%; proteins = 7.88g%; carbohydrates = 69.96 g%; fat = 0.60g%; ashes = 1.27g%; and fibers = 7.70g%).

Surgical procedures were carried out at the Laboratory of Animal Experimentation, School of Dentistry, Federal University of Bahia (Salvador, Brazil). The animals, average of 2 months old, were submitted to general intraperitoneal anesthesia (Zoletil50®; 0.1 mL/1000 g) and had their back shaven and one standardized excisional wound measuring 1 × 1 cm created on the dorsum of each animal with a scalpel. No sutures were performed.

The animals were divided then into six subgroups as follows (Table 1): group 1, control (standard diet; $n = 5$); group 2, control (DBR; $n = 5$); group 3, standard diet + polarized light (λ400–2000 nm; 20 J/cm², $n = 5$); group 4, standard diet + polarized light (λ400–2000 nm; 40 J/cm², $n = 5$); group 5, DBR + polarized light (λ400–2000 nm 20 J/cm², $n = 5$); group 6, DBR + polarized light (λ400–2000 nm; 40 J/cm², $n = 5$).

When appropriate, the wounds were illuminated with a polarized light source (Bioptron®; λ400–2000 nm; 20 or 40 J/cm²; 40 mW; 2.4 J/cm² per minute; ϕ ± 5.5 cm; Bioptron AG, Monchaltorf, Switzerland). The illumination was carried out using a specially devised device in order to obtain better concentration of the light of the Bioptron® on the area to be treated. The focal distance was kept at 10 cm. For all the experimental groups, the first application of the treatment was carried out immediately after the surgical procedure and repeated every 24 h during the experimental period of 7 days according to recommendations of the manufacturer. Euthanasia was performed on postoperative day 8.

Specimens were taken and processed to wax, cut, and stain with hematoxylin and eosin (H&E) and Sirius Red. These were then analyzed under light microscopy by two experienced pathologists. The analysis included re-epithelialization, inflammatory infiltrate, and fibroblastic proliferation. Sirius Red–stained slides were used to perform descriptive analysis of the collagen deposition.

RESULTS

Undernourished

Controls. In undernourished subjects (untreated controls), ulcerations covered by crusts of variable thickness were observed (Fig. 1). Underlying this area, an extensive area of granulation tissue containing hyperemic neo-capillaries was observed (Fig. 2); a moderate number of young fibroblasts and

TABLE 1. PARAMETERS USED FOR IRRADIATION OF THE EXPERIMENTAL GROUPS

Groups	*Wavelength (λ, nm)*	*Focus diameter (ϕ, cm)*	*Intensity (mW)*	*Dose per session (J/cm²)*	*Treatment dose (J/cm²)*
Control					
Nourished	—	—	—	None	None
Unnourished	—	—	—	None	None
Polarized Light					
Nourished	400–2000	±5.5	40	20	140
Nourished	400–2000	±5.5	40	40	280
Unnourished	400–2000	±5.5	40	20	140
Unnourished	400–2000	±5.5	40	40	280

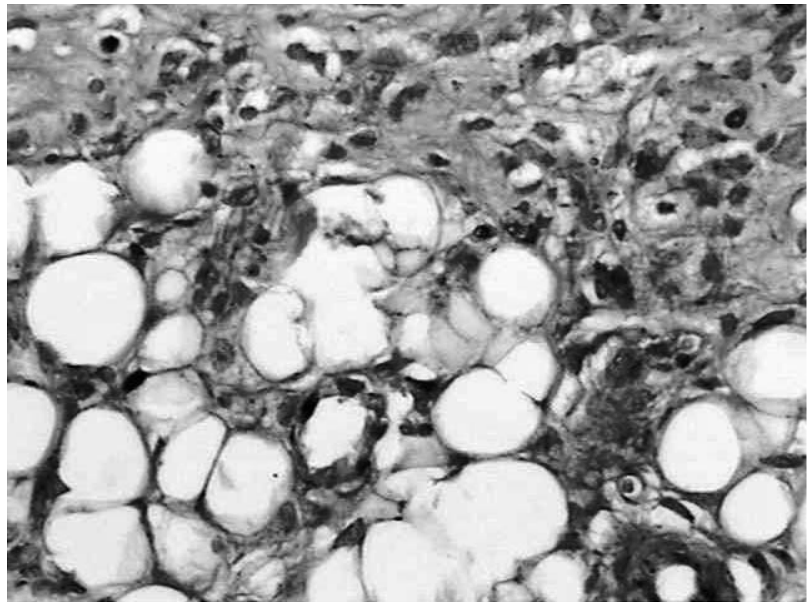

FIG. 1. Photomicrography of the dermis showing extensive area of granulation tissue evidencing the presence of mostly congested blood vessels, fusiform and/or oval young fibroblasts, and mixed inflammatory infiltrate and collagen fibers. H&E, approximately ×100.

intense mixed inflammatory infiltrate were dispersed in large amounts on an irregularly distributed collagen matrix in maturation, as evidenced by Sirius Red staining. In one of the specimens, the collagen fibers were not remarkably positive by Sirius Red staining, demonstrating that the tissue was quite immature. Granulation tissue reached the hypodermis. In two other specimens, areas of hemorrhagic exudate were observed. Most specimens showed areas of necrosis, and in a few, interstitial edema was present. On most of the specimens, immature multinucleated adipocytes were seen deeper into the wound (Fig. 3).

Polarized light. When illuminated with 20 J/cm,^{2} wounds exhibited a surface covered by epithelium, usually keratinized, showing acanthosis and inter-papillary atrophies, and an absence of skin appendices and cytoplasmatic vacuolization. Epithelial "pavementing" (or margination) was observed in most specimens, and this was covered by a crust. Underlying this area, a dense granulation tissue was observed, which contained young fibroblasts parallel to the surface, as well as neocapillaries and moderate inflammatory infiltrate (predominantly mononuclear) extending sometimes to the hypodermis (Fig. 4). The granulation tissue showed a more discreet number of fibroblasts, and collagen matrix was more organized. One case showed extensive ulceration filled in by granulation tissue, a moderate number of young fibroblasts, hyperemic and delicate neo-capillaries (Fig. 5), discrete to intense mixed lymphoplasmocitary inflammatory infiltrate, and intense interstitial edema. In this case, the collagen matrix was disorganized, but mature. In another three cases, cellular elements were dispersed in a collagen matrix, and were a little more organized and mature. In one case, the collagen deposit was very organized and in an advanced phase of maturation. All specimens

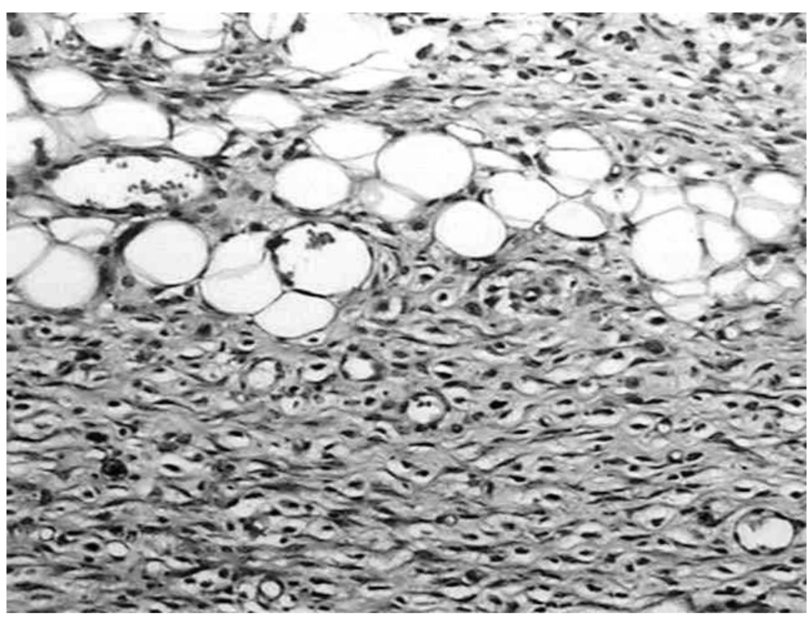

FIG. 2. Photomicrography of the dermis showing intense collagen deposition in maturation and organization. The presence of mature collagen fibers on the dermis adjacent to the wounded site can be seen. H&E, approximately ×100.

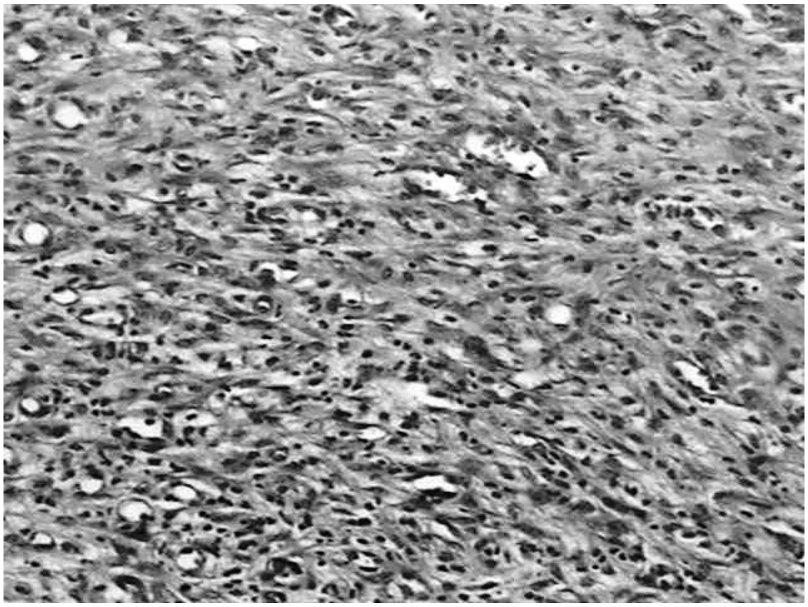

FIG. 3. Photomicrography of the wound showing extensive ulceration covered by a crust of varied length, the presence of granulation tissue rich in congested newly formed blood vessels, fibroblasts, and leukocytes down into the wound. H&E, approximately ×40.

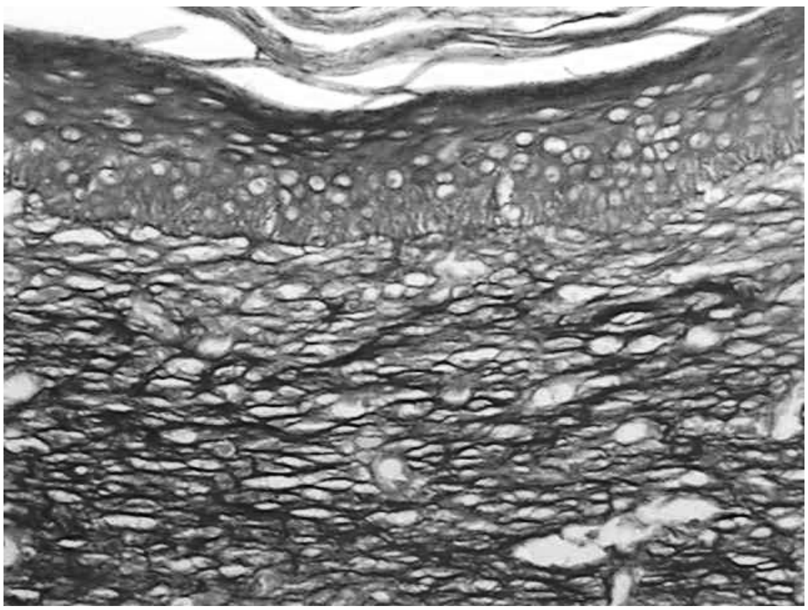

FIG. 4. Photomicrography of the dermis showing large numbers of parallel fusiform fibroblasts, newly formed and mostly congested blood vessel sprouts, and collagen matrix. Rare inflammatory cells can be seen. H&E, approximately ×200.

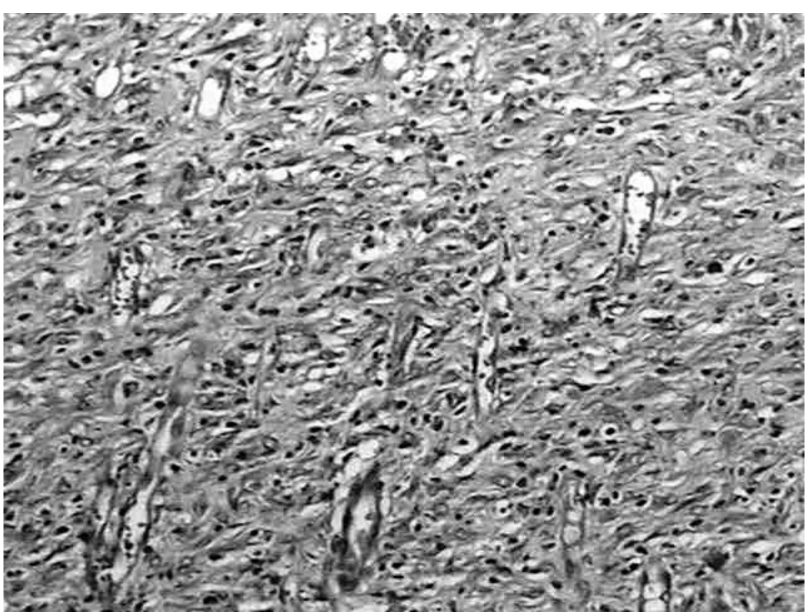

FIG. 5. Photomicrography of the wound completely covered by epithelium showing irregular acanthosis, large amounts of granulation tissue rich in blood vessels, fibroblasts, and moderate amount of mixed inflammatory infiltrate. H&E, approximately ×100.

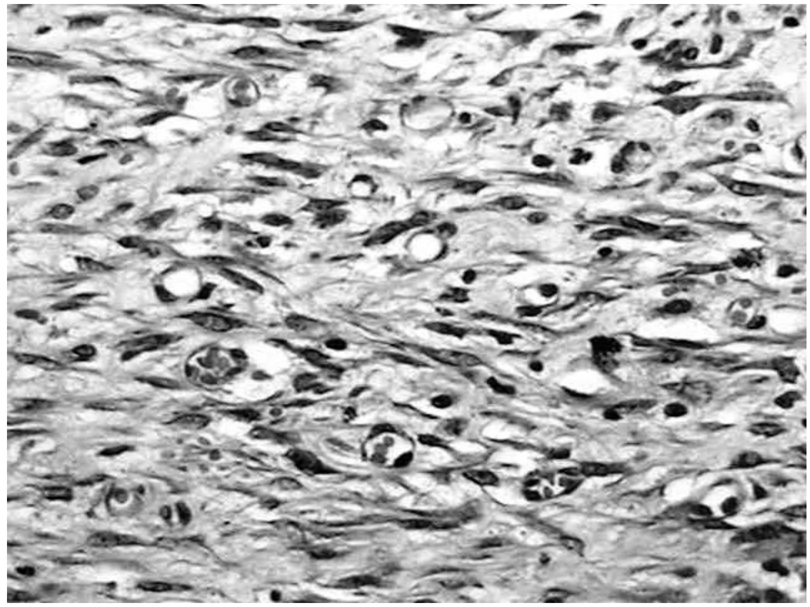

FIG. 6. Photomicrography showing the dermis replaced by granulation tissue and the presence of young fibroblasts, newly formed blood vessels, and discrete neutrophilic inflammatory infiltrate. Collagen deposition is more evident at the wound surface. H&E, approximately ×100.

showed the presence of multi-vacuolated adipocytes, either focused or unevenly distributed on the hypodermis. Raising the dose to 40 J/cm^2 resulted in a keratinized epithelium on all wounds, covered by a crust sometimes exhibiting pseudoepitheliomatous hyperplasia. Underlying this area, a dense granulation tissue was seen, which contained young fibroblasts parallel to the surface, neo-capillaries, and moderate to intense mononuclear inflammatory infiltrate. No hyperemia was found. These cellular elements were distributed in an organized collagen matrix in advanced maturation. Adipocytes were seen in fewer specimens.

Nourished

Controls. Animals fed with standard diet showed, at the end of the experimental period, wounds partially covered by typical epithelium and a lack of skin appendices. All specimens showed, superficially, an extensive area of eosinophilic coagulum, here referred to as "crust." Subjacent to the epithelium, granulation tissue was seen, which contained young fibroblasts parallel to the surface and producing a collagen matrix (Fig. 6). Blood vessel sprouts (usually hyperemic and containing neutrophils), and a moderate mixed and diffuse inflammatory infiltrate were also observed. Those cellular elements were distributed among bundles of mature collagen fibers regularly disposed, as evidenced by Sirius Red stain (Fig. 7).

Polarized light. When illuminated by polarized light at 20 J/cm^2, specimens showed ulceration covered by a thick crust. Underlying this area, granulation tissue showed a large amount of blood vessels and hyperemic neo-capillaries, a large number of young fibroblasts parallel to the surface, and a discrete or

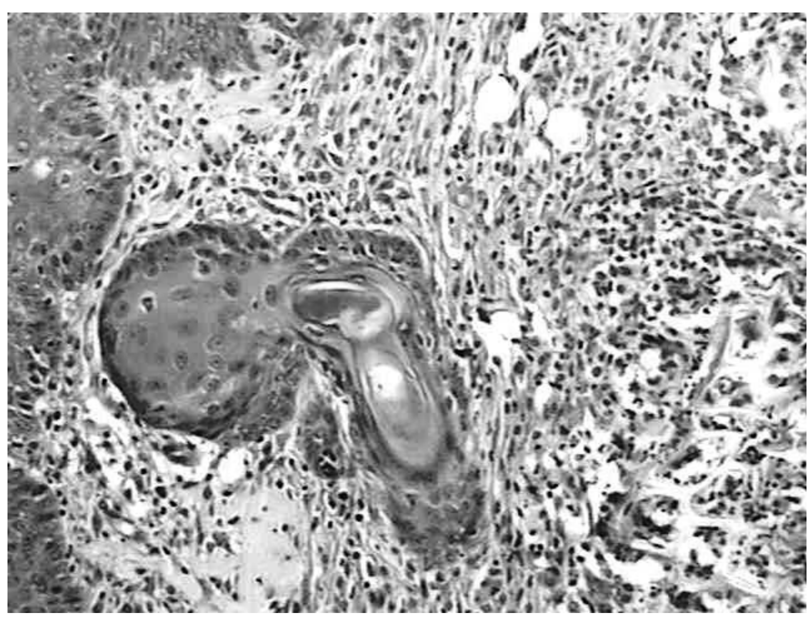

FIG. 7. Photomicrography showing the wound covered by epithelium, which is supported by connective tissue rich in collagen fibers that parallel are distributed in relation to the wound surface. H&E, approximately ×100.

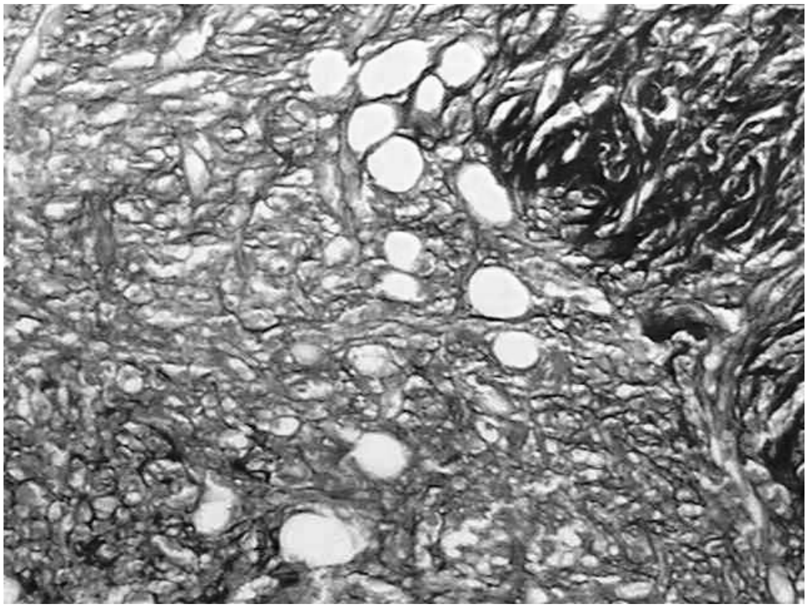

FIG. 8. Photomicrography of the deeper part of the dermis showing large amounts of young fibroblasts distributed in a mostly delicate collagen mesh of fibers invading the fatty layer. H&E, approximately ×200.

moderate mixed inflammatory infiltrate that sometimes distended the fatty layer. No edema was seen. All these elements were dispersed in a more organized and more mature collagen matrix than the groups previously described (Fig. 8). Adipocytes were seen at the wound surface in fewer specimens. Raising the dose to 40 J/cm^2 resulted in an area of ulceration covered by a crust of variable thickness. In one specimen, epithelial pavementing was observed in almost the entire wounded site. Underlying the area of ulceration, an extensive area of granulation tissue was seen, which contained neo-capillaries, young fibroblasts, hyperemic blood vessels, and moderate mixed inflammatory infiltrate dispersed irregularly and distributed in a collagen matrix discretely marked by Sirius Red staining. This aspect probably represented an intermediate phase of maturation (Fig. 9). Edema was seen in one specimen. The granulation tissue distended the fatty layer of the hypodermis. In other areas, free from inflammation, tissue showed vacuolar degeneration.

DISCUSSION

Nutritional deficiencies have a significant effect on wound healing (altering the regeneration of the tissue); the inflammatory reaction; and immunological function. In other words, such deficiencies can interfere at any point of the healing process.[5] Malnutrition will result in major changes in the process of protein synthesis of the scar and will stimulate a larger lysis of collagen.[4] Changes in the diet are necessary following extensive surgical procedures in the oral cavity due to surgical

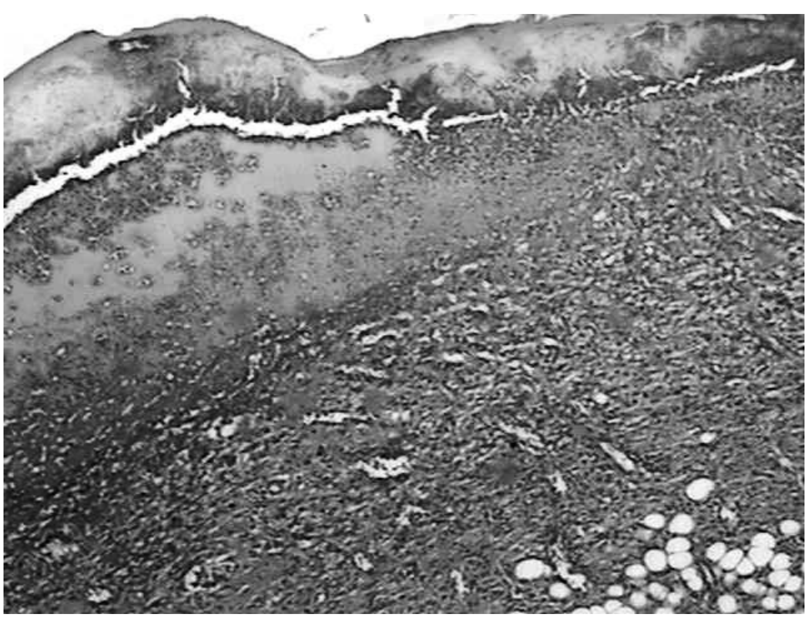

FIG. 9. Photomicrography of the deep part of the dermis showing fusiform fibroblasts, matrix rich in parallel collagen fibers, blood vessel sprouts, and a discrete mixed inflammatory infiltrate invading a rich fatty layer. H&E, approximately ×100.

trauma. When impairment of the oral function is intense, it can be necessary to use parenteral feeding. The use of dietary supplements is not always necessary, however, after small surgeries if the patient has an appropriate diet.[8]

A considerable reduction in levels of nutrients, especially proteins, is often observed in patients suffering from severe trauma or consumptives diseases, which may lead to deficiencies in collagen synthesis.[4] A delay in differentiation and proliferation of fibroblasts at wound sites is also observed. On the other hand, in patients with a diet rich in proteins faster healing of wound is found.[4,9]

Due to the effects of malnutrition on tissue healing, the present study used an animal model to simulate severe undernourishment and to assess the effect of phototherapies on wound healing. The use of light to stimulate wound healing is still very controversial in the literature. However, most authors agrees that laser therapy possesses biomodulatoy effect in wound healing, improving scar formation and accelerating the process.[10]

Comparison of the present results with those of other reports is not easy, as very few studies on this topic have been carried out. However, Galvão et al.[1] used the same undernourishment model to study the healing process in the rat.

The DBR diet is a model, developed in Brazil, to induce severe malnutrition, or marasmus, which is still a common disease affecting lower class populations living in deprivation. The disease is one of three forms of serious protein-energy malnutrition and can be considered an adaptation to insufficient energy intake. Marasmus results from a negative energy balance. This imbalance can result from a decreased energy intake, increased energy expenditure, or both (such as that observed in acute or chronic disease, where sufferers adapt to an energy deficit with a decrease in physical activity, lethargy, a decrease in basal energy metabolism, slowing of growth, and finally weight loss).

Most studies on the effects of phototherapies on the healing process have attributed the observed effects to several treatment parameters and properties of the light source used. Monochromaticity is one of the properties of laser light that has been suggested as an important factor in the final results of treatment. But it seems that it is not the main factor, as previous studies pointed out positive biomodulatory effects using different wavelengths.[11–13]

Karu et al.[11] suggested that coherence is not important for photobiological effects, because both coherent and incoherent light have been shown effective. Belkin and Schwartz[14] suggested that coherent light is not necessary, as most biomodulatory effects are obtained with the use of non-coherent light at an appropriate wavelength.

Many previous studies have investigated the effect of coherence on the biological effects of phototherapies. Studies using cells in cultures showed no difference in biological response between cultures treated with laser light (i.e., coherent light) and those treated with a non-coherent light source. Additionally, there has been no elucidation of the behavior of coherence as light passes through tissues.[15]

Polarization, however, is neglected in most of reports of laser therapy.[24] This is why this form of phototherapy was used in the present investigation. Polarization of light causes brighter random intensity gradients, which can enhance the effect of light coherence when tissue is illuminated. On scattering tissues, coherence length plays the main role, as this parameter determines the depth of tissue where coherent properties of light can be potentially manifested. This manifestation is dependent on attenuation, that is, spatial coherence. This may explain why coherence is not important for biological response on cell monolayers, on thin layers and cell suspensions, and on tissue surface.[16–18] But the effect of coherence can be observed deeper in the tissues, so absorption of low-intensity light by biological tissue is purely of a non-coherent nature.[16–18] The effect of light therapy, therefore, is related to electric and magnetic polarization, but not to wavelength.[19]

It is well known that light polarization remains unchanged through a thin layer of cells, but that polarization is lost after a penetration of approximately 1 mm. Optical penetration in skin is affected by the strong scattering produced (mainly) by collagen fibers.[20] Linear polarization can be preserved, without the complete loss of polarization, up to 1.2 mm in normal human skin.[21]

Incoherent light, emitting polarized light, is able to induce biostimulative effects on living cells similar to low-level lasers. The Bioptron® lamp combines visible light at λ480–700 nm and infrared light at λ700–2000 nm, and it is a low-power light source (similar to the low-level laser), but it is polychromatic and incoherent. Several mechanisms are responsible for the photobiostimulating effects of the electromagnetic spectrum present in this polychromatic light source. These lead to the same final photoresponse, but start the cascade of metabolic events at different cellular levels.

Several studies have demonstrated that polarized light has a significantly beneficial effect on wound healing due to faster epithelialization, less exudation, improved quality of early scar tissue formation, quicker wound closure, and increased tensile strength. Polarized light was also found to trigger human cellular and humoral defenses, and increase the release of growth factors, cytokines, and collagen synthesis.[31–38] The use of polarized light may also affect local peripheral vasodilatation, which may enhance the blood flow of skin and the delivery of oxygen to the wounded area, facilitating the transport of nutrients to the site.[22–30]

With regard to the protocol used in this study, illumination was performed immediately after surgery and at 24-h intervals for 7 days as recommended by the manufacturer. The macroscopic analysis of wound healing in subjects fed either diet is aligned with a previous report found in the literature.[1] During the removal of specimens, it was evident that, in undernourished animals, the wound was fragile and showed a tendency to dehiscence, which was different from wounds in regularly fed and illuminated animals. This clear weakness of the wound is due to a poorer quality of the tissue in undernourished animals.

Analysis of nourished animals at a dose of 20 J/cm^2 showed that epithelialization was incomplete in most cases. In undernourished animals illuminated with polarized light at a dose of 20 J/cm^2, no complete epithelialization was detectable at the end of the experimental time, but a more advanced epithelial pavementing was observed with illumination at a dose of 40 J/cm^2.

When analyzing the inflammatory infiltrate, nourished controls presented moderate chronic inflammation; undernourished ones presented moderate inflammatory mixed infiltrate.

Medeiros and Freire[31] suggested that animals on low-protein diet show unfavorable disturbances in wound contraction and inflammation due to low levels of proteins and attributed to nutritional status an important role in the closing of open wounds. Results on our present study also indicate that protein deficiency had a negative effect on the evolution of the inflammatory response. When analyzing the experimental groups, a positive influence of phototherapy is evident, as nourished animals illuminated with polarized light showed an inflammatory infiltrate that was moderate and chronic, resulting in a faster resolution of inflammation.

In the present study fibroblasts proliferated in all groups. The use of appropriate doses, wavelengths, potency, density, and time of exposure positively influenced fibroblastic proliferation and the production of collagen. When illumination was used at a dose of 20 J/cm^2 on nourished or undernourished subjects, treatment was most effective. In all cases, collagen fibers were well organized and heavily marked by Sirius Red stain, which represents an increased production of collagen fibers and better organization of the healing tissue. Nourished subjects illuminated with 40 J/cm^2 showed a more regular organization and weaker marking by Sirius Red stain, which indicates that treatment was more effective when the tissue was deficient in some way.

Differences in collagen at the healing wound were found between the control groups. Nourished subjects presented a more organized pattern of collagen fibers than undernourished animals, suggesting earlier maturation and larger deposition of fibers. This result is in agreement with a previous study[4] which indicated that nutritional deficiencies have a major effect on wound healing, by altering regeneration of tissue, inflammatory reaction, and immunological function. Corsi et al.[4] suggested that malnutrition would cause alterations in synthesis of proteins at the scar, as well as stimulate a larger lysis of collagen.

Better results were found in the nourished and undernourished groups illuminated with polarized light at a dose of 20 J/cm^2. This group showed collagen fibers parallel to the surface, and these were strongly marked by Sirius Red stain, which means that there was a larger deposition of collagen fibers and a better organization of tissue. In nourished animals illuminated at a dose of 40 J/cm^2, an irregular organization of fibers was seen. Despite being strongly marked, the fibers did not present a good organization.

In conclusion, the polarized and incoherent light of the Bioptron® had a positive effect on wound healing, which was more evident on nourished than undernourished subjects. Nutritional status influenced the progression of healing as well as the quality of the healed tissue. The use of the polarized light resulted in a positive biomodulatory effect, most evident in nourished subjects at a dose of 20 J/cm^2.

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