Photomedicine and Laser Surgery
Volume 24, Number 3, 2006

Pp. 389–396

Effects of Laser Therapy in CO_2 Laser Wounds in Rats

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ABSTRACT

Objective: **The aim of this study was to assess the effects of laser therapy and its possible dose dependency on the healing of CO_2 laser surgical wounds.** ***Background Data:*** **Several reports from our group and others have indicated that light therapies may improve healing, depending on wavelength, dose, intensity of the light, and both local and systemic conditions.** ***Methods:*** **Circular surgical wounds were created on the dorsum of Wistar rats, which were separated into three groups (A, B, and C). Group A acted as control and had no additional treatment. Groups B and C were irradiated with GaAlAs λ685-nm laser light, either with 20 J/cm² (Group B) or 40 J/cm² (Group C). The animals were humanely killed at the end of the experimental period; specimens were taken and routinely processed to wax and stained with Hematoxylin and Eosin, Sirius Red, and alpha–Smooth Muscle Actin (αSMA).** ***Results:*** **Laser-irradiated groups showed a healing process characterized by a more prominent fibroblastic proliferation, with young fibroblasts actively producing collagen; no myofibroblasts were found. No statistically significant differences were observed when the different doses were compared.** ***Conclusion:*** **It may be concluded that, using this methodology, laser therapy has a positive effect in wound healing produced by CO_2 laser, and the dose has no influence on the treatment.**

INTRODUCTION

WOUND HEALING is a complex process that involves several tissue responses, and many factors may delay or quicken it, amongst them the use of different types of lasers. It is well known that the improper use of the CO_2 laser causes a delay in the healing process because of the thermal damage that follows irradiation of living tissues. On the other hand, several reports have indicated that light therapies may improve healing, depending on wavelength, dose, intensity of the light, and both local and systemic conditions.[1–6]

The effects of laser therapy on wound healing have been extensively studied, and conflicting results have been reported. Many reports demonstrated a positive influence of several types of lasers on healing,[7–12] and others have found no effect at all.[13] This is a consequence of the different protocols used and of the settings of the laser therapy.[14,15]

Laser therapy is characterized by its ability to induce photobiological processes without significant thermal effect.[16–18] Laser therapy has also been used for pain relief, as an anti-inflammatory and as an anti-edematous agent.[19,20]

On the other hand, the CO_2 laser is an ablative laser widely used to cut, coagulate, or vaporize tissues. This laser is largely used in several specialized medical areas, as well as in dentistry, because of its affinity with the water content of the tissues.[18,21–24] Several benefits of its usage over conventional techniques have been previously reported and include local hemosthasis, which results in a dry and clean operative field; decontamination of infected wounds, because of the high temperature generated locally; reduced pain and edema, because of the cauterization of nerve endings and the sealing of lymphatic vessels on the wounded site; and less scarring and wound contraction, due to the small number and orientation of myofibroblasts. Additionally, this technique reduces post-operative complications as well as morbidity following surgery. These aspects also result in a reduction of costs for both the patient and the professional.[2,16,21,25–30]

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The aim of the present study was to assess the effect of laser therapy on the healing process of CO_2 laser wounds using two different doses.

METHODS

Eighteen young healthy male Wistar rats, weighing 300 g on average, were kept in individual cages, in natural conditions of temperature and brightness, fed with standard laboratory food pellets and given water *ad libitum* during the experimental period. The animals were submitted to both the surgical procedure and laser therapy at the Laboratory of Animal Experimentation of the School of Dentistry of the Federal University of Bahia, Brazil. Under general anesthesia (intramuscular injection of 0.3 ml/Kg of Zoletil®), the animals had their back manually shaven and washed with saline, and a 2% alcoholic iodine solution was applied to the surface, which was then dried with sterile gauze. One standardized circular wound (φ 1.5 cm^2) was created on the dorsum of each animal using a CO_2 laser (Sharplan 20C®, λ10,600 nm, 4 W, Superpulse, φ ~0.2 mm, 5 mm/sec). The animals were divided into three groups (A, B, and C) with six animals in each (six wounds). The animals in Group A acted as controls and received no further treatment. In groups B and C, the animals were treated with laser therapy (Thera Lase®; DMC Equipamentos, São Paulo, Brazil; GaAlAs, λ685 nm, CW, 40 mW, φ~0.06 mm) either with 20 or 40 J/cm^2. The animals were manually restrained for irradiation. The laser probe was applied on contact mode, perpendicularly to the wound surface, in four points placed 0.5 mm off the margins of the wound, fractionating the dose of each session. The equipment used automatically adjusted irradiation time according to the intensity. Laser therapy was performed immediately after laser surgery and repeated at 48-h intervals during 7 consecutive days. No additional treatment was provided to any group. Eight days after wounding, the animals were sacrificed and specimens taken. The specimens were coded, kept on 10% formalin solution during 24 h, and routinely processed to wax cut and stained with hematoxylin and eosin (H&E), Sirius red stains, and immunostained with alpha–Smooth Muscle Actin (α-SMA) and examined under light microscopy by an experienced pathologist at the Laboratory of Oral Pathology of the School of Dentistry of the Federal University of Bahia. The pathologist was not informed of the meaning of the coding of the specimens. The following parameters were used for histological analysis: edema, crust, neovascularization, fibroblastic proliferation, collagen deposition and distribution, and reepithelialization. Hyperemia, fibrinous exudate, inflammatory infiltrate, and the presence of myofibroblasts were graded as follows: absent (0), mild (1), moderate (2), or severe (3).

RESULTS

Descriptive and semi-quantitative report of light microscopy of H&E-stained specimens was performed. Sirius red–stained specimens were used to describe collagen fibers, and immunohistochemistry for α-SMA allowed description of myofibroblasts.

On control specimens, the cutaneous wound was characterized by the presence of intensely vascularized granulation tissue. In 33.3% of the cases, the presence of a mononuclear inflammatory infiltrate was seen. In 66.7%, the inflammatory infiltrate was of mixed cellularity. The intensity of the inflammatory infiltrate was variable, and was considered moderate in 66.7% of the cases and severe in 33.3%. Neutrophils were predominantly seen on the surface of the wound or associated to the presence of a coagulated fibrinous exudate, which was observed on two specimens. Subjacent connective tissue showed a smaller number of fibroblasts when compared to the experimental groups (Fig. 1). A discrete deposition of collagen fibers was also observed in this group, and these were located parallel to the wound surface (Fig. 2). Immunomarking for α-SMA showed myofibroblasts sparsely distributed close to the wound surface (Fig. 3).

On animals treated with laser therapy (20 J/cm^2), the wound was characterized by the presence of an inflammatory infiltrate of mixed cellularity of severe intensity in 66.7% of the cases. On 50% of the specimens, eosinophilic amorphous areas, and neutrophils and lymphocytes were observed and considered as areas of fibrinous exudation (Fig. 4). The granulation tissue was largely populated by young fibroblasts (Fig. 5). Sirius red stain evidenced large amount of collagen fibers (Fig. 6). No myofibroblasts were detected by α-SMA immunomarking.

After increasing the dose to 40 J/cm^2, the wound was covered by a neutrophilic serous infiltrate, and beneath it, a highly vascularized granulation tissue was observed. Inflammatory response was characterized by a mixed cellularity inflammatory infiltrate of variable intensity. Congested and dilated blood vessels whose lumer were rich in neutrophils were also evident, as were areas of fibrinous exudation. Intense fibroblastic proliferation was evidenced, and these cells were mainly young. No immunomarked cells were seen in this group (Fig. 7).

Statistical analysis

After the qualitative analysis, the data were statistically analyzed by a Mann-Whitney test. The results showed significant differences, when comparing both laser-treated groups and their controls, for fibrinous exudate and quantity of myofibroblasts, but no differences were found between the two laser groups (Tables 1 and 2).

DISCUSSION

Despite the fact that the effects of laser therapy as a biomodulator of both cell responses and wound healing have already been reported by both *in vivo* and *in vitro* studies,[1,3,4,6–12] its effects on the healing of CO_2 laser wounds have not been described. Because of this, the findings of the present study are difficult to compare.

The use of laser therapy for wound healing was initially described as early as 1960. Because of its ability to either stimulate or inhibit tissue responses, the term "biostimulation" was changed to "biomodulation."[32] Several reports have shown that major components of the healing process are affected by several wavelengths, which include: fibroblastic proliferation,

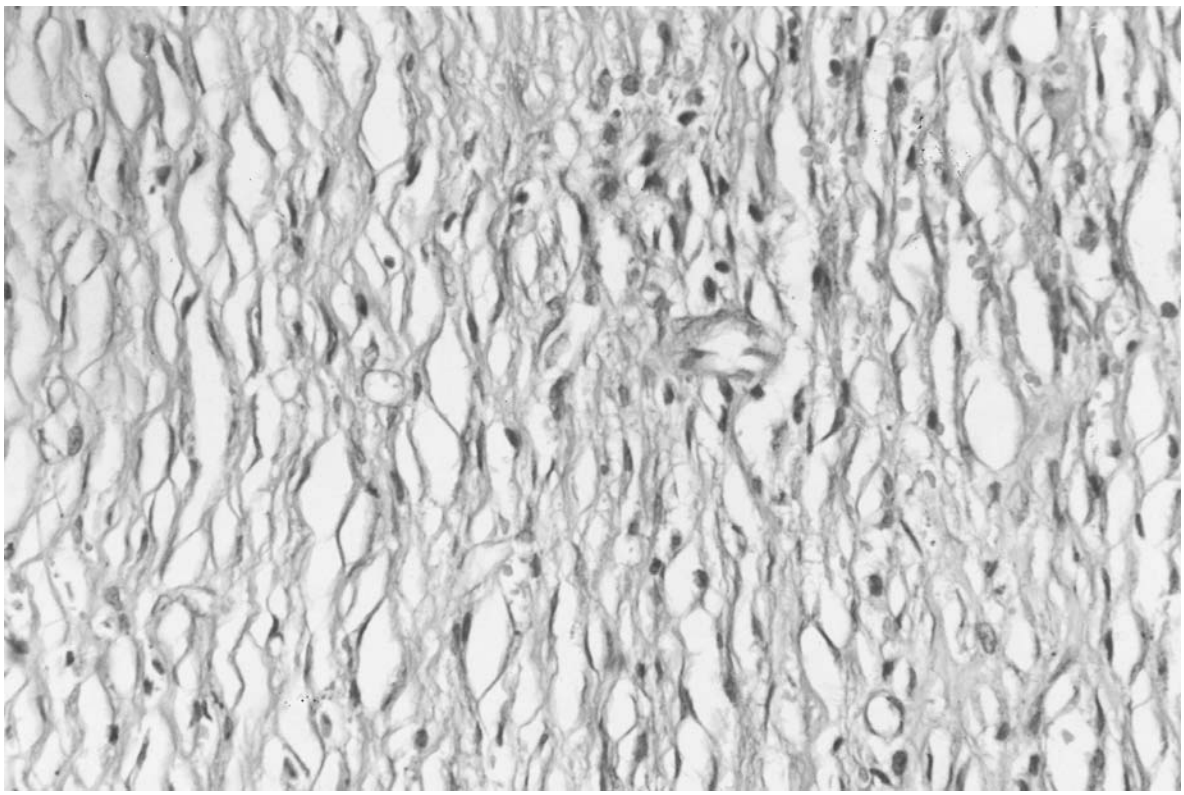

FIG. 1. Photomicrograph of group A showing fibrous connective tissue and absence of inflammatory infiltrate (Hematoxylin-Eosin stain; original magnification, ×200).

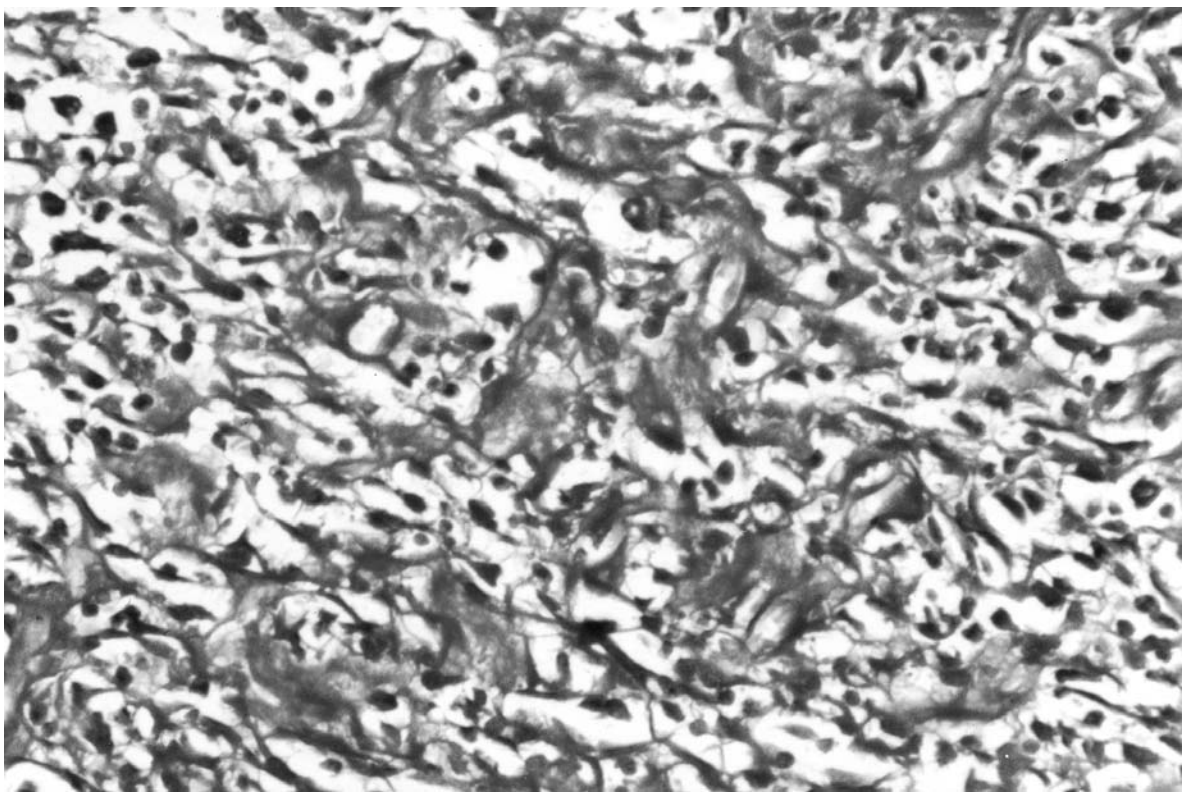

FIG. 2. Photomicrograph of group A showing discrete deposition of collagen matrix (immunohistochemistry by Sirius Red stain; original magnification, ×400).

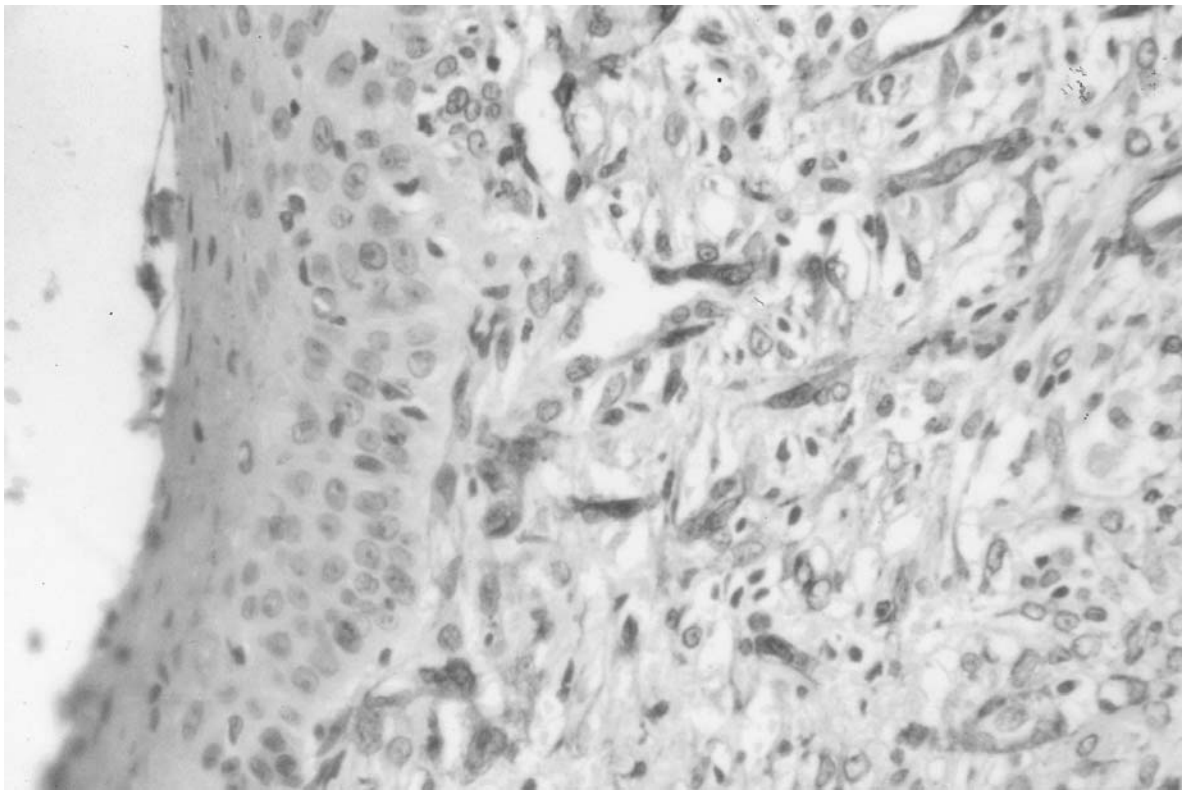

FIG. 3. Photomicrograph of group A showing positive stain of myofibroblasts for α-SMA immunohistochemistry in wound lateral edge (Streptavidin-Biotin immunoperoxidase stain; original magnification, ×400).

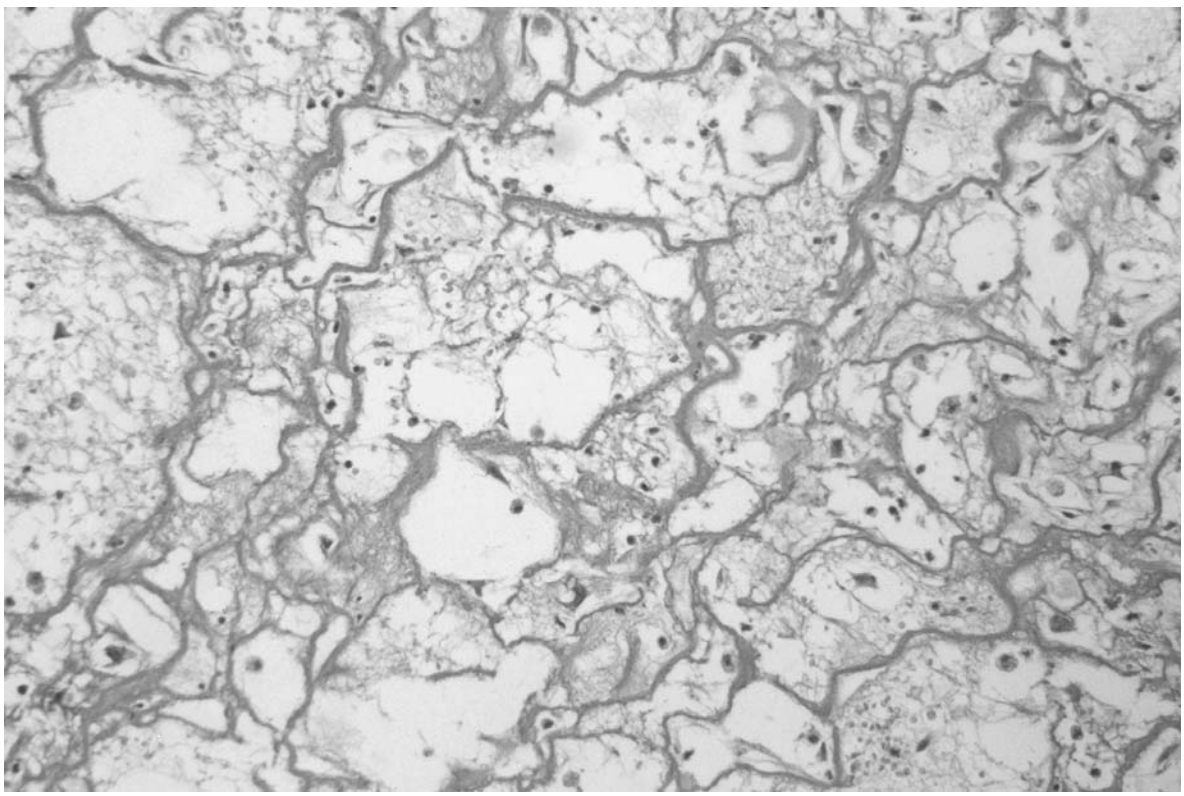

FIG. 4. Photomicrograph of group B. Fibrinous inflammatory exudate was observed (Hematoxylin-Eosin stain; original magnification, ×100).

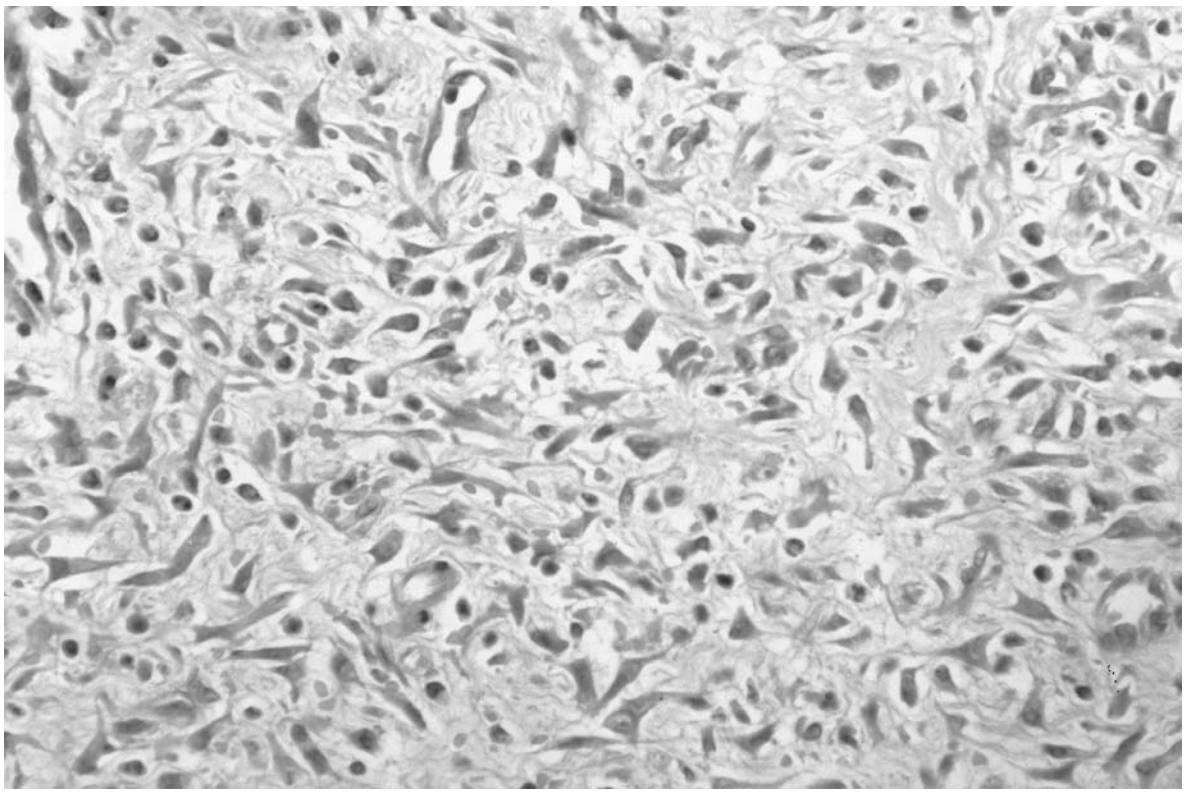

FIG. 5. Photomicrograph of group B showing granulation tissue characterized by intense proliferation of young fibroblasts and inflammatory cells (Hematoxylin-Eosin stain; original magnification, ×200).

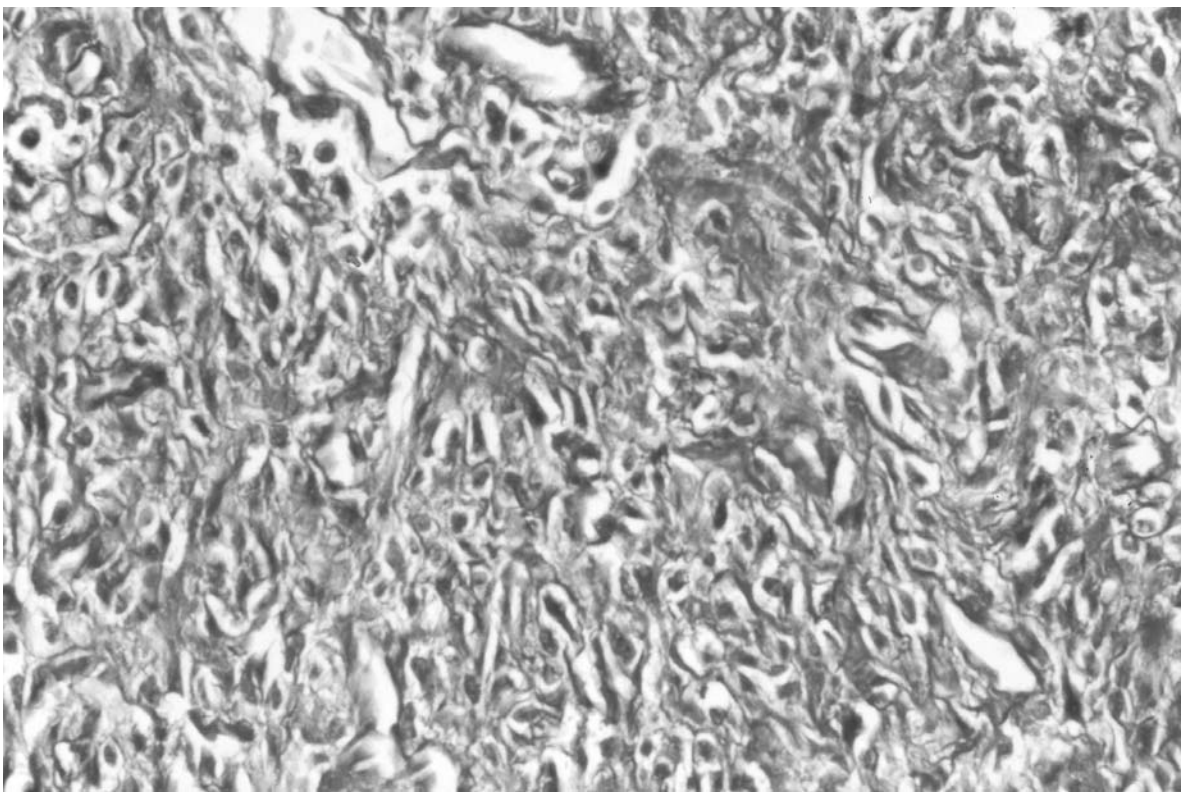

FIG. 6. Photomicrograph of group B showing oblique deposition of collagen fibers (immunohistochemistry by Sirius Red stain; original magnification, ×400).

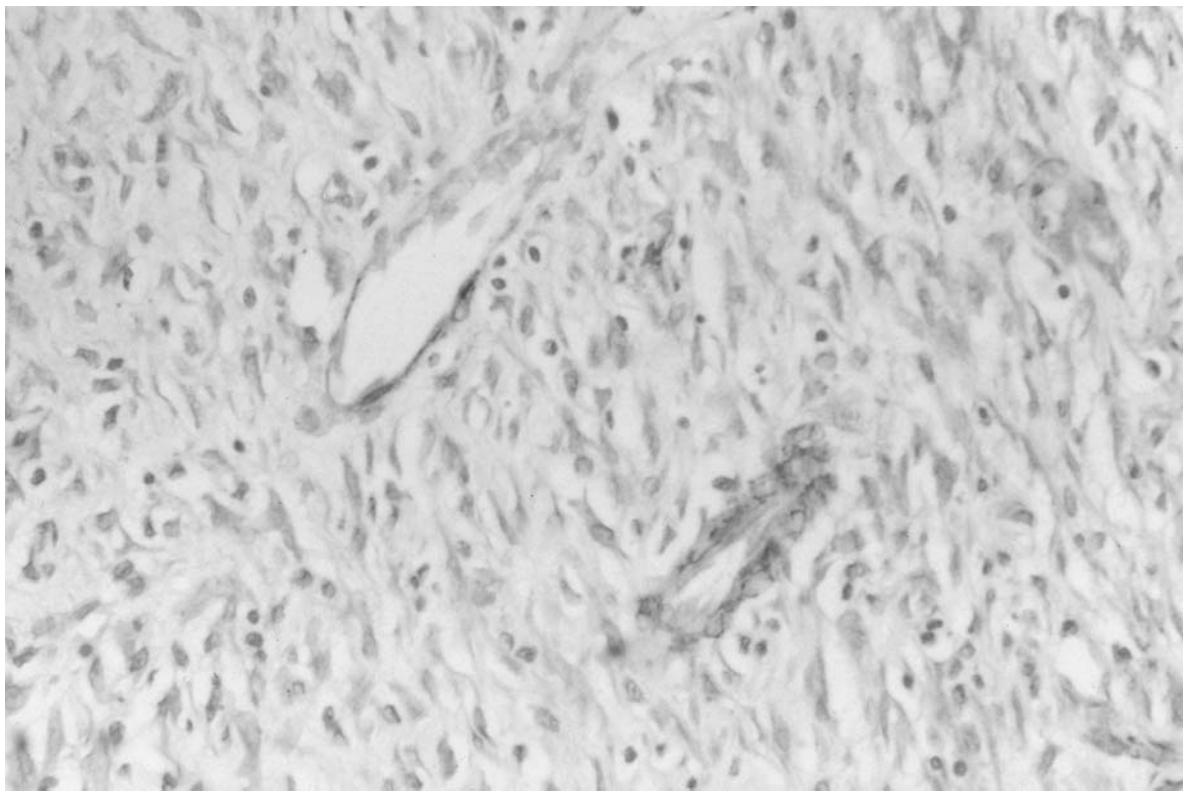

FIG. 7. Photomicrograph of group C. No myofibroblasts were stained by α-SMA immunohistochemistry. Positive stain of internal control represented by vessel wall, (Streptavidin-Biotin immunoperoxidase stain; original magnification, ×400).

proliferation of keratinocytes, collagen synthesis and deposition, and increased angiogenesis.[1,3,4,6,8]

Collagen synthesis and other components of the connective tissue are important for the healing process at early stages. However, this process has to be self-controlled in order to prevent the formation of hypertrophic scars.

The positive biomodulation of laser therapy on fibroblastic proliferation[1,6,33] and on collagen synthesis and deposition[6] are well described in current literature. In the present investigation, a telling number of fibroblasts was observed on irradiated subjects when compared to their controls. These cells were predominantly young and very active in collagen production. Despite the fact that the collagen organization observed in the present study was not to the same as a previous report from our group,[6] it was similar to another report,[7] which suggested that laser therapy influences collagen synthesis but does not significantly affect collagen organization.

One reported advantage of the use of the CO_2 laser on soft tissue is the production of a more aesthetic scar and less wound contraction, these aspects being attributed to the presence of a small number of myofibroblasts.[2,5,26] Further to these findings, in our study these cells were not found on CO_2 laser wounds treated with laser therapy.

TABLE 1. FIBRINOUS EXUDATE ON CO_2 LASER CUTANEOUS WOUNDS

	Group					
	C		*L20*		*L40*	
Parameter	*n*	*%*	*n*	*%*	*n*	*%*
Fibrinous exudate						
Absent	4	66.7	—	—	—	—
Mild	2	33.3	5	83.3	2	33.3
Moderate	—	—	—	—	2	33.3
Severe	—	—	1	16.7	2	33.3

C, control; L20, laser therapy 20 J/cm^2; L40, laser therapy 40 J/cm^2; comparison between C and L20, $p = 0.041$; C and L40, $p = 0.009$.

TABLE 2. MYOFIBROBLASTS ON CO_2 LASER CUTANEOUS WOUNDS

	Group					
	C		*L20*		*L40*	
Parameter	*n*	*%*	*n*	*%*	*n*	*%*
Myofibroblasts						
Absent	—	—	6	100	6	100
Mild	—	—	—	—	—	—
Moderate	6	100	—	—	—	—
Severe	—	—	—	—	—	—

C, control; L20, laser therapy 20 J/cm²; L40, laser therapy 40 J/cm²; comparison between C and L20, $p = 0.002$; C and L40, $p = 0.002$.

Dose dependency of the biomodulatory effects of laser therapy was described by several authors.[1,3,4,6,33] Previous reports suggested that higher doses usually do not result in visible effects or show inhibitory results on cells.[33] In this study, the changes of dose had no detectable effect. This finding further supports the idea that smaller doses are effective. However, it has to be considered that a determined wavelength used with a same dose may result in different responses, depending on the type of irradiated tissue or on the pathology being treated.[33] This may indicate that fibroblasts and myofibroblasts may react differently to a given parameter as seen in the present investigation.

It has also been suggested that laser therapy produces some systemic effects,[34,35] which would explain the absence of differences between irradiated wound and contra-lateral wounds which were used as controls on other reports.[14] Some reports commented on by Túner and Hode,[15] did not show the effects of laser therapy. These results may be explained by the use of inappropriate parameters for the treatment.

The mechanisms by which laser light affects tissues are still not well established but it has been suggested that the effects may be due to the stimulation of vitamin C on cells,[36] the stimulation of photoreceptors of the respiratory chain of mitochondria,[37] changes on the level of cell ATP or cAMP and the stability of the cell membrane.[8]

CONCLUSION

The results of the present study suggest that the use of GaAlAs λ685nm laser light had a positive biomodulatory effect on the proliferation of fibroblasts and a negative effect on the proliferation of myofibroblasts using doses of 20 or 40 J/cm² on CO_2 laser wounds. This could be very interesting in cases where aesthetics and functionality are important for the success of the scar treatment.

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