

EFFECTS OF THE VISIBLE AND NEAR INFRARED POLARIZED LASER RADIATION ON SKIN WOUNDS REPAIR

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ABSTRACT

The effects of low-intensity linearly polarized visible (632.8 nm) and near infrared (797 nm) laser radiation were studied on healing of skin wounds created in the back of rats. It is shown that the relative orientation of the electric field has an essential role on the healing process, in the case of red light irradiation. On the other hand, no morphological difference was observed among the lesions irradiated by near infrared radiation with respect to the polarization orientation.

INTRODUCTION

The use of low intensity visible and near infrared laser radiation to stimulate wounds healing and tissue regeneration is a current field of research that rouses interest in specialists and the general public. In this case, the radiation intensity is so low that the resulting biological effects are due directly to the radiation-tissue interaction and not to the heat generation. Although this therapy is largely empirical in the clinical practice, several authors have reported beneficial effects on wound healing in animal models [1-3] as well as there is suggestive evidence of laser biostimulation in tissue culture [4-6]. Nicola and coworkers [2] showed that the coherence and polarization of laser light play important roles in wound repair, while Colver and Priestly suggested that no significant effects can be obtained by He-Ne low power irradiation on components of wound healing *in vitro* [7]. Despite of large number of studies published in the literature, results are some times conflicting, and only few has offered scientific support for these effects [8-10]. In this work it is reported an experiment to study the influence of linearly polarized, low intensity, He-Ne and GaAlAs laser radiation on the healing process of skin wounds artificially created in rats skin.

MATERIAL AND METHODS

We used a group of 20 male adult Lewis strain rats divided in two groups of 10 animals weighing about 300 grs. The source of light for the first group was a He-Ne laser (UNIPHASE, USA) mounted in a convenient set up, with wavelength of $\lambda = 632.8$ nm, 10 mW of output power and beam diameter about 2 mm. The emission from the probe was modified to ensure an uniform exposure of the wound by inserting optical components: a Glan-Thompson polarizing prism with a precision disk used as holder to rotate it in 90° and thus to get linearly polarized light; a convergent lens ($f = 7$ cm) and a neutral density filter 0.04 for $\lambda = 632.8$ nm. At last, an objective was used with $f = 5$ cm and ratio 2:1 to obtain an expanded beam of 6 mm. The source of light used in the second group of animals was a GaAlAs diode-laser (SDL 2382) which was operated at $\lambda = 797$ nm, and its 99.9 % linearly polarized output beam was adjusted to an intensity of 3 mW/cm^2 .

The animals were anaesthetized by ether inhalation and had the back shaved. Three round burnings measuring about 6 mm in diameter were produced at the end of the spinal column of each animal using a cylindrical bras rod cooled to 77 K. 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. After last application, the lesion #1 was illuminated with the He-Ne or GaAsAl laser polarization aligned with

the rat spinal direction, the lesion #2 with the perpendicular relative orientation, and the lesion #3 was not irradiated (control). The total dose was 1 J/cm^2 per irradiation corresponding to an exposition time of 3 minutes. The animals were irradiated on the 3rd, 7th, 10th and 14th day. After each irradiation four rats were killed to obtain the morphological information. On the 17th day the last rats were killed. After sacrifice, the wounds were removed and fixed in Bouin's liquid overnight. They were then dehydrated in graded ethanol followed by clearing in xylem. The specimens were then embedded in paraffin and cut at $5 \mu\text{m}$. The sections were stained with hematoxylin and eosin and observed with a light microscope.

RESULTS

The morphological analysis showed that the rate of closure of the wound changes among the groups. At 17 days post wounding the control skin (lesion #3) was not completely reepithelized. The dermis was still infiltrated by a great number inflammatory cells as well as cell debris (Fig. 1).

The influence of linearly low-intensity laser irradiation on the healing process of skin wounds has a dependence on the relative orientation between the electric field polarization and the sample preferential direction when a He-Ne laser is used. The rate of closure was significantly increased in the lesion #1, when the laser polarization is aligned with the rat spinal direction. On the 17th day post-wounding the lesion #1 was completely healed when compared to lesion #2 and #3, which showed a poor degree of healing by this time. In these specimens the skin surface that had been injured was completely recovered by an epithelial layer that appeared to be thicker than normal epidermis. The repaired dermis was formed by a loose connective tissue composed mainly by large fibroblasts. The cytoplasm of these cells was large and basophilic indicating a high metabolic activity (Fig.2). The epidermis of lesion #2 which was illuminated by a He-Ne with the laser polarization aligned with the perpendicular relative orientation, was also repaired on the 17th day post-wounding. Although the subjacent dermis contained very active fibroblasts like in lesion #1, a moderate inflammatory process was still present (Fig. 3) indicating that the repair process was not completely finished.

No difference was observed between the lesion #1 and lesion #2 when the source of light was the GaAlAs diode-laser. On the 17th day post-wounding, both were recovered by a new epidermis. In both lesions the epithelial layer was thicker when compared with the epithelial layer of the normal skins. The subjacent dermis was formed by a thick layer of a loose connective tissue whose fibroblasts appear to be metabolic actives (Figs. 4 and 5).

DISCUSSION

Abergel et al. [6] demonstrated that He-Ne and GaAs low power laser irradiation enhanced procollagen synthesis in human skin fibroblast. Alternatively, Colver and Priestly asserted that there were no significant effects of He-Ne irradiation on wound healing [7]. Lubart et al [11] reported that the coherent irradiation is not essential on fibroblast proliferation, however, Mester and collaborators [12] found that the effect of incoherent light was 0,74% when compared to that of the laser with respect to the immunosuppressive effect of human lymphocytes. With plano-polarization of corresponding plane, an efficiency of 80% was achieved.

Although non polarized and/or non coherent light are made responsible for many biological effects, Nicola and coworkers [2] showed that coherence and polarization plays an important role in the wound healing. Our results also suggest that the polarization component of radiation is factor essential, if the exposure is visible light. This argument is in conformity with the Maxwell's theory for the optical properties of surfaces which states that the energy deposition efficiency in a microroughness interface depends on the electrical field polarization component [8]. Nevertheless, if

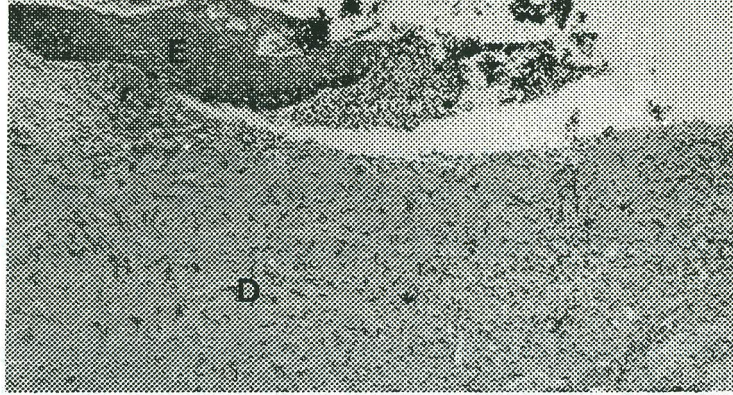


Fig. 1- Photomicrograph from a not irradiated skin (lesion #3) showing an injured area 17 days post-wounding. A large area of the dermis is still devoid of epithelial layer. Cells debris are observed near the edge of damaged epithelium. E- epithelium, D- dermis. HE x 40.

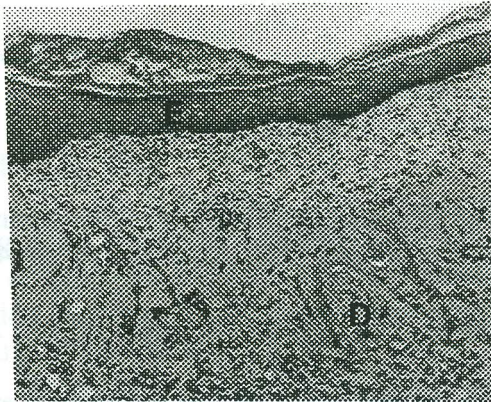


Fig.2- Photomicrograph from an irradiated skin (lesion #1) showing a wounded area 17 days post-wounding. It is possible to observe that the injured area is completely recovered by an health epithelial layer (E). The repaired connective tissue of the superficial dermis is formed by a loose connective tissue rich in active fibroblasts. HE x 40.

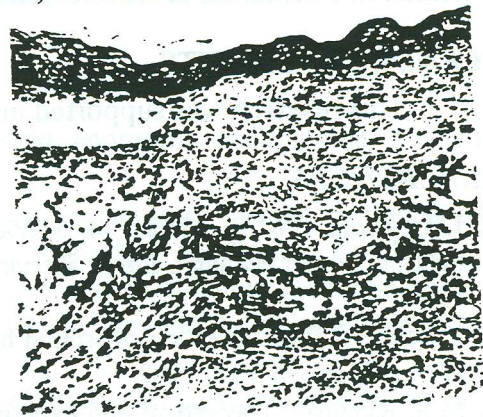
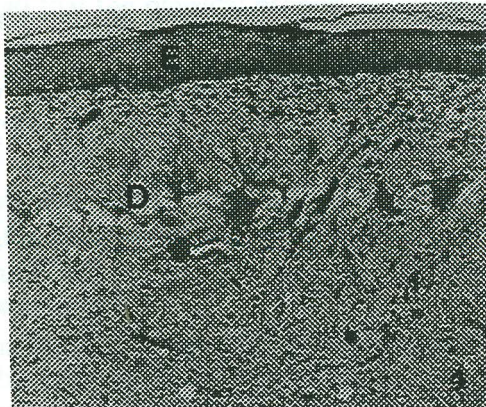


Fig.3- Photomicrograph from an irradiated skin (lesion #2) showing a wounded area 17 days post wounding. Although the epithelial layer is completely regenerated (E) the subjacent dermis is not completely recuperated and the deep dermis continues to be infiltrated by a large number of inflammatory cells. D- dermis. HE x 40.



Figs. 4 and 5- Photomicrographes from an irradiated skin showing a wounded area 17 days post wounding. No morphological difference was noted between lesion #1 (fig.4) and lesion #2 (fig. 5). The epithelial layer (E) which recovered the scar is completely regenerated. The subjacent dermis (D) was formed by a thick layer of a loose connective tissue whose fibroblasts appear to be metabolic actives. HE x 62,5

the exposure is near-infrared light, the component of polarized radiation is not relevant factor on the wounds healing process. It is probable that this finding is due to a higher depth penetration of the light in the skin at the wavelength 632.8 nm [13].

CONCLUSION

This study demonstrated that the relative orientation between the direction of the linearly polarized electric field and the spinal direction is an important factor on the healing process of inflammatory lesions created in the end of the spinal column of Lewis rats if the exposure is He-Ne laser, however, there was no morphological difference between the irradiated lesions with respect to polarization orientation at wavelength 797 nm.

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