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Healing of Surgical Wounds Made with λ970-nm Diode Laser Associated or Not with Laser Phototherapy (λ655 nm) or Polarized Light (λ400–2000 nm)

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Abstract

Objective: The aim of this study was to analyze the effect of two phototherapies, laser and polarized light, on diode laser (970λ nm) wounds. ***Background Data:*** Lasers have been used in surgery, and some wavelengths may cause thermal damage to the tissue and affect healing. Several studies have shown that some wavelengths are effective in improving healing. Coherent and noncoherent light have been successfully used on the modulation of biological phenomena of several origins. ***Animals and Methods:*** Thirty-one Wistar rats were divided into 3 groups (GI to GIII). A 20-mm×2-mm wound was created on the dorsum of each animal with a diode laser (Sirolaser, Sirona®, Bensheim, Germany). Group GI acted as control. On GII, laser light (λ655 nm, 30 mW, ϕ ~3 mm, 12 J/cm^2) was used and on GIII illumination with polarized light (λ400–2000 nm, 40 mW, ϕ ~5.5 cm, 12 J/cm^2) was used, every other day (GII) or daily (GIII) for 7 days. The animals were killed at 0, 7, and 14 days after surgery. Specimens were taken, routinely processed, stained and imunnomarked [HE (hematoxylin–eosin), sirius red, α-smooth muscle actin (SMA)], and underwent histological analysis. ***Results:*** GII showed better response at day 14 when re-epithelialization was in a more advanced stage. The number of myofibroblasts was significantly different over the healing time (7 to 14 days); this number was smaller than that observed on G1. On GIII at day 7, the number of myofibroblasts was significantly higher than for GII. At day 14, a more pronounced deposition of collagen matrix was also seen, and inflammation was discrete and more advanced for GIII. ***Conclusion:*** The results of the present study showed that the effect of the use of laser light was more evident at early stages of healing and that the use of polarized light improved the resolution of the inflammatory reaction, increased the deposition of collagen, increased the number of myofibroblasts, and quickened re-epithelialization during the experimental time.

Introduction

The use of mono- and/or polychromatic light sources is growing among health care workers. Several studies have shown that phototherapies at different wavelengths present positive effects and advantages over most conventional therapies.

High-power lasers have been used as tools on many surgical procedures and have been shown to be effective, safe, and advantageous when compared to other surgical techniques.[1,2] Some lasers may also kill bacteria.[5] On the other hand, many surgical wavelengths may cause thermal damage to the tissue and affect healing in many ways.[1,4]

Several light sources operating at low energy levels at both the visible or infrared spectrum have been shown to be effective in treating inflammatory conditions, in the reduction of edema and pain,[11] and in improving wound healing and repair.[12] The use of this therapy does not cause cell damage since no thermal effect is caused by the light.[14] These days, both coherent and noncoherent light sources have been successfully used in the modulation of biological phenomena of several origins.[3]

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Table 1. Criteria Used for Histomorphometric Analysis

Criterion	*Score*		
Re-epithelialization	**Absent**	**Present** Covering < 50% of the wound **Present** Covering > 50% of the wound **Present** Covering 100% of the wound with irregular thickness **Present** Covering 100% of the wound with regular thickness	
	Discrete	*Moderate*	*Intense*
Accute inflammation	Presence of < 25% of neutrophyls on the wounded site	Presence of 25–50% of neutrophyls on the wounded site	Presence of > 50% of neutrophyls on the wounded site
Mixed inflammation	Presence of < 25% of mono- or polymorphnuclear leukocytes on the wounded site	Presence of 25–50% of mono- or polymorphnuclear leukocytes on the wounded site	Presence of > 50% of mono- or polymorphnuclear leukocytes on the wounded site
Chronic inflammation	Presence of < 25% of chronic inflammatory cells on the wounded site	Presence of 25–50% of chronic inflammatory cells on the wounded site	Presence of > 50% of chronic inflammatory cells on the wounded site
Granulation tissue	Discrete number of fibroblasts, collagen fibers, and inflammatory cells	Moderate number of fibroblasts, collagen fibers, and inflammatory cells	Intense number of fibroblasts, collagen fibers, and inflammatory cells
Neoangiogenesis	Newly formed blood vessels with smaller number than seen on healthy tissue	Newly formed blood vessels with similar number seen on healthy tissue.	Newly formed blood vessels with greater number than seen on healthy tissue
Fibroblastic proliferation	Presence of < 25% of fibroblasts	Presence of < 50% of fibroblasts	Presence of > 50% of fibroblasts
Collagen deposition	Presence of < 25% of collagen matrix	Presence of < 50% of collagen matrix	Presence of > 50% of collagen matrix
Myofibroblast proliferation	Presençe of < 25% actin-positive cells, excluding vessels, walls, and lumen on the wounded site	Presence of 25–50% actin-positive cells, excluding vessels, walls, and lumen on the wounded site	Presence of > 50% actin-positive cells, excluding vessels, walls, and lumen on the wounded site

This study aimed to assess, histologically, the effects of the use of two types of phototherapies, laser (λ650 nm) or polarized light (λ400–2000 nm), on the healing of cutaneous surgical wounds created with a λ970-nm diode laser on the dorsum of rats.

Animal and Methods

This study was approved by the animal ethics committee of the Universidade do Vale do Paraíba - UNIVAP (no: a043/cep/2007). Thirty-one young adult ($\sim$90 days old) male Wistar rats ($\sim$350 g) were kept under natural conditions of light, humidity, and temperature at the animal house of the Instituto de Pesquisa e Desenvolvimento of UNIVAP during the experimental period. The animals were fed with standard laboratory pelted diet and had water ad libidum. The animals were kept in individual metallic cages on a day–night light cycle with controlled temperature during the experimental period. Under general anesthesia (1% atropine, 20 mL, Fagra®, 0.04 mL/100 g;[21] 10 mL of 10% Cetamina® (Syntec, São Paulo, Brazil), 0.1 mL/100 g and 10 mL of 2% Cloridrato de Xilazina®, (Syntec, São Paulo, Brazil), 0.1 mL/100 g)[22] the dorsum was shaved and a 20-$\times$2-mm wound was created using a λ970-nm diode laser (4 W, ϕ $\sim$320-μm fiber (Sirona®, Bensheim, Germany). The animals were given antibiotics immediately after surgery (Pentabiótico®, Fort Dodge®, São Paulo, Brazil, 0.02 mL/100 g). The animals were then randomly distributed into 3 groups (G1, GII, GIII). Group I acted as untreated control. Each group was then subdivided into 3 subgroups according to animal death time: immediate ($n=5$), day 7 ($n=3$), and day 14 ($n=3$). Subjects of GII and GIII were further treated with two types of phototherapies (laser or polarized light). Animals of GII were treated with laser light λ655 nm, 30 mW, ϕ $\sim$3 mm (Kondortech®, São Carlos, São Paulo, Brazil), 12 J/cm^2]. Laser light was applied transcutaneously and fractioned on 6 contact points at the wound margins (6$\times$2 J/cm^2). The treatment was carried out at every other day during the

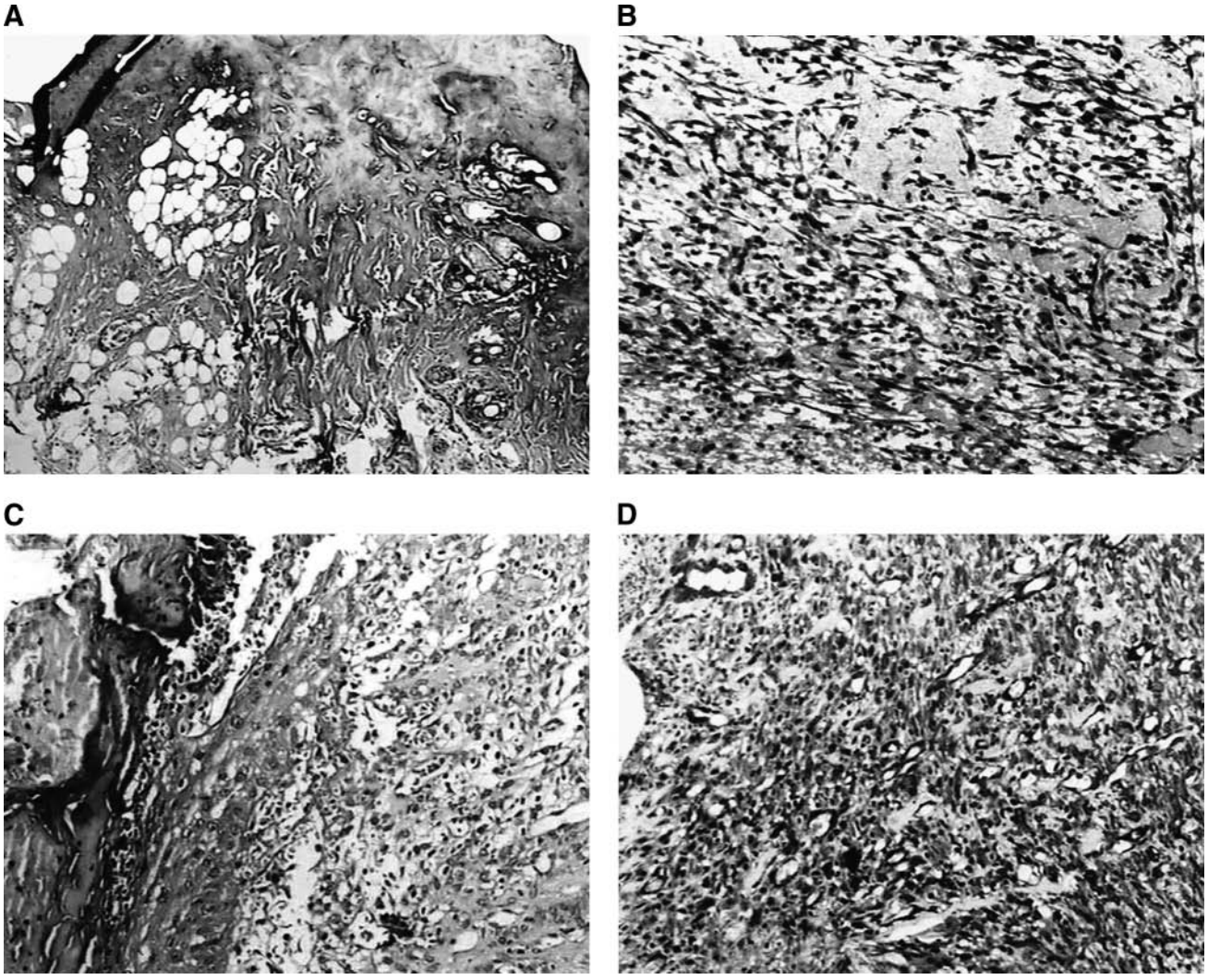

FIG. 1. (**A**) Photomicrograph of specimen from group GI showing no cell viability on the region and areas of tissue necrosis ranging from the epithelium down to the hypodermis (HE, $\times$40). (**B**) Seven days after surgery, α-smooth muscle actin (α-SMA) immunomarking evidenced discrete presence of myofibroblasts (α-SMA, $\times$40). (**C**) At day 14, the wound showed small epithelial migration in less than 50% of the area (HE, $\times$100) and (**D**) a marked number of myofibroblasts (α-SMA, $\times$40).

experimental periods (7 or 14 days). Subjects of GIII were daily illuminated with a polarized light source (λ400–2000 nm, 40 mW, ϕ ~5.5 cm (Bioptron®, Wollerau, Switzerland); focal distance of 10 cm; dose per session 12 J/cm^2). Animal death occurred immediately and at 7 and 14 days after surgery and was carried out with an overdose of general anesthetics. A 16-cm^2 specimen was removed and fixed in 10% formalin for 24 h. The specimens were routinely processed to wax, cut (5 or 3μm), and stained (HE, sirius red) or immunomarked (monoclonal antiactin, α-smooth muscle actin (Sigma-Aldrich®, St. Louis, MD, USA) at the Oral Pathology Laboratory of the School of Dentistry of the Federal University of Bahia. Each specimen underwent histological analysis carried out by an experienced pathologist in a double-blind manner. Three slides were made of each specimen. The criteria used for this analysis were adapted from one used previously by our team (Table 1).[23] When applied, each criterion was scored as absent, discrete, moderate, or intense according to the percentage of the phenomena observed. The percentage of occurrence of each score was analyzed using Minitab 15® software. Statistical analysis was carried out using the Fischer exact test or Kruskall–Wallis test, both at a significance level of 5%.

Immediately after surgery, control (GI) specimens showed tissue necrosis extending down to the hypodermis (Fig. 1A); there were some piknotic nuclei and collagen fibers far from the wounded site. Seven days after surgery the wound was covered by a thick crust and no re-epithelialization could be seen. Inflammatory reaction (acute, chronic, and mixed) was discrete. The presence of granulation tissue was intense at this stage, and this was rich in blood vessels. Large numbers of fibroblasts and intense deposition of collagen matrix were also observed. The number of myofibroblasts was discrete in 100% of the cases (Fig. 1B). At day 14, the wound was still covered by a thick crust and re-epithelialization was not seen in 33.3% of the cases. For 66.67% of these cases, the epithelium covered less than 50% of the wound (Fig. 1C). Inflammatory reaction was discrete and of varied type; the amount of granulation tissue was moderate and moderately vascularized. A marked number of fibroblasts and intense deposition of collagen matrix could also be observed at this time. Myofibroblasts were markedly seen on most specimens (66.67%) (Fig. 1D).

On laser-irradiated subjects (GII), at day 7, ulceration was observed and was covered by a crust of variable thickness with the presence of necrotic remnants. Epithelium was absent in all cases. Inflammatory reaction varied from discrete to moderate and was acute, chronic, or mixed. An intense deposition of collagen was observed on the wounded site, and angiogenesis varied from moderate to intense. There was a large number of fibroblasts placed parallel to the wound surface. Collagen deposition was moderate at this stage. The number of myofibroblasts was discrete on all subjects (Fig. 2A). At day 14, the presence of a crust of varied thickness was noticed, and the inflammatory reaction was discrete, chronic, and of mixed cellularity. Epithelium was not seen on 20% of the specimens. In 40% of the cases in which epithelium was present, it covered less than 50% of the wounded site. For 20% of the cases, epithelial recovery covered more than 50% of the wound, and for 20% of the cases 100% of the wound was covered by epithelial tissue (Fig. 2B). Deposition of granulation tissue was either discrete or intense and showed

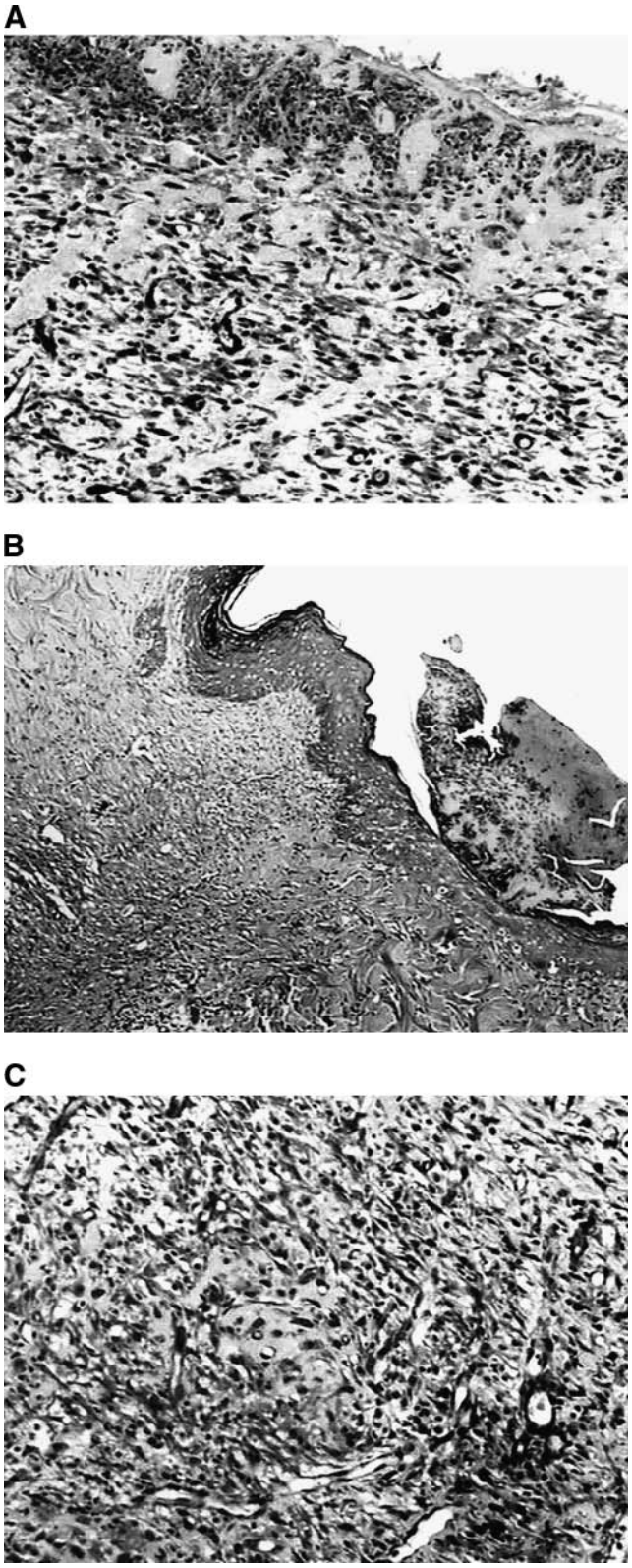

FIG. 2. (**A**) Photomicrograph of specimen from group GII showing a discrete number of myofibroblasts (SBI + α-SMA, ×40). (**B**) At day 14, epithelialization was observed in more than 50% of the wounded site (H&E, ×40) and (**C**) a moderate amount of myofibroblasts (SBI + α-SMA, ×100).

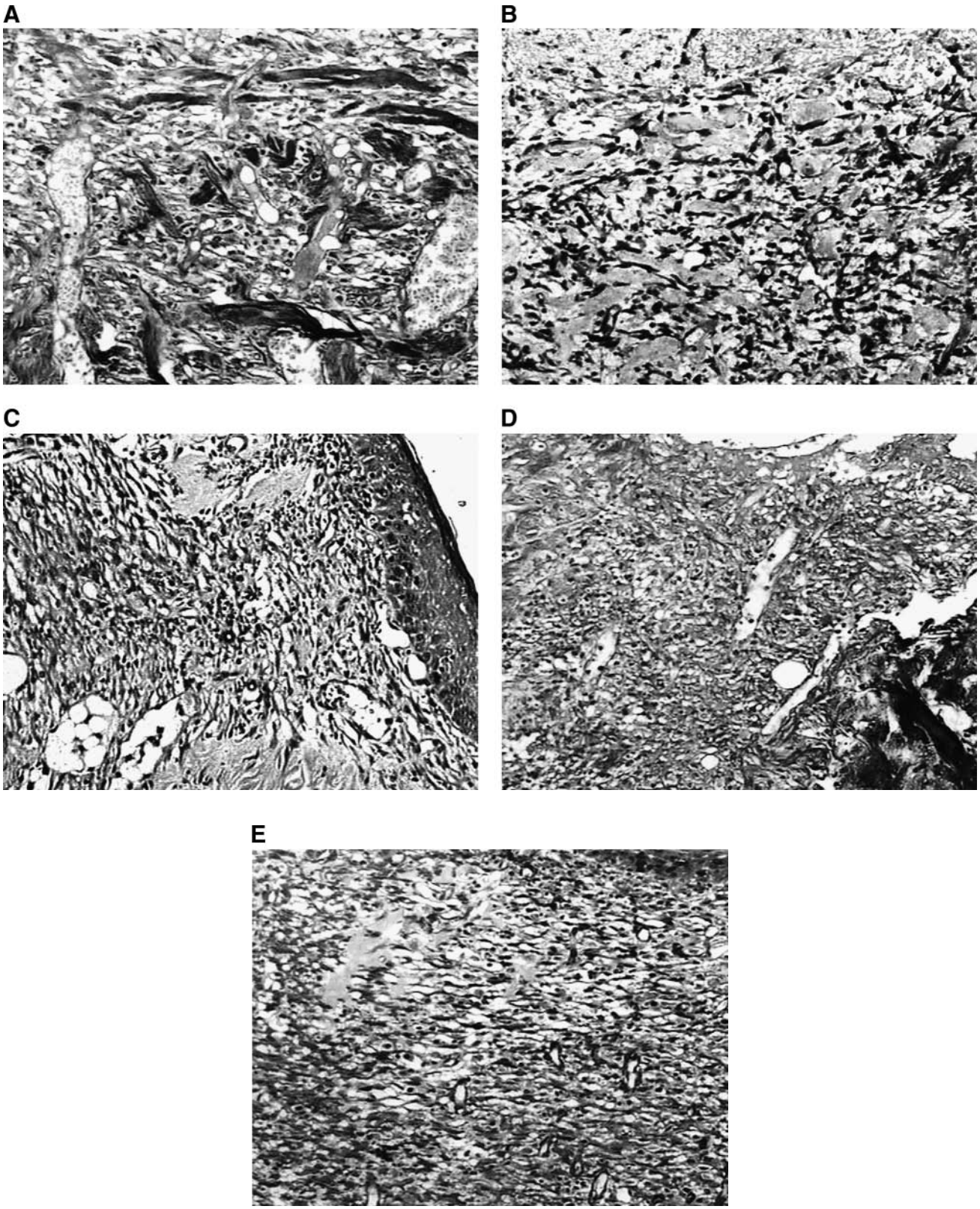

FIG. 3. (**A**) Photomicrograph of specimen from group GIII showing moderate deposition of collagen fibers (sirius red, ×100) and (**B**) a discrete number of myofibroblasts (SBI + α-SMA, ×100). (**C**) At day 14, re-epithelialization was observed on 100% of the wound (H&E, ×100), (**D**) intense deposition of collagen matrix (sirius red, ×100), and (**E**) marked number of myofibroblasts (SBI + α-SMA, ×40).

moderate angiogenesis. A large number of fibroblasts and moderate deposition of collagen matrix were also seen at this stage. A moderate amount of myofibroblasts was seen on 80% of the cases (Fig. 2C).

On subjects illuminated with polarized light, at day 7 after surgery, the presence of a thick crust and necrotic debris was seen on 60% of the cases; on the other 40%, no necrosis tissue was seen. Re-epithelialization was not seen on 80% of the wounds and covered less than 50% of the wound on 20% of the subjects. A discrete chronic inflammatory reaction was seen and was either chronic or of mixed cellularity. There was intense deposition of granulation tissue associated with intense angiogenesis. There was a large number of fibroblasts and moderate deposition of granulation tissue (Fig. 3A). A moderate amount of myofibroblasts was seen on 80% of the cases (Fig. 3B). Tissue edema was seen in some specimens. At day 14, 60% of the wounds showed crusting. Re-epithelialization was seen on 100% of the wounds on 60% of the subjects. For 20% of the cases, the epithelium was observed covering less than or more than 50% of the wounded site (Fig. 3C). The amounts of granulation tissue and angiogenesis were moderate. Inflammation was discrete and chronic on 60% of the cases. The number of fibroblasts was large, and intense deposition of collagen matrix was seen at this stage (Fig. 3D); both were parallel distributed to the wound surface. The number of myofibroblasts was larger in 80% of the subjects (Fig. 3E). A summary of the results is given in Table 2.

Statistical analysis showed significant differences in the number of myofibroblasts at day 7 between the two phototherapies; larger numbers were seen when the polarized light source was used (GIII, $p = 0.04$). At day 14, significant differences were seen between subjects illuminated (GIII) and irradiated (GII) ($p = 0.05$). Time was also important. Significant differences ($p = 0.01$) were seen between 0 and 7 days on irradiated (GII) and illuminated subjects (GIII) for re-epithelialization. There were also significant differences in the number of fibroblasts on illuminated subjects (GIII, $p = 0.04$). At day 14, significant differences were seen in the number of myofibroblasts on all groups: control (GI, $p = 0.02$), laser (GII, $p = 0.04$), and polarized light (GIII, $p = 0.04$). The overall analysis of the results (Kruskal–Wallis test) showed that, at day 7, the number of myofibroblasts was higher on illuminated subjects (GIII); this number was significantly different from both controls (GI, $p = 0.04$) and laser-treated subjects (GII, $p = 0.04$). At the end of the experimental period, the criterion re-epithelialization was more important also on illuminated subjects (GIII); the findings were significantly different from both control (GI, $p = 0.04$) and laser-treated subjects (GII, $p = 0.04$). Illuminated subjects also showed higher expression of collagen deposition when compared with laser-treated subjects (GII, $p = 0.04$).

Table 2. Summary of the Results of the Computerized Morphometry

		7 days			*14 days*		
Time criterion	*Grade*	*Control (GI), %*	*Laser (GII), %*	*LP (GIII), %*	*Control (GI), %*	*Laser (GII), %*	*LP (GIII), %*
Re-epithelialization[a]		100	100	80	33.33	20	0
		0	0	20	66.67	40	20
		0	0	0	0	20	20
		0	0	0	0	20	40
		0	0	0	0	0	20
Inflammation	Acute[a]	0	0	0	0	0	0
		33.33	0	0	33.33	0	0
		0	0	0	0	0	0
		0		20	0	0	0
	Mixed[a]	0	0	0	0	0	0
		33.33	20	40	33.33	60	20
		0	60	0.00	0	0	0
		0	0	0	0	0	0
	Chronic[a]	0	0	0	0	0	0
		33.33	20	40	33.33	40	60
		0	0	0	0	0	20
		0	0	0	0	0	0
Fibroblastic proliferation[a]		0	0	0	0	0	0
		0	0	0	0	0	0
		0	0	40	0	0	0
		100	100	60	100	100	100
Collagen deposition[a]		0	0	0	0	0	0
		33.33	40	20	0	20	0
		0	60	80	33.33	60	20
		66.67	0	0	66.67	20	80
Myofibroblast proliferation[a]		0	0	0	0	0	0
		100	100	20	0	20	0
		0	0	80	33.33	80	20
		0	0	0	66.67	0	80

[a]In the order seen in Table 1.

Discussion

Optimization of wound-healing processes with light sources is widely described in the literature.[8,10] This optimization is due to the effect of several wavelengths on the cells, and this is particularly seen on cells under some type of stress. However, the effects of light on thermally damaged tissues are not well reported in the literature.

Surgical laser wounds involve a different healing process, especially for wounds produced by wavelengths that cause heating of the tissues. This heating results in cell damage of different intensities depending on the distance from the impact site. These changes are mainly seen within the first week of the repair. The lack of cell viability on the thermally damaged area is considered the main factor in delaying re-epithelialization.[6] The literature also reports the presence of fewer myofibroblasts on this lesion. This aspect is the cause of minimal wound contraction and scarring.[31]

In the present study, we found that wounds treated with laser phototherapy (GII) showed better response at day 14 when re-epithelialization was in a more advanced stage. It was also found that the number of myofibroblasts on these subjects was significantly different over the healing time (7 to 14 days). However, this number was smaller than that observed on controls, as previously reported. It has been reported that the use of high doses does not improve the proliferation of these cells. It is possible that such higher doses may further stress the cells and reduce migration and proliferation, viability, and mitochondrial activity. Our results suggest that smaller doses (4 to 8 J/cm^2) may be more effective in inducing an increase of myofibroblastic proliferation.

The positive effect on myofibroblastic proliferation was also found on illuminated subjects at early stages of the healing process. At day 7, the number of myofibroblasts was significantly higher than on laser-treated subjects.[19] An overall tendency for a more advanced process was also detectable in this group.[3] The tendency for an increase in the number of myofibroblasts was also seen at the end of the experimental period, as well as a more pronounced deposition of collagen matrix. Inflammation was discrete and more advanced on illuminated subjects.[3] The overall observations of the present study are suggestive of more advanced repair on illuminated subjects when compared wih control and laser-treated subjects.

There is an overall tendency to use polychromatic light sources for the treatment of wounds. This use increases as positives results are reported elsewhere. It is important to note that the benefits reported in the literature are very similar to those reported when monochromatic light sources are used. [3]There are also reports that the use of polychromatic noncoherent light possesses beneficial effects for wound healing, mainly for fibroblasts and myofibroblasts, as found in the present study.[19] It is very important that specific protocols be observed owing to the differences in many parameters observed in the polarized light sources currently marketed.[3]

When using thermal lasers such as those in the present study, the observed delay in healing, owing to cellular damage, is caused by heat.[2] However, this negative effect may be minimized by the use of certain wavelengths, including polarized light. In the present study, we found a significant increase in the number of myofibroblasts at early stages, more advanced re-epithelialization during the experimental time, and increased collagen deposition on illuminated subjects.

The results of the present study have shown that the effect of using laser light was more evident at early stages of the healing and that using polarized light improved the resolution of the inflammatory reaction, increased the deposition of collagen, increased the number of myofibroblasts, and quickened re-epithelialization during the experimental time.

Author Disclosure Statement

No competing financial interests exist.

References

1. Azevedo, L.H. (2005). Processo reparacional em tecidos cutâneo e oral de ratos submetidos à incisão com lasers de CO_2 e Diodo, e com bisturi elétrico e convencional. Uma análise morfométrica. 67 p. Thesis (PhD, Dentistry). Faculdade de Odontologia da Universidade de São Paulo, São Paulo, Brazil.
2. D'arcangelo, C., Di Maio, F.D.N., Prosper I, G.D., Conte, D., Baldi, M., and Caputi, S. (2007). A preliminary study of healing of diode laser versus scalpel incisions in rat oral tissue: a comparison of clinical, histological, and immunohistochemical results. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 103, 764–773.
3. Pinheiro, A.L., Meireles, G.C., Carvalho, C.M., de Barros Vieira, A.L., dos Santos, J.N., and Ramalho, L.M. (2006). Biomodulative effects of polarized light on the healing of cutaneous wounds on nourished and undernourished Wistar rats. Photomed. Laser Surg. 24, 616–624.
4. Rizzo, L.B., Ritchey, J.W., Higbee, R.G., Bartels, K.E., and Lucroy, M.D. (2004). Histologic comparison of skin biopsy specimens collected by use of carbon dioxide or 810-nm diode lasers from dogs. J. Am. Vet. Med. Assoc. 225, 1562–1566.
5. Pinheiro, A.L., Cavalcanti das Neves, J., Lisboa de Castro, J.F., et al. (2002). Comparison of the CO_2 laser and chlorohexidine on the descontamination of infected cutaneous wounds: a histologic study in rats. J. Clin. Laser Med. Surg. 20, 123–127.
6. Romanos, G.E., Henze, M., Banihashemi, S., Parsanejad, H.R., Winckler, J., and Nentwig, G.H. (2004). Removal of epithelium in periodontal pockets following diode (980 nm) laser application in the animal model: an in vitro study. Photomed. Laser Surg. 22, 177–183.
7. Mison, M.B., Steficek, B., Lavagnino, M., Teunissen, B.D., Hauptman, J.G., and Walshaw, R. (2003). Comparison of the effects of the CO_2 surgical laser and conventional surgical techniques on healing and wound tensile strength of skin flaps in the dog. Vet. Surg. 32, 153–160.
8. Gál, P., Vidinský, B., Toporcer, T., et al. (2006). Histological assessment of the effect of laser irradiation on skin wound healing in rats. Photomed. Laser Surg. 24, 480–488.
9. Aimbire, F., Albertini, R., Pacheco, M.T.T., et al. (2006). Low-level laser therapy induces dose-dependent reduction of TNF levels in acute inflammation. Photomed. Laser Surg. 24, 33–37.
10. Medrado, A.R., Pungliesi, S., Reis, S.R., and Andrade, Z.A. (2003). Influence of low level laser therapy on wound healing and its biological action upon myofibroblasts. Laser Surg. Med. 32, 239–244.

11. Ferreira, D.M., Zângaro, R.A., Balbin Villaverde, A., et al. (2005). Analgesic effect of He–Ne (632.8 nm) low-level laser therapy on acute inflammatory pain. Photomed. Laser Surg. 23, 177–181.
12. Nascimento, P.M., Pinheiro, A.L., Salgado, M.A., and Ramalho, L.M. (2004). A preliminary report on the effect of laser therapy on the healing of cutaneous surgical wounds as a consequence of an inversely proportional relationship between wavelength and intensity: histological study in rats. Photomed. Laser Surg. 22, 513–518.
13. Rabelo, S.B., Villaverde, A.B., Nicolau, R.A., Salgado, M.A.C., Melo, M.S., and Pacheco, M.T.T. (2006). Comparison between wound healing in induced diabetic and nondiabetic rats after low-level laser therapy. Photomed. Laser Surg. 24, 474–479.
14. Matić, M., Lazetić, B., Poljacki, M., Duran, V., and Ivkov-Simić, M. (2003). Low level laser irradiation and its effect on repair processes in the skin. Med. Pregl. 56, 137–141.
15. Karu, T. (1999). Primary and secondary mechanisms of action of visible to near-IR radiation on cells. J. Photochem. Photobiol. B: Biol. 49, 1–17.
16. Amat, A., Rigau, J., Waynant, R.W., Ilev, I.K., and Anders, J.J. (2006). The electric field induced by light can explain cellular responses to electromagnetic energy: a hypothesis of mechanism. J. Photochem. Photobiol. B. 82, 152–160.
17. Karu, T. (2003). Tuan Vo-Dinh. In: Low-Power Laser Therapy. Biomedical Photonics Handbook. Boca Raton, FL: CRC Press, pp. 1–25.
18. Enwemeka, C.S. (2006). The place of coherence in light induced tissue repair and pain modulation. Photomed. Laser Surg. 24, 457.
19. Pinheiro, A.L., Pozza, D.H., Oliveira, M.G., Weissmann, R., and Ramalho, L.M. (2005). Polarized light (400–2000 nm) and non-ablative laser (685 nm): a description of the wound healing process using immunohistochemical analysis. Photomed. Laser Surg. 23, 485–492.
20. Al Watban, F. (2006). Polychromatic LED in oval full-thickness wound healing in non-diabetic and diabetic rats. Photomed. Laser Surg. 24, 10–16.
21. Schanaider, A., and Silva, P.C. (2004). Uso de animais em cirurgia experimental. Acta. Cir. Bras. 19, 441–447.
22. Massone, F. (1994). Anestesiologia Veterinária: Farmacologia e Técnicas. Rio de Janeiro, Brazil: Guanabara Koogan, p. 107.
23. Meireles, G.C., Santos, J.N., Chagas, P.O., Moura, A.P., and Pinheiro, A.L. (2005). Effectiveness of laser photomodulation at 660 or 780 nanometers on the repair of third-degree burns in diabetic rats. Photomed. Laser Surg. 26, 47–54.
24. Hawkins, D., and Abrahamse, H. (2006). Effect of multiple exposures of low-level laser therapy on the cellular responses of wounded human skin fibroblasts. Photomed. Laser Surg. 24, 705–714.
25. Hawkins, D., and Abrahamse, H. (2006). The role of laser fluence in cell viability, proliferation, and membrane integrity of wounded human skin fibroblasts following helium–neon laser irradiation. Laser Surg. Med. 38, 74–83.
26. Eichler, M., Lavi, R., Friedmann, H., Shainberg, A., and Lubart, R. (2007). Red light-induced redox reactions in cells observed with TEMPO. Photomed. Laser Surg. 25, 170–174.
27. Amat, A., Rigau, J., Nicolau, R.A., et al. (2004). Effect of red and near-infrared laser light on adenosine triphosphate (ATP) on the luciferine–lucifrase reaction. J. Photochem. Photobiol. A: 168, 59–65.
28. Karu, T.I., and Kolyakov, S.F. (2005). Exact action spectra for cellular responses relevant to phototherapy. Photomed. Laser Surg. 23, 355–361.
29. Grigor'ian, A.S., Grigor'iants, L.A., and Kasparov, A.S. (2006). Experimental–morphological study of the effects of diode laser scalpel with different irradiation parameters (wavelength 0.97 mm) on oral mucosa. Stomatologiia (Mosk). 85, 8–13.
30. Robbins, J.B., Reinisch, L., and Ellis, D.L. (2003). Wound healing of 6.45-micron free electron laser skin incisions with heat-conducting templates. J. Biomed. Opt. 8, 594–600.
31. Zeinoun, T., Nammour, S., Dourov, N., Aftimos, G., and Luomanen, M. (2001). Myofibroblasts in healing laser excision wounds. Lasers Surg. Med. 28, 74–79.
32. Dua, R., and Chakraborty, S. (2005). A novel modeling and simulation technique of photo-thermal interactions between lasers and living biological tissues undergoing multiple changes in phase. Comput. Biol. Med. 35, 447–462.
33. Wilder-Smith, P., Arrastia, A.M.A., Liaw, L.H., and Berns, M. (1995). Incision properties and thermal effects of three CO_2 lasers in soft tissue. Oral Surg. Oral Med. Oral Pathol. 76, 685–691.
34. De Freitas, A.C., Pinheiro, A.L., De Oliveira, M.G., and Ramalho, L.M. (2002). Assessment of the behavior of myofibroblasts on scalpel and CO_2 laser wounds: Na immunohistochemical study in rats. Photomed. Laser Surg. 20, 221–225.
35. Grbavac, R.A., Veeck, E.B., Bernard, J.P., Ramalho, L.M., and Pinheiro, A.L. (2006). Effects of laser therapy in CO_2 laser wounds in rats. Photomed. Laser Surg. 24, 389–396.
36. Hawkins, D., and Abrahamse, H. (2005). Biological effects of helium–neon laser irradition on normal and wounded human skin fibroblasts. Photomed. Laser Surg. 23, 251–259.
37. Houreld, N., and Abrahamse, H. (2007). In vitro exposure of wounded diabetic fibroblast cells to a helium–neon laser at 5 and 16 J/cm^2. Photomed. Laser Surg. 25, 78–84.
38. Al-Watban, F., and Andres, B.L. (2006). Polychromatic LED in oval full-thickness wound healing in non-diabetic and diabetic rats. Photomed. Laser Surg. 24, 10–16.
39. Hawkins, D., and Abrahamse, H. (2007). Influence of broad-spectrum and infrared light in combination with laser irradiation on the proliferation of wounded skin fibroblasts. Photomed. Laser Surg. 25, 159–169.
40. Al-Saadi, M.H., Nadeau, V., and Dickinson, M.R. (2006). A novel modeling and experimental technique to predict and measure tissue temperature during CO_2 laser stimuli for human pain studies. Lasers Med. Sci. 21, 95–100.
41. Kolkman, R.G.M., Steenbergen, W., and Leeuwen, T.G.V. (2006). In vivo photoacustic imaging of blood vessels with a pulsed laser diode. Lasers Med. Sci. 21, 134–139.

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