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Effects of 1047-nm Neodymium Laser Radiation on Skin Wound Healing

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ABSTRACT

Previous research in our laboratory has shown that the polarization component of the electrical field plays an important role on the healing process of inflammatory lesions created in the end of the spinal column of Lewis rats, using a He-Ne laser at $\lambda = 632.8$ nm. It is well known that polarization is lost in a turbid medium, such as living tissue. However, the Nd:YLF wavelength ($\lambda = 1{,}047$ nm) allows more polarization preservation than $\lambda = 632.8$ nm, and the Nd:YLF laser beam has been used in clinical trials as a biostimulating agent. In this work, we investigated the influence of a low-intensity, linearly polarized Nd:YLF laser beam on skin wound healing, considering two orthogonal directions of polarization. We have considered a preferential axis as the animals' spinal column, and we aligned the linear laser polarization first parallel, then perpendicular to this direction. Burns of about 6 mm in diameter were created with liquid N_2 on the back of the animals, and the lesions were irradiated on days 3, 7, 10, and 14 postwounding, D = 1.0 J/cm². Lesions 1 and 2 were illuminated using Nd:YLF pulsed laser radiation. Lesion 1 was irradiated with linear polarization parallel with the rat spinal column. Lesion 2 was irradiated using the same protocol, but the light polarization was aligned with the perpendicular relative orientation. Control lesions were not irradiated. We have taken photographs from the wound areas on the 3rd, 7th, 10th, 14th, and 17th postoperative day for a biometrical analysis. The results have shown that lesion 1 healed faster than the control lesions ($p < 0{,}05$), which presented a smaller degree of healing after 14 days postwounding.

INTRODUCTION

Low-intensity laser therapy (LILT) has been used in many experiments since the 1960s to examine the influence of laser radiation on the healing process of wounds. Lasers used in wound healing have been shown to speed up the healing process in leg ulcers and burns wounds. Lasers used in this capacity have been demonstrated to improve skin healing capabilities.[5] However, despite a large number of studies published in the literature, results are frequently conflicting, and very few have presented scientific explanation.[1–4] In some studies, an increase in the wound healing rate of closure after LILT *in vivo*[6–11] has been observed while others found no change.[12–15] In those experiments, however, polarization effects were not considered. Nicola and collaborators[16] have suggested that coherence and polarization of laser light might play an important role in the process of wound repair, but the effect of the polarization components was not considered in that investigation. According to Maxwell's equations for the optical properties of surfaces, the efficiency of energy deposition in a microroughness interface depends on the electrical field polarization component.[17] Considering a linearly polarized beam, this efficiency will depend on the roughness parameters for p-polarized light and will not depend on such parameters for s-polarized light.

Previous research in our laboratory has shown that the polarization component of the electrical field plays an important role in the healing process of inflammatory lesions created in the end of the spinal column of Lewis rats, using a He-Ne laser at $\lambda = 632.8$ nm.[18] It is well known that polarization is lost in a turbid medium, such as living tissue. However, the neodymium laser wavelength ($\lambda = 1.047 \mu m$) allows more polarization preservation than $\lambda = 632.8$ nm,[19] and indeed this laser has been used in clinical trials as a biostimulating agent.[20] In this

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work, we have investigated the influence of a low-intensity, linearly polarized Nd:YLF laser beam on skin wound healing, considering two orthogonal directions of polarization.

MATERIALS AND METHODS

Animals

A group of 12 male adult Lewis rats weighing about 300 g were used in this experiment. The animals were anesthetized by ether inhalation. After anesthesia, the surgical site was shaved. Three round burns measuring about 6 mm in diameter were created aseptically at the end of the spinal column on the back of each animal during 3 consecutive days using a cylindrical brass rod cooled to 77 K. The contact was kept for 5 sec. The application was made twice a day with an interval of 5 min for a total of 3 days.[17] Animals were singly housed in solid-bottomed cages, and food and water were allowed on an *ad libitum* schedule.

Laser system

The study was performed using a homemade Nd:YLF multimode, partially coherent, pulsed laser beam with a wavelength of 1,047 nm, with repetition rate of 7 Hz, pulse duration of 300 μsec, and energy per pulse of 1.3 mJ. The laser was delivered directly to the skin. The output was measured continuously using an LM-P10i detector (Coherent, USA). The laser beam was aligned to cover the entire wound area, including the boundaries.

Treatment experimentation

Because of interindividual variability in regeneration, each experimental animal acted as its own control, and treated and untreated (control) burns were made in the same animal. After the last nitrogen application on skin, lesions 1 and 2 were illuminated using Nd:YLF pulsed laser radiation. The laser polarization was aligned with the rat spinal direction in lesion 1 and with the perpendicular relative orientation in lesion 2. Lesion 3 was not irradiated (control). The total dose per irradiation was about 1 J/cm^2 corresponding to an exposition time of 2 min. The lesions were irradiated on the 3rd, 7th, 10th, and 14th day postwounding. After each irradiation, we took photographs from the wound areas at a constant distance. The last pictures were taken on the 17th postoperative day. The wound diameter of all rats was measured from the pictures using a caliper.

Statistical analysis

The size reduction of control and irradiated lesions was calculated for days 3, 7, 10, 14, and 17 postwounding. The differences between control and treated groups were analyzed using the analysis of variance (ANOVA) test. Significance was accepted at $p < 0.05$.

RESULTS

Figure 1 exhibits the percentage of wound healing acceleration. The percentage of wound healing acceleration in size reduction relative to control was calculated following Al-Watban and Zhang.[9] On the 17th postoperative day, this percentage was 71% for lesion 1 and 12% for lesion 2. The changes of wound size for the three lesions during the entire experiment period can be seen in Figure 2. It shows a rapid decrease of the wound size in lesion 1 compared with lesions 2 and 3 (control). These results indicate that lesion 1 healed faster than other lesions, which presented a smaller degree of healing 17 days postwounding.

The results of statistical analysis revealed no significant differences in the size reduction of the wound between control and experimental groups for days 3 and 7. For the 10th, 14th, and 17th postoperative day, statistical analysis showed no significant differences between lesions 1 and 2 and between lesions 2 and 3; however, significant differences ($p < 0.05$) were observed between lesions 1 and 3 (Table 1).

DISCUSSION

Despite the large number of publications on the study of the action of the low-power light (LPL), the mechanism is still not clear. Lubart and collaborators reported that coherent irradiation

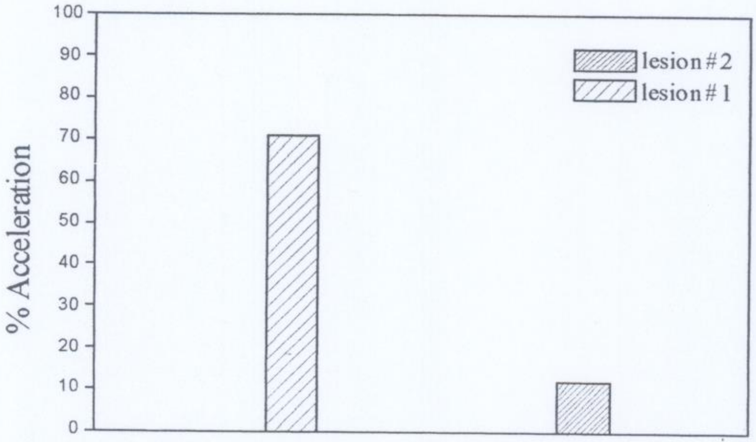

FIG. 1. Percentage of acceleration of lesion 1 and lesion 2 with respect to lesion 3 (control).

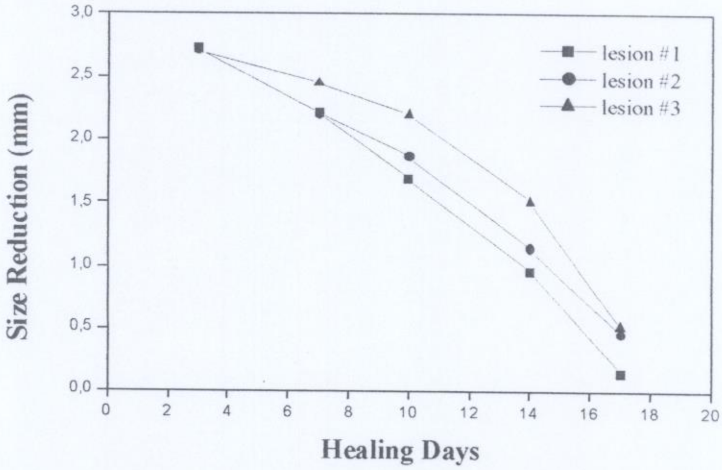

FIG. 2. Linear graph showing the changes of wound size for irradiated and control lesions.

is not essential on fibroblast proliferation.[21] Rigau et al. showed no increase in the number of fibroblasts following LPL exposure, but revealed significant changes in metabolic rates when compared with unirradiated controls.[22] Loevschall and A-Bindslev demonstrated an increased incorporation of tritiated thymidine in cultured human oral fibroblasts after LPL irradiation.[23] These experiments did not consider the polarization effects. Mester and co-authors were the first to compare the effects of monochromatic polarized and nonpolarized normal light with those of laser light with respect to the immunosuppressive effect of human lymphocytes. They found that the effect of incoherent light was 0.74% when compared to that of the laser. With plano-polarization of corresponding plane, an efficiency of 80% was achieved.[24] Bolton et al. reported that the proliferate response of fibroblasts was greatest in the cultures exposed to supernatants from macrophages treated with the 95% polarized light source when compared to 14% polarized light irradiation.[25]

It is well known that, if cell cultures are illuminated, the polarization remains unchanged through the thin layers of cells. However, if a turbid medium, highly scattering (such as living tissue), is irradiated, the polarization is lost after a penetration of a millimeter or so. 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 skin without the complete loss of linear polarization.[26] Recently, Vitkin and Studinski have suggested the important influences of medium optical properties on the polarization preservation in diffusive scattering from *in vivo* turbid biological media.[27]

Although nonpolarized and/or noncoherent light are made responsible for many biological effects, Nicola and collaborators showed that coherent and polarized light plays an important role in wound healing.[16] Another study demonstrated that the direction of He-Ne polarized light affects the healing process, particularly the formation of connective tissue of the dermis, consisting in an important factor for the healing acceleration of inflammatory lesions.[18]

In this work, we suggest that the polarization component of radiation is an essential healing factor for the wavelength of 1,047 nm. Our results indicated an accelerated healing process of inflammatory lesions created in the end of the spinal column of rats by low-intensity Nd:YLF polarized laser beam whose direction of polarization was aligned with the vertebral column of the animals. We propose that this electrical field component polarization plays an important role in skin wound repair.

Table 1. Statistical Analysis Results Using ANOVA Test

	Wound diameter (mm)		
Day	*Lesion 1*	*Lesion 2*	*Lesion 3*
3	2.67 ± 0.34	2.67 ± 0.34	2.70 ± 0.29
7	2.26 ± 0.36	2.24 ± 0.31	2.48 ± 0.31
10	1.75 ± 0.35*	1.94 ± 0.43	2.24 ± 0.39
14	0.96 ± 0.29*	1.12 ± 0.31	1.48 ± 0.30
17	0.19 ± 0.29*	0.46 ± 0.29	0.54 ± 0.17

*$p < 0.05$ vs. control (lesion 3).

CONCLUSION

LILT at the appropriate dosimetric parameters can speed up the healing process in burn wounds. The macroscopic analysis of skin wound healing in this study showed that lesions irradiated with laser linear polarization parallel with the rat spinal column healed faster than nonirradiated lesions on days 14 and 17 postwounding. Our investigation of the biostimulating effects of the Nd:YLF polarized laser beam enables us to conclude that the relative direction of the laser polarization plays an important role in the acceleration of wound healing in rats at $\lambda = 1,047$ nm.

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