Comparison of linear polarization degree in health and wounded rat skin

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ABSTRACT

Low-intensity laser therapy (LILT) with adequate wavelength, intensity, and dose can accelerate tissue repair. However, there is still disperse information about light characteristics. Several works indicate that laser polarization plays an important role on the wound healing process. This study was conducted to verify the degree of linear polarization in normal and pathological rat skin samples. Artificial burns about 6 mm in diameter were created with liquid N_2 on the back of the animals. The degree of polarization was measured in normal and pathological skin samples. It was verified that linearly polarized light can survive in the superficial layers of skin and it can be more preserved in skin under pathological condition when compared with health skin. The present study supports the hypothesis that polarized laser radiation can be used to treat open wounds and improve the healing.

Keywords: low intensity laser therapy, linear polarization degree, wound healing

1. INTRODUCTION

One of the most interesting and controversial therapy is the low intensity laser therapy, largely used in clinical treatment of various pathological conditions such as wound healing and the control of pain and inflammation. Low intensity laser radiation produces increase in temperature less than 0.5 °C, so the effects are not thermal. The most used lasers are He-Ne, GaAs and GaAsAl. The physical model and the biological reasons for the effectiveness in laser treatment have not made clear. According to Karu[1], using monochromatic red to near infrared radiation, the cytochrome c oxidase is a possible photoacceptor, which starts the cascade of metabolic events at the level of the respiratory chain of the mitochondria through photochemical events. However, Smith proposed that infrared radiation starts that cascade by photophysical effects on the membranes[2]. Despite the widespread clinical application of low intensity laser therapy, there are still little information about dependence of the effect on the irradiation dose, wavelength, regime and intensity, and the exact mechanism of photobiomodulation has not yet been established. Furthermore, knowledge of the optical parameters of the tissue is important for all kinds of phototherapy.

It is well known that the polarization is lost in a turbid medium, such as living tissue. However, earlier results obtained in our laboratory showed that polarized laser light plays an important role in order to hasten the healing of lesions created by the application of liquid nitrogen over the skin of rats when compared to lesions irradiated with non-polarized laser light[3]. More recently, we demonstrated that the polarization component of the electrical field is an important factor in the healing process of inflammatory lesions created in the end of the spinal column of Lewis rats when exposed by He-Ne laser[4]. Since studies revealed that human granulation tissue varied in many aspects in comparison with normal skin[5], the current study sought to measure the degree of linear polarization in normal and wounded skin samples, and thus, to verify if polarization can contribute on cutaneous wound healing acceleration.

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2. MATERIAL AND METHODS

Male adult Lewis rats of similar ages and weights were used in this study. The animals were anaesthetized by ether inhalation and had the back shaved. Burns measuring about 6 mm in diameter were inflicted on the end of the spinal column of each animal using a cylindrical brass rod cooled to -196°C. The contact was kept for five seconds. The application was made twice a day with an interval of five minutes for a total of three days.

Discs of skin about 8mm in diameter were removed and mounted in a metal holder. Afterwards, they were frozen in isopentane and maintained at liquid nitrogen (-196°C). Thin sections of health and burned tissue were cut parallel to the skin surface in the desired thickness of 10µm, 20µm, 30µm, 40µm and 50µm using a HM 500 OM freezing microtome (MICRON LABORGERÄTE GmbH, Germany) at -21°C and fixed in glass slides at room temperature. The samples were stored at -5°C and taken to room temperature two hours before the measurements. Later, the glass slides were stained with toluidine blue for morphological analysis of the specimens. The morphological analysis was accomplished using a Nikon Labophot AFX-II light microscope.

In order to measure the degree of linear polarization of the samples, we used a He-Ne laser (UNIPHASE, USA) mounted in a convenient set up, with wavelength of λ= 632.8 nm, 10 mW of output power and beam diameter about 2 mm. The emission from the probe was modified to ensure an uniform irradiation: to get linearly polarized light a Glan-Thompson polarizing prism with a precision disk as a holder to rotate it in 90°, a convergent lens (f=7cm), and a neutral density filter 0.04 for λ= 632.8nm were used. At last, an objective was used with f= 5cm and ratio 2:1 to obtain an expanded beam of 6mm. An additional 10GT04AR.14 Glan-Thompson polarizer (NEWPORT, USA) was placed on the optical axis. The samples were inserted between the light source and the second polarizer. Light emerging from the sample was collected by LM-1 detector probe (COHERENT, USA) through the second polarizing element.

Measurements were made for incident polarization orientations ranging from 0 to 90° with respect to the optical axis, in 15° increments.

3. RESULTS

Degree of polarization is a term used to quantify the amount of light that is polarized relative to the total amount of light that is both polarized and unpolarized. A degree of polarization value equal to 1 corresponds to completely polarized light, whereas a degree of polarization value equal to 0 corresponds to completely unpolarized light[6].

The degree of linear polarization was measured by placing the sample between the two linear polarizers and measuring the transmitted light intensity in two conditions: I_{max} was taken with the polarizers in parallel and I_{min} was taken with the polarizers in orthogonal. The degree of linear polarization is then:

$$P_L = \left| \frac{I_{max} - I_{min}}{I_{max} + I_{min}} \right|$$

Figure 1 shows the degree of linear polarization in health and burned rat skin.

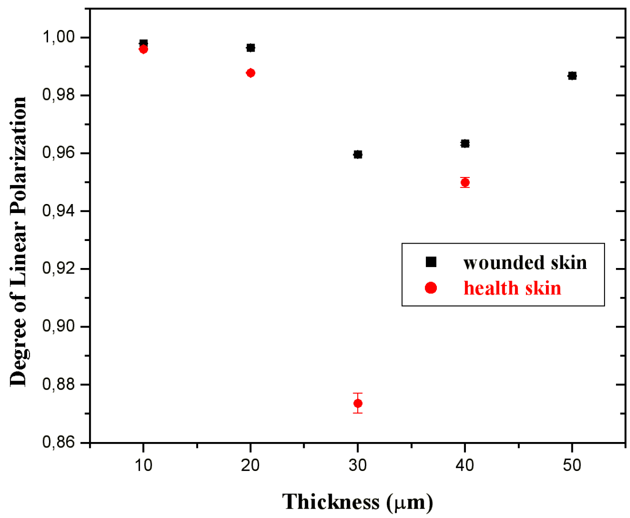

Figure 1: Degree of linear polarization for health and burned skin.

We can observe from figure 1 that the linear polarization is more preserved in skin under pathological condition when compared with health skin. Also, it is possible to note that the degree of linear polarization decreased at 30μm and increased again at 40μm. The morphological analysis of the specimens showed that the epidermis-dermis interface is found at 30μm. The normal rat epidermis is typically about 40-50μm in thickness.

4. DISCUSSION

The He-Ne laser and semiconductor laser can be used for biostimulation in the therapy of trophic ulcers healing, pain management, dental diseases, or acupuncture. For all these applications, accurate information about the space distribution of light in normal skin and pathologically changed skin is required. Advancement of phototherapy is based on greater knowledge of the optical properties of the tissue and thus the possibility to influence them.

Literature is still scarce about the influence of laser-induced biomodulation of skin wound repair with respect to polarization. It is well known that the light polarization remains unchanged through a thin layer of cells, however, if we illuminate a highly scattering medium, such as living tissue, the polarization is lost after a penetration of a millimeter or so. The optical penetration in skin is affected by the strong scattering produced principally by collagen fibers[7], and linearly polarized incident on the skin will be rapidly depolarized by dermal collagen. However, the epidermis and initial papillary dermis apparently allow penetration of linearly polarized light with modest depolarization[8]. Sankaran and collaborators concluded that linearly polarized light preserves its propagation properties through longer penetration depths than circularly polarized light in biological tissues at λ= 632,8 nm[6]. In fact, linear polarization can be preserved over 2,5 transport paths in the red and near infrared wavelength ranges. Therefore, light can travel a distance of 1,2 mm in the human normal skin without the complete loss of linear polarization[9].

Our previous histological analysis of burns showed that the injured area was devoid of epidermis. In the subjacent dermis was observed a variety of inflammatory cells and cell debris. Neutrophils were the predominating cells although monocytes, macrophages, and giant cells were also present . The blood vessels were dilated and most of them fulfilled with blood cells[4]. Wound healing is a controlled biological process involving a series of complex cellular interactions. Following inflammation, the wound matrix is gradually replaced by granulation tissue followed by a long process where collagen accumulates and restores tensile strength[5]. Transmittance in granular tissue was about 2,5 times higher than that in normal skin according to Kolárová et al.[10]

In the present study the degree of linear polarization in health and wounded skin was investigated. Our results indicate that linearly polarized light can survive in the superficial layers of skin, and thus, to contribute on cutaneous wound healing acceleration. Furthermore, it can be more preserved in skin under pathological condition, like a burn, when compared with health skin.

Ripley et al. observed that the optical properties of fibroid were found to be lower than myometrium, and this was attributed to the differences in both anatomy and vascularity[11]. For this reason, we could suggest that optical properties of normal and under pathological condition skin should be taken into account so that appropriate parameters of radiation can be selected for optimal dosimetry in low intensity laser therapy.

5. CONCLUSION

It was verified that linearly polarized light can survive in the superficial layers of skin. It can be more preserved in skin under pathological condition burn when compared with health skin. In this way, polarized light could be used to contribute on treatment of open wounds in clinical trials.

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