Histological study of wound healing in rats following He-Ne and GaAlAs laser radiation

Martha S. Ribeiro[a], Denise M. Zezell[a], Edison P. Maldonado[a], Nilson D.Vieira Jr.[a], Karin Carbone[b], Cleusa M.R. Pellegrini[b] and Telma M.T. Zorn[b]

[a]Departamento de Materiais Optoeletrônicos, Instituto de Pesquisas Energéticas e Nucleares, CNEN/SP, C.P. 11049, 05422-970, São Paulo, SP, Brazil
[b]Departamento de Histologia e Embriologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, 05508-900, São Paulo, SP, Brazil

ABSTRACT

The influence of low-intensity linearly polarized visible (λ= 632.8nm) and near infrared (λ= 797nm) laser radiation on healing of skin wounds was compared histologically. Three round lesions measuring about 6 mm in diameter were created at the end of the spinal column of 20 rats divided in two groups of 10 animals by burning the rat back skin with liquid nitrogen. Lesions #1 and #2 were illuminated using He-Ne or GaAlAs laser radiation. The laser polarization was aligned with the rat spinal direction in lesion #1, lesion #2 with the perpendicular relative orientation and lesion #3 was not irradiated (control). The animals were irradiated with a total dose of 1 J/cm^2 on day 3, 8, 11 and 14. After each irradiation, two rats from each group were killed to obtain morphological information. On day 17 the last rats were killed. The irradiated lesions presented a significant acceleration on the wound repair compared to the control. The results showed that the relative orientation of the electric field has an essential role on the healing process if exposure is visible light. On the other hand, no morphological difference was observed between illuminated lesions by near infrared radiation with respect to polarization orientation.

Keywords: low-intensity laser therapy, polarized light, wound healing, visible and near infrared radiation.

1. INTRODUCTION

There is currently much interest in the use of low intensity visible and near infrared laser radiation to stimulate wounds healing and tissue regeneration. In this case, the radiation intensity is so low that the resulting biological effects are due directly to the radiation 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 coworkes[2] showed that the coherence and polarization of laser light play an important role in wound repair while Colver and Priestly suggested that were no significant effects following 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 a few has offered scientific support for these effects[8-10]. For this reason, an experiment was performed in which the influence of linearly polarized low intensity He-Ne and GaAlAs lasers radiation on the healing process of skin wounds artificially created in rats skin was investigated.

2. 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 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 exposure of the wound by inserting optical components: a Glan-Thompson polarizing

For further author information
M.S.R. (correspondence): email: mribeiro@net.ipen.br
Telephone: 5511-8169313; Fax: 5511-8169315
T.M.T.Z.: email: temtzorn@usp.br
Telephone: 5511-8187260; Fax: 5511-8187309

Part of the EUROPTO Conference on Effects of Low-Power Light on Biological Systems • Stockholm, Sweden • September 1998
SPIE Vol. 3569 • 0277-786X/98/$10.00

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 λ= 632.8 nm. At last, an objective was used with f= 5cm 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 λ= 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 3^{rd}, 7^{th}, 10^{th} and 14^{th} day. After each irradiation four rats were killed to obtain the morphological information. On the 17^{th} 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 (lesion #3) was not completely reephithelized. **Fig. 1** shows the dermis still infiltrated by a great number inflammatory cells as well as cell debris.

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 (**Fig.2**). On the 17^{th} 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 ticker 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. In **Fig. 3** it can observe 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 17^{th} day post-wounding. Although the subjacent dermis contained very active fibroblasts like in lesion #1, a moderate inflammatory process was still present 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 17^{th} day post-wounding, both were recovered by a new epidermis. In both lesions the epithelial layer was ticker 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**).

4. 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 immunosupressive effect of human lymphocytes. With plano-polarization of corresponding plane , an efficiency of 80% was achieved. Also, Bolton et al.[13] investigated the effects of different levels of polarization on the release of growth factors from the U-937 macrophage-like cell line. The proliferative response was greatest in the cultures exposed to supernatants from macrophages treated with the 95% polarized light source when compared with the 14% polarized light source.

Although non polarized and/or non coherent light are made responsible for many biological effects, Nicola and coworkes[2] showed that coherence and polarization plays an important role in the wound healing. Our results suggest that the polarization component of radiation is factor essential too, 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 the exposure is near-infrared light, the component of polarized radiation is not relevant factor on the wounds healing process. It seems probable that this finding is due to a higher penetration of the light in the skin at the wavelength 797nm[14].

5. CONCLUSION

This study demonstrated that the polarization component of the electical field is an important factor in 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, no morphological differences were observed between the irradiated lesions with respect to polarization orientation at wavelength 797nm.

6. ACKNOWLEDGMENTS

This research was supported in part by grant from Conselho Nacional de Pesquisa e Desenvolvimento (CNPq), Third World Academy of Sciences (TWAS), grant #96-190 RG/PHYS/LA and Fundação de Amparo à Pesquisa do estado de São Paulo (FAPESP).

7. REFERENCES

1. 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 2134[A], pp. 448-450, 1994.
2. J. Becker, "Biostimulation of wound healing in rats by combined soft and middle power lasers", *Biomed. Tech.* **35**, pp. 98-101, 1990.
3. 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.
4. 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.
5. 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.
6. R.P. Abergel, C.A. Meeker, T.S. Lam, R.M. Dwyer, M.A. Lesavoy and J. Uitto, "Control of connective tissue metabolism by lasers: recent developments and future prospects", *Dermatol. Surg.* **11**, pp. 1142-1150, 1984.
7. G.B. Colver and G.C. Priestley, "Failure of a helium-neon laser to affect components of wound healing in vitro", *Br. J. Dermatol.* **121**, pp. 179-186, 1989.
8. 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.
9. 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.
10. M. Fenyö, "Theoretical and experimental basis of biostimulation by laser irradiation", *Opt. Laser Tech.* **16**, pp. 209-215, 1984.
11. R. Lubart, H. Friedmann, I. Peled and N. Grossman, "Light effect on fibroblast proliferation", *Laser Ther.* **5(2)**, pp. 55-57, 1993.
12. E. Mester, S. Nagylucskay, W. Waidelich, S. Tisza, P. Greguss, D. Haina and A. Mester, "Effects of direct laser radiation on human lymphocytes", *Arch. Dermat. Res.* **263**, pp. 241-245, 1978.
13. P. 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.
14. J. Marshall , J.K. Haywood, "The Biological Nature of the Hazard" in *Medicine Laser Safety*, H. Moseley and J.K. Haywood eds, United Kingdom, pp 1-8, 1986.

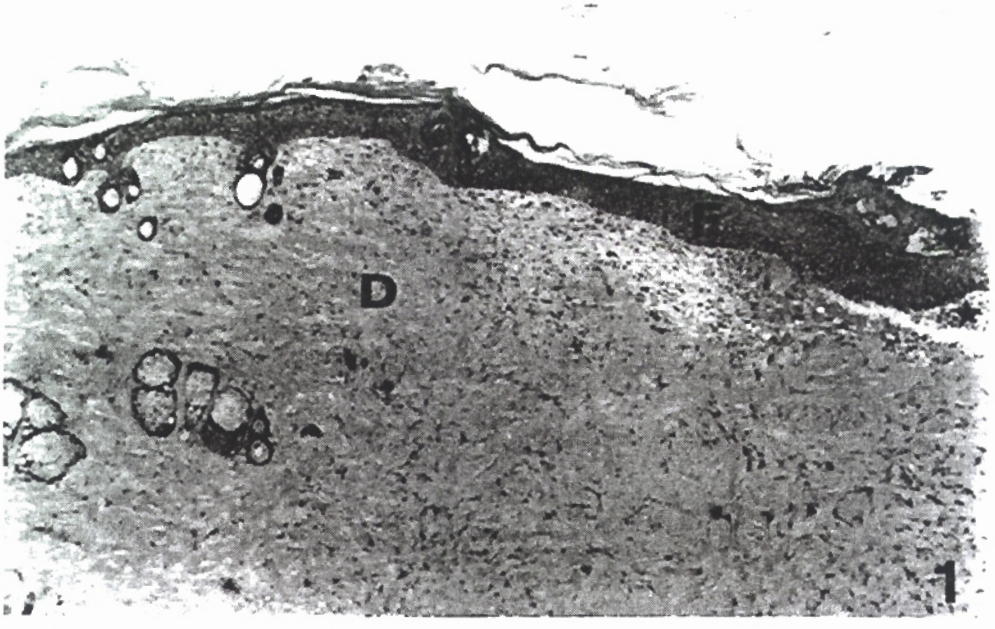


Fig. 1- Photomicrograph from a not irradiated skin (lesion #3) showing the lesion 17 days post-wounding. Cells debris are observed near the edge of damaged epithelium (★). E- epithelium, D- dermis. HE x 40.

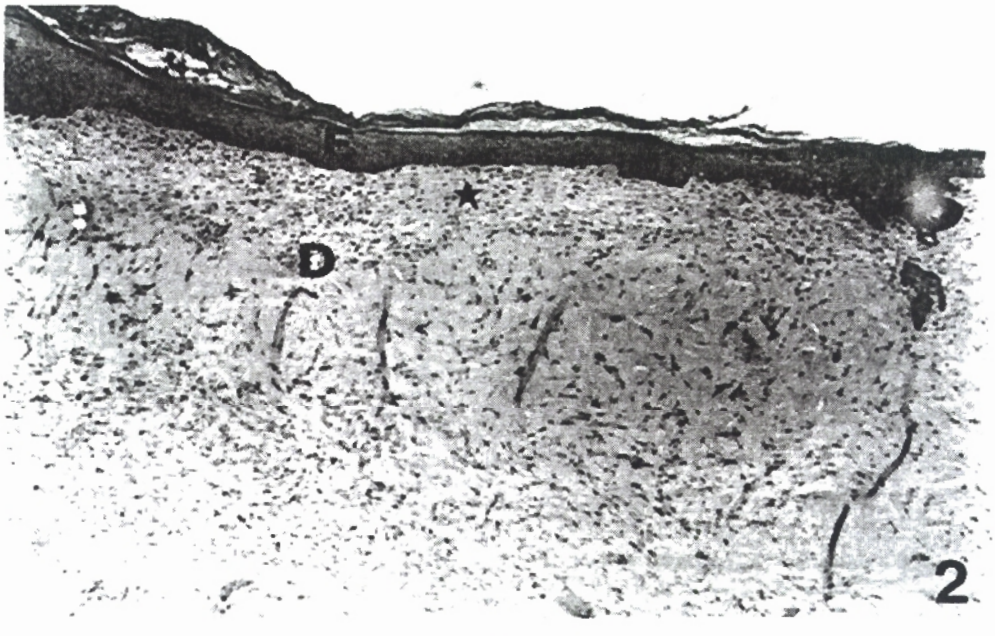
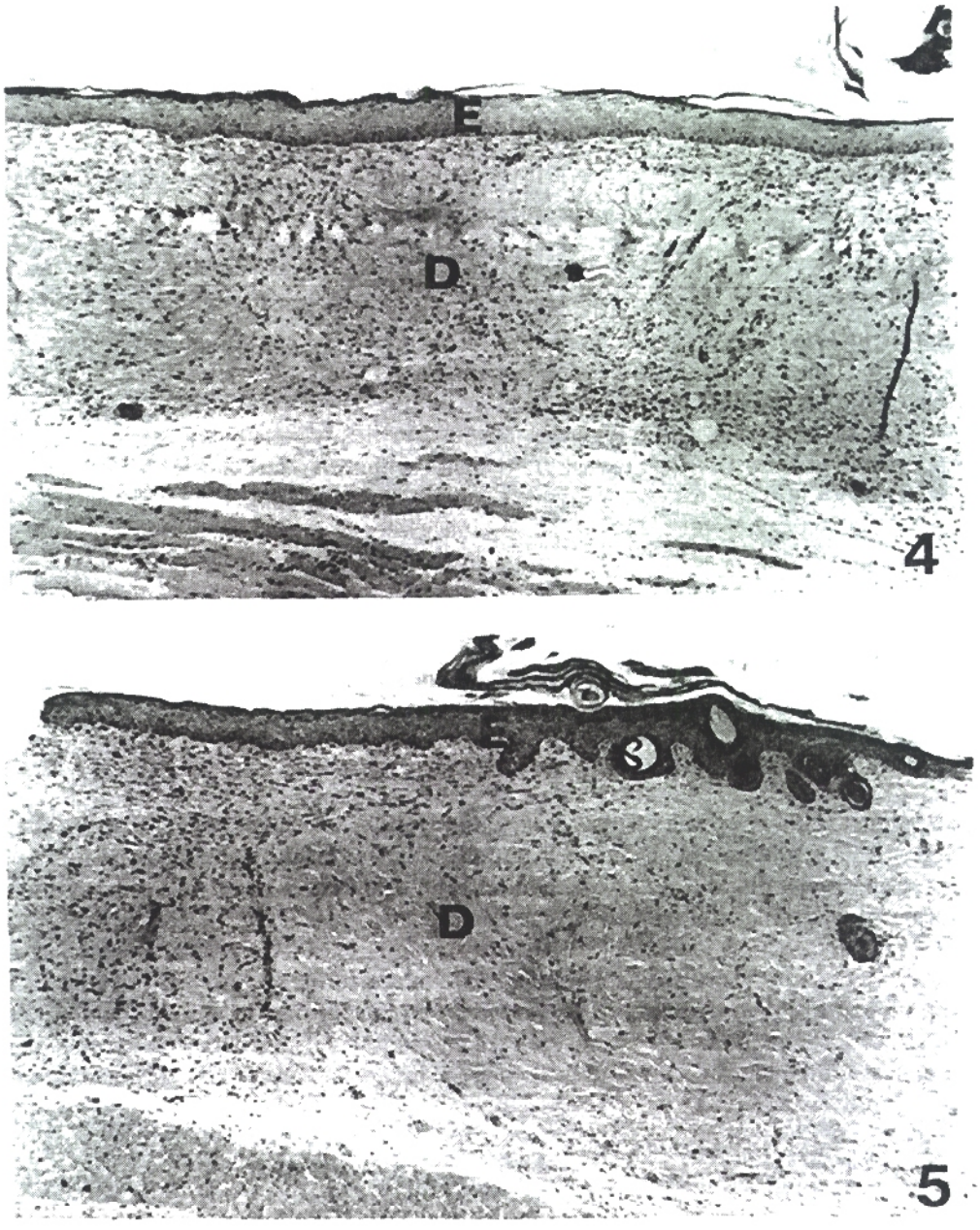


Fig.2- Photomicrograph from an irradiated skin (lesion #1) showing a wounded area 17 days post-wounding. Observe that the injured area is completely recovered by an health epithelial layer (E). The superficial dermis (★) was repaired by a loose connective tissue rich in active fibroblasts. D- dermis.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

Fig.3- Photomicrograph from an irradiated skin (lesion #2) showing a wounded area 17 days post wounding. The epithelial layer is completely regenerated (E), however, 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 remarkable morphological differences were 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 40.