"Effects of He-Ne polarized laser radiation on skin wounds repair: a morphological study"

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ABSTRACT:

This study was undertaken to investigate the influence of low level s- and p-polarized visible He- Ne laser irradiation (λ= 632.8 nm, I= 5 mW/cm^2) on healing of skin wounds. Three lesions about 6 mm in diameter were induced on back of rats with liquid nitrogen: one was irradiated by He-Ne s-polarized radiation, other by He-Ne p-polarized and the third lesion was not irradiated. Two rats were killed after each irradiation to get sections of tissue for morphological analysis. The wound illuminated by p-polarized He-Ne laser was completely healed after 17 days when compared to other lesions which showed a poor degree of healing.

Key words: low level therapy, wounds healing, polarized light, He-Ne laser and visible radiation

1. INTRODUCTION:

Low level laser therapy (LLL) has been used in many experiments since the 60 decade to examine the influence of laser radiation on the healing process of wounds. However, despite of a large number of studies published in the literature, results are many times conflicting, and only a few has presented scientific argument[1-4]. Some papers have suggested the increase in the wound healing rate of closure alter LLL exposure in rats[5-7] while others found no change[8-9]. In these experiments the polarization effects were not considered. A more recent paper showed that the coherence and polarization of laser light play an important role in wound repair but the effect of the polarization component was not considered[10]. According to the Maxwell's equations to optical properties of surfaces, the energy deposition efficiency in a microroughness interface depends on the electrical field polarization component[11]. Considering a linearly polarized beam, this efficiency will depend of the roughness parameters to p-polarized light and it will not depend of such parameters to s-polarized light. For this reason, an experiment was performed in which the effects of the s- and p- polarization components of laser light on wounds healing artificially created in rats skin were investigated.

2. MATERIAL AND METHODS:

We used a group of 10 male adult Lewis strain rats weighing about 300 grs. The source of light was a He-Ne laser (UNIPHASE, USA) with a wavelength of λ=632.8 nm, 10 mW in output power and beam diameter about 2 mm, mounted in a convenient set up. 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 s- and p-polarized light; a convergent lens (f= 7 cm) and a neutral density filter 0.04 for λ= 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.

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The animals were anesthetized by ether inhalation. After anesthesia the back of rats was 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 brass 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[10]. After the last application, the lesion # 1 was illuminated with s-polarized He-Ne laser, the lesion # 2 was irradiated with p-polarized radiation 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 two 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 μm. The sections were stained with hematoxylin and eosin and observed with a light microscope.

3. 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 was not completely reepithelized (Fig. 1) .The dermis was still infiltrated by a great number inflamatory cells as well as of cell debris (Fig.2). The rate of closure was significantly increased in p-polarized irradiated wounds. On the 17th day post-wounding the lesion # 2 was completely healed when compared to the other lesions which showed a poor degree of healing by this time. In these specimens the skin surface that had been injuried was completely recovered by an epithelial layer that appeared to be thicker than normal epidermis (Fig. 3). The repaired dermis was formed by a loose connective tissue composed mainly by large fibrolasts. The cytoplasm of these cells was large and basophylic indicating a high metabolic activity (Figs 3 and 4). The epidermis of lesion # 1 was also repaired on the 17th day post-wounding (Fig.5). Although the subjacent dermis contained very active fibroblasts like in lesion # 2, a moderate inflammatory process was still present (Fig. 6) indicating that the repair process was not completely finished.

4. DISCUSSION:

There is a rich literature about the biostimulating effects of low power light, but information is quite disperse. Lubart et al[12] reported that the coherent irradiation is not essential on fibroblast proliferation. Rigau et al[13] showed no increase in the number of fibroblasts following LLL exposure, but revealed significant changes in metabolic rates when compared with unirradiated controls. Loevschall's study[14] demonstrated an increased incorporation on tritiated thymidine in cultured human oral fibroblasts after LLL irradiation. These experiments did not consider the polarization effects. Mester and coworkers[15] 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.. Bolton et al[16] reported that the proliferative 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.

Although non polarized and/or non coherent light are made responsible for many biological effects, Nicola et al[10] showed that coherent and polarized light plays an important role in the wound healing. We suggest that the polarization component of radiation is essential factor too, if the exposure is visible light. This argument is in according with the Maxwell's theory for the optical properties of surfaces[11] .

5. CONCLUSION:

Our results indicated an accelerated healing process of inflammatory lesions created in the end of the spinal column of Lewis rats by p-polarized low energy He-Ne laser. We propose that this electrical field component polarization enhances fibroblast collagen production. Quantitative autoradiography with ^{3}H proline is in progress to confirm this increased collagen synthesis.

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REFERENCES:

1. T. Karu, "Photobiology of low-power laser effects", *Health Phys.* **56(5)**, pp. 691-704, 1989.
2. T. Karu, "Interactions of monochromatic visible light and near infrared radiation with cells: currently discussed mechanisms", *Laser-Tissue Interaction VI*, S.L. Jacques and A. Katzir, eds., Proc. SPIE 2391, pp. 576-586, 1995.
3. M. Fenyö, "Theoretical and experimental basis of biostimulation by laser irradiation", *Opt. Laser Tech.* **16**, pp. 209-215, 1984.
4. K. Kamikawa and T. Ohnishi, "Essential mechanisms of low power laser effects", *Laser Applications in Medicine and Surgery*, G. Galetti, L. Bolognani and G. Ussia, eds., pp. 11-18, Bologna:Monduzzi, 1992.
5. J. Becker, "Biostimulation of wound healing in rats by combined soft and middle power lasers", *Biomed. Tech.* **35**, pp. 98-101, 1990.
6. J.S. Kana, G. Hutschenreiter, D. Haina and W. Waidelich, "Effect of low-power density laser radiation on healing of open skin wounds in rats", *Arch. Surg.* **116**, pp. 293-296, 1981.
7. S. Rochkind, M. Rousso, M. Nissan, M. Villarreal, L. Bar-Nea and D. G. Rees, "Systemic effects of low-power laser irradiation on the peripheral and central nervous system, cutaneous wounds, and burns", *Lasers Surg. Med.* **9**, pp. 174-182, 1989.
8. A. Kass, "Does the light of soft lasers or of combination soft and middle-power lasers improve wound healing in rats? Continuing studies", *Biomed. Tech.* **35**, pp. 154-157, 1990.
9. G. Anneroth, G. Hall, H. Ryden, L. Zetterqvist, "The effect of low-energy infrared laser radiation on wound healing in rats", *Br. J. Oral Maxillofac. Surg.* **26**, pp. 12-17, 1988.
10. J.H. Nicola, E.M.D. Nicola, M. Simões and J.R. Paschoal, "The role of polarization and coherence laser light on wound healing", *Laser Tissue Interaction V*, S.L. Jacques, ed., Proc. SPIE 2134A, pp. 448-450, 1994.
11. M.S. Ribeiro, "Theoretical and experimental study of the polarized laser radiation non-thermal effects in living tissues", *Thesis of Master Degree*, IPEN, São Paulo, Brazil, 1991.
12. R. Lubart, H. Friedmann, I. Peled and N. Grossman, "Light effect on fibroblast proliferation", *Laser Ther.*, **5(2)**, pp. 55-57, 1993.
13. J. Rigau, M.A. Trelles, R.G. Calderhead and E. Mayayo, "Changes in fibroblast proliferation and metabolism following *in vitro* Helium-Neon laser irradiation", *Laser Ther.* **3(1)**, pp. 25-33, 1991.
14. H. Loevschall and D. Arenholt-Bindslev, "Effect of low level diode laser irradiation of human oral mucosa fibroblasts *in vitro*", *Lasers Surg. Med.* **14**, pp. 347-354, 1994.
15. E. Mester, S. Nagylucskay, W. Waidelich, S. Tisza, P. Greguss, D. Haina and A Mester, "Effects of direct laser radiation on human lymphocytes", *Arch. Dermatol. Res.* **263**, pp. 241-245, 1978.
16. P. A. Bolton, M. Dyson and S. Young, "The effect of polarized light on the release of growth factors from the U-937 macrophage-like cell line", *Laser Ther.* **4(1)**, pp. 33-37, 1992.

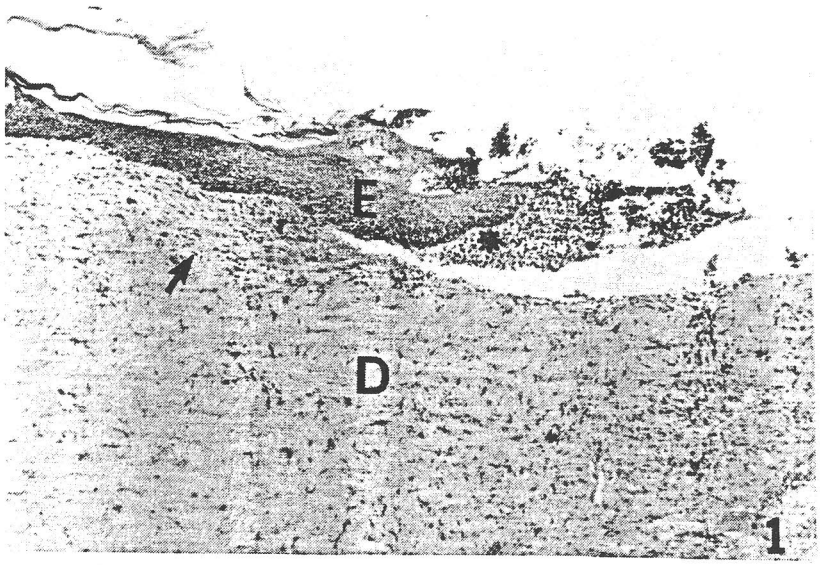

Fig. 1- Photomicrograph from a not irradiated skin (lesion # 3) showing a injuried area 17 days post-wounding. Observe that a large area of the dermis is still devoid of epithelial layer. Note a loose connective tissue (arrow) below the already repaired epithelium. Cells debris are observed near the edge of injured epithelium (*). E- epithelium; D- dermis. Hematoxilin and eosin. X 40.

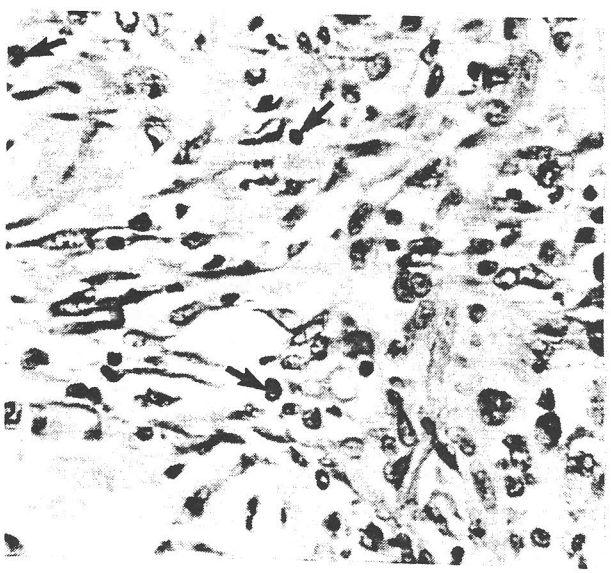

Fig.2-High magnification showing an area of dermis below a not completely repaired area. Observe an increased concentration of inflammatory cell in this area (arrows). Hematoxilin and eosin. X 400.

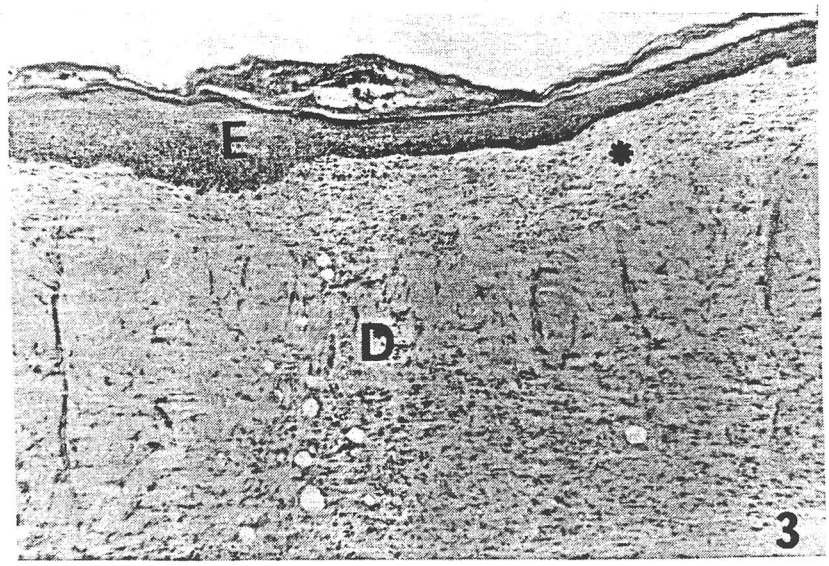

Fig. 3- Photomicrograph from a irradiated skin (lesion # 2) showing a wounded area 17 days post-wounding. Compared with the figure 1 it is possible to observe that the wounded area is completely recovered by an health epithelial layer (E). The repaired connective tissue of the superficial dermis (*) is formed by a loose connective tissue rich in active fibroblasts. D- dermis. Hematoxilin and eosin. X 40.

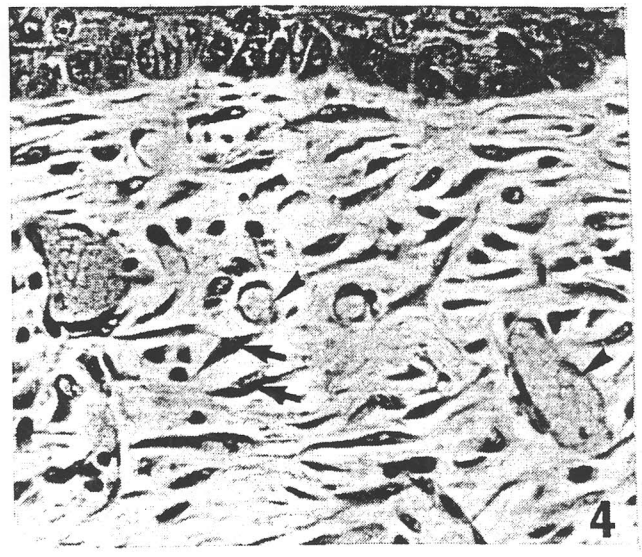

Fig.4- High magnification showing the loose connective tissue from a repaired area. Observe that the fiibroblasts (arrows) are big and show morphological characteristics of high metabolic activity. Many small blood vessels are also present in this region (arrows head). Hematoxilin and eosin. X 400.

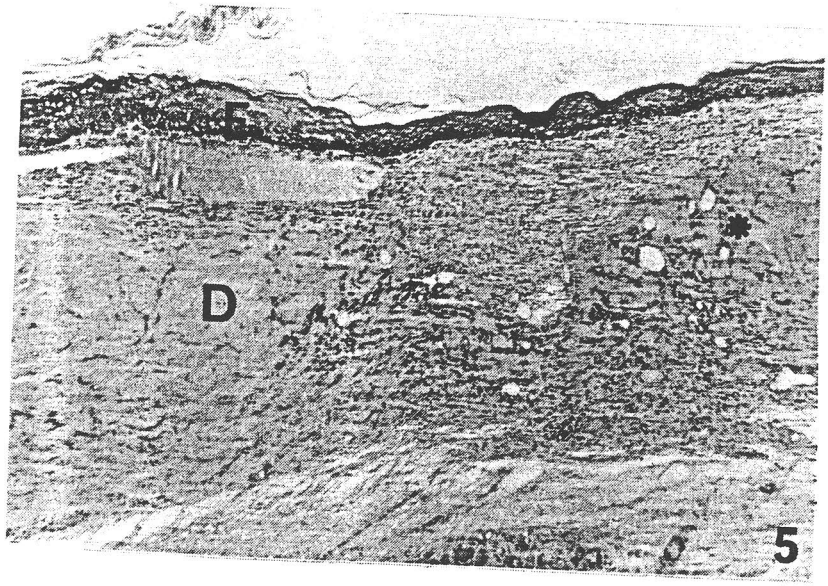

Fig. 5- Photomicrograph from a irradiated skin (lesion # 1) showing a wounded area 17 days post-wounding. Observe that although the epithelial layer is completely regenerated (E) the subjacent dermis is not completely recuperated and the deep dermis continue to be infiltrade by a large number of inflammatory cells (*). D-dermis. Hematoxilin and eosin. X 40.

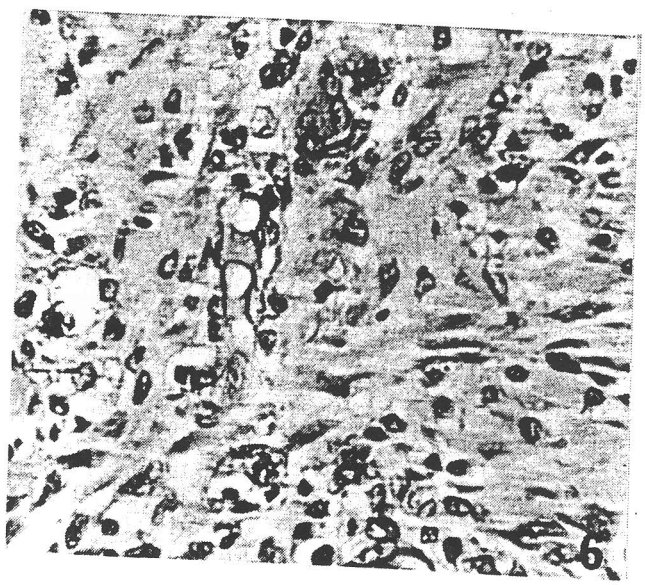

Fig 6- High magnification of an area of the deep dermis situated just below a repaired area from an animal killed 17 days after the lesion and after 4th irradiation. Observe that there are still many inflammatory cells infiltrated in the connective tissue. Hematoxilin and eosin. X 400.