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Effects of Laser Photobiomodulation on Cutaneous Wounds Treated with Mitomycin C: A Histomorphometric and Histological Study in a Rodent Model

Nicole R. Silva Santos, Ph.D.,[1] Jean N. dos Santos, Ph.D.,[2] João B. Macedo Sobrinho, Ph.D.,[3] Luciana M.P. Ramalho, Ph.D.,[4] Carolina M. Carvalho, Ph.D.,[1] Luiz G.P. Soares, D.D.S.,[1] and Antônio L.B. Pinheiro, Ph.D., MDentSci[1]

Abstract

Aim: The aim of the present study was to assess histologically the effect of Laser Photobiomodulation (LPBM) on skin wounds treated with Mitomycin C (MMC). ***Background Data:*** Wound healing occurs because of a competitive mechanism between the synthesis and lyses of collagen. Therefore, any factor that increases the lyses or reduces the synthesis of collagen may result in changes in the healing process. MMC is an antineoplastic drug that inhibits fibroblast proliferation, collagen synthesis, and neoangiogenesis. LPBM has been shown to stimulate wound healing, increasing the production of collagen, fibroblastic proliferation, and angiogenesis. ***Materials and Methods:*** Forty-eight Wistar rats were randomly distributed into 4 main groups ($n = 12$): G1 − control (G1a − 7 d and G1b − 14 d); G2 − MMC (G2a − 7 d and G2b − 14 d); G3 − MMC + λ660 nm laser (G3a − 7 d and G3b − 14 d); and G4 − MMC + λ790 nm laser (G4a − 7 d and G4b − 14 d). Under general anesthesia, one excisional wound was created on the dorsum of each animal. Two ml of MMC solution was applied to the wound 4 h after surgery for 5 min. LPBM was performed on groups G3 (λ690 nm; 20 J/cm^2; 30 mW; $\Phi = 2$ mm) and G4 (λ790 nm; 20 J/cm^2; 40 mW; $\Phi = 2$ mm), starting immediately after the application of the MMC and repeated every other day during the experimental period. Laser light was applied transcutaneously at 4 equidistant points on the wound margin (4×5 J/cm^2, 20 J/cm^2/session). The specimens were routinely cut and processed to wax. The slides were stained with HE and Sirius red. Computerized hystomorphometry was performed. ***Results:*** LPBM resulted in reduced inflammation and an increase in both fibroblast proliferation and collagen deposition. ***Conclusion:*** The use of LPBM improves wound healing in subjects treated with MMC.

Introduction

The lack of integrity of the skin or the mucous membrane is called a wound. A wound represents the interruption, anatomical or functional, of the continuity of the tissue that is followed by damage or cellular death. Wound healing occurs because of a competitive mechanism between the synthesis and lyses of collagen. Therefore, any factor that increases the lyses or reduces the synthesis of collagen may result in changes in the healing process.[1]

The body uses several tissue reactions in order to recover its integrity. There are several mechanisms involved in the healing of wounds, including the differentiation, proliferation, and migration of the cells and their interaction with the matrix, or the replacement of the lost tissue by a less-differentiated one. These two mechanisms are known as regeneration and wound healing.[2–4.]

Most living tissues heal by the second mechanism, which is characterized by the formation of granulation tissue and contraction of myofibrolasts. There are several hypotheses on the origin of myofibroblasts, among them their differentiation from fibroblasts or stem cells. This cell is contractile and possesses characteristics of both the fibroblast and the smooth-muscle cell.[5]

There are several conditions that may affect the healing process. These include systemic or local disorders, as well as the use of some drugs.[1] Mitomycin C (MMC) is an antineoplastic antibiotic. It is an alkylating agent that inhibits DNA synthesis by forming a cross linkage of strands of double helix so that the neoplastic cells cannot proliferate. It inhibits cell

[1]Laser Center, School of Dentistry, Federal University of Bahia (UFBA), Salvador, Brazil.
[2]Oral Pathology, Federal University of Bahia (UFBA), Salvador, Brazil.
[3]Laser Center, IAPPEM, Salvador, Brazil.
[4]Oral Medicine, Federal University of Bahia (UFBA), Salvador, Brazil.

Table 1. Animal Distribution on the Experimental and Control Groups

Group	n	Treatment	Time of animal death (d)
G1a	6	Control	7
G1b	6	Control	14
G2a	6	Mytomicin C	7
G2b	6	Mytomicin C	14
G3a	6	λ660 nm laser	7
G3b	6	λ660 nm laser	14
G4a	6	λ790 nm laser	7
G4b	6	λ790 nm laser	14

division, fibroblast proliferation, protein and collagen synthesis, and neoangiogenesis. This has been demonstrated in vitro and in vivo using the rat model.[6–11] MMC decreases fibrosis in wounds treated with its topical form.[7] When used topically, this agent appears to be well tolerated, with no apparent side effects when applied to skin-incision sites.[10,11] Topical MMC applied for 5 min in a laser-induced injury of the posterior glottis in rabbits significantly prevented gross injury and subsequently increased collagen content and fibroblast proliferation compared with sham-treated controls.[8] There are still uncertainties regarding the dose effects of MMC on wound healing and its effect on different types of wounds, since an awareness of the mechanisms involved in wound healing is very important for understanding the possible effects of therapeutic methods that may help or improve the process. The healing of skin wounds requires the participation of several cell lineages, and each one of these cells may react differently to the therapeutic used.[11]

The incessant search for methods to minimize pain, to minimize or quicken the inflammatory response, and to simulate cell function and proliferation without harming the tissues are highlighted for those using light sources.[2] Laser Photobiomodulation (LPBM) is used in many biomedical sciences to promote tissue regeneration, and it has been shown to possess advantages such as the control of the pain, stimulation of the healing process, anti-inflammatory action, and increase in the production of collagen on the fibroblastic proliferation, as well as an increase in the local microvascularization. Most studies on the effects of phototherapies on the healing process have attributed the observed effects to several treatment parameters and the properties of the light source used.[12,13] The aim of the present study was to assess histologically the effect of LPBM on skin wounds treated with MMC.

Materials and Methods

Following the approval of the Animal Experimentation Ethics Committee of the School of Dentistry at the Federal University of Bahia, 48 young adult male Wistar rats weighing approximately 275 g were obtained from the Centro de Criação de animais da Faculdade de Medicina Veterinária da Universidade Federal da Bahia. The animals were kept at the School's animal experimentation laboratory in individual plastic cages bedded with wood chips and maintained at 22°C in a day/night light cycle. The animals were fed with a standard pelted laboratory diet and had water ad libidum. After regular quarantine, the animals were randomly distributed into four main groups ($n = 12$): G1 − control (G1a − 7 d and G1b − 14 d); G2 − MMC (G2a − 7 d and G2b − 14 d); G3 − MMC + λ660 nm laser (G3a − 7 d and G3b − 14 d); and G4 − MMC + λ790 nm laser (G4a − 7 d and G4b – 14 d) (Table 1). Under general anesthesia (Tiopental®, Cristália, Itapira, Brazil, 50 mg/kg), the animals' backs were shaved and cleaned with a 2% chlorohexidine solution. One excisional wound measuring 1 cm^2 was created on the dorsum of each animal with an adapted scalpel containing two parallel blades. The depth of the wound was controlled by the length of the bevel of the scalpel blades. Two ml of MMC solution (MITOCIN®, 0.5 mg/ml, Bristol Myer Squibb, São Paulo, Brazil) was dripped on sterilized gauze covering the wound 4 h after surgery. The gauze was removed after 5 min, and the area was irrigated with 20 ml of saline solution. The wounds were left to heal by secondary intention; neither suture nor dressings were used in any case. Bleeding was controlled by compression with sterile gauze pads. The animals from each group were then divided into two subgroups according to the time of death (Table 1).

LPBM was performed on groups G3 (λ660 nm; 20 J/cm^2; 30 mW; $\Phi \sim 2$ mm, Kondortech, São Carlos, SP, Brazil) and G4 (λ790 nm; 20 J/cm^2; 40 mW; $\Phi \sim 2$ mm, Kondortech, São Carlos, SP, Brazil), starting immediately after the application of the MMC and repeated every other day until the day

Table 2. Criteria Used for Light Microscopy

Criterion	Score		
Edema[a]	Absent	Present	
Crust formation[a]	Absent	Present	
Neoangiogenesis[a]	Absent	Present	
Epithelial pavementing	Partial (<50%)	Complete (>50%)	
Hyperemia[b]	Discrete presence (<25%) of congested blood vessels	Moderate presence (25–50%) of congested blood vessels	Intense presence (>50%) of congested blood vessels
Fibrinous exsudate[b]	Discrete presence (<25%) of edema	Moderate presence (25–50%) of edema	Intense presence (>50%) of edema
Inflammatory infiltrate[a]	Discrete presence (<25%) of infl. cells	Moderate presence (25–50%) of infl. cells	Intense presence (>50%) of infl. cells
Fibroblastic proliferation[b]	Discrete presence (<25%) of fibroblasts	Moderate presence (25–50%) of fibroblasts	Intense presence (>50%) of fibroblasts

[a]Criterion set as Absent (0) or Present (1).
[b]Criterion set as Absent (0), Discrete (1), Moderate (2), or Intense (3).

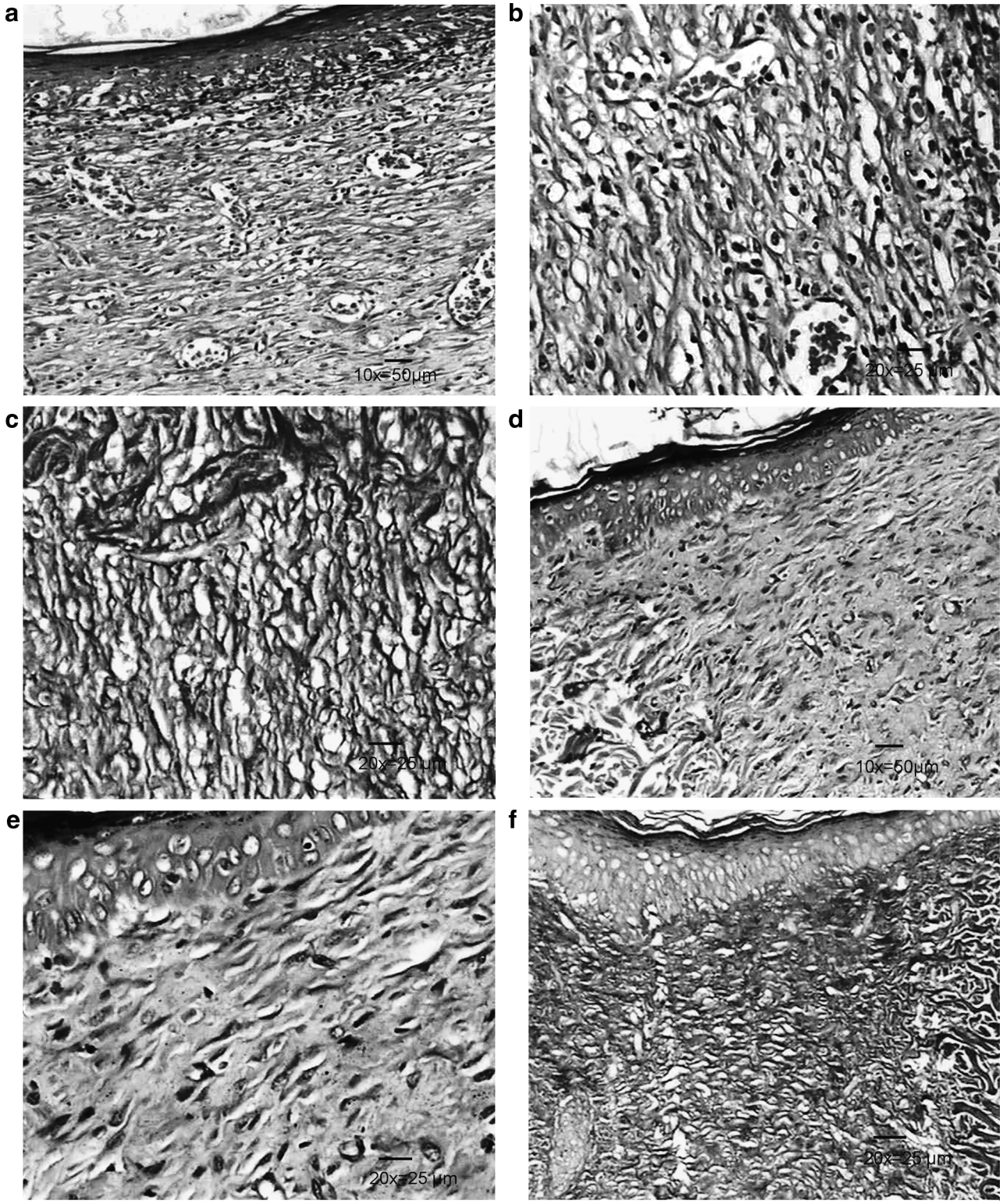

FIG. 1. (**a**) Photomicrography of the control specimen 7 d after surgery, showing epithelial pavementing and inflammatory infiltrate varying from discrete to moderate. HE, approximately×40. (**b**) Areas of chronic inflammatory infiltrate, congested blood vessels, and interstitial edema were also seen at this stage. HE, approximately×100. (**c**) Intense collagen deposition and the presence of fibroblasts were also detectable. Sirius red, approximately×100. (**d**) On day 14, epithelial pavementing and a discrete inflammatory reaction were seen. HE, approximately×40. (**e**) Fibroblastic proliferation was also observed at this stage. HE, approximately×100. (**f**) Collagen deposition varied from moderate to intense and fibroblasts were seen at the end of the experimental period. Sirius red, approximately×100.

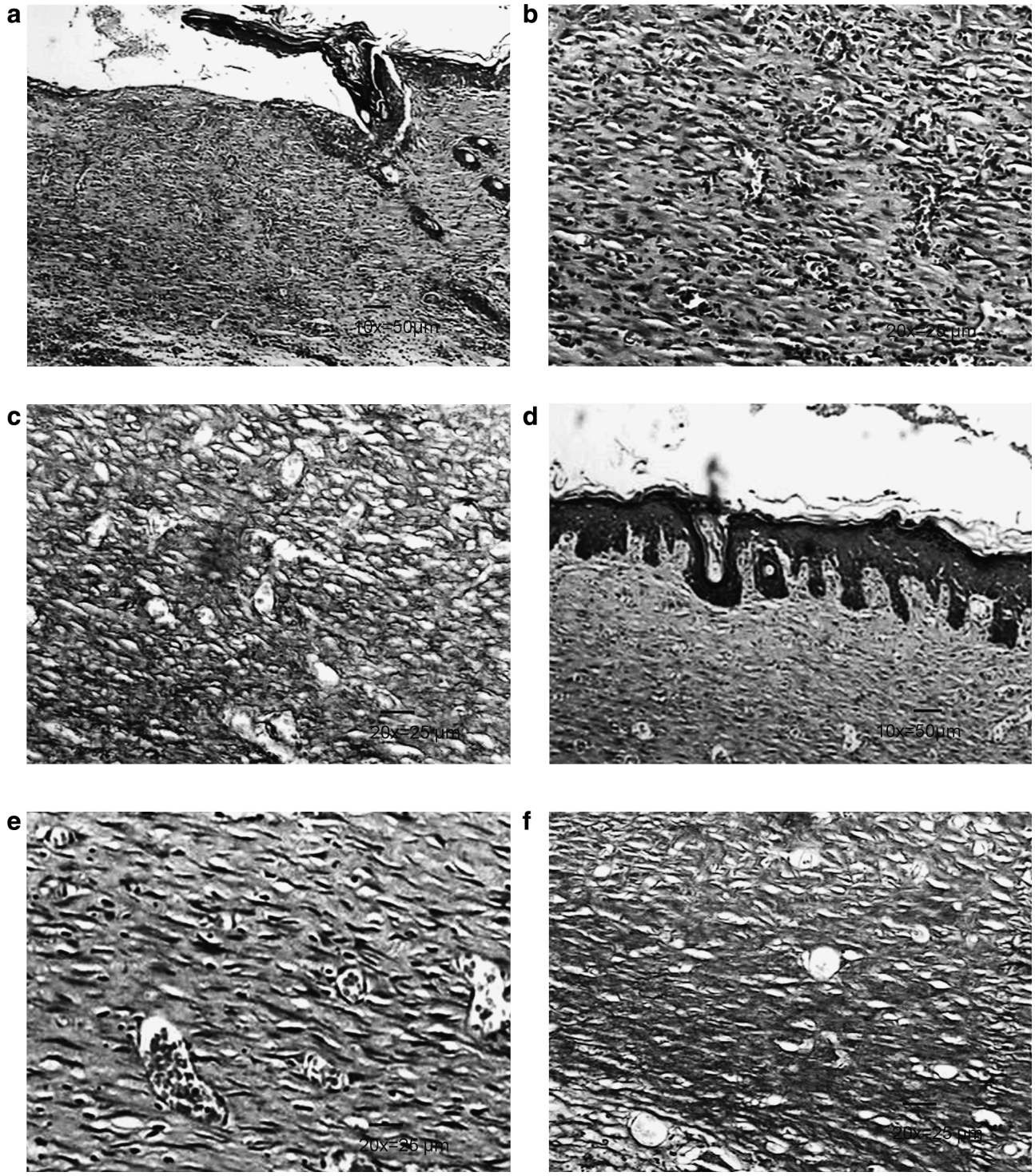

FIG. 2. (**a, b**) Photomicrography of the MMC-treated specimen 7 d after surgery, showing an intense chronic inflammatory reaction and the presence of crusting. HE, approximately×40,×100. (**c**) Intense fibroblast proliferation and discrete deposition of collagen matrix were observed at this stage. Sirius red, approximately×100. (**d**) On day 14, epithelial pavementing and a moderate inflammatory reaction on the dermis were seen. HE, approximately×40. (**e**) A chronic inflammatory reaction, fibroblasts, and congested blood vessels were seen at this stage. HE, approximately×100. (**f**) A discrete collagen deposition, fibroblasts, and congested blood vessels were observed at this stage. Sirius red, approximately×100.

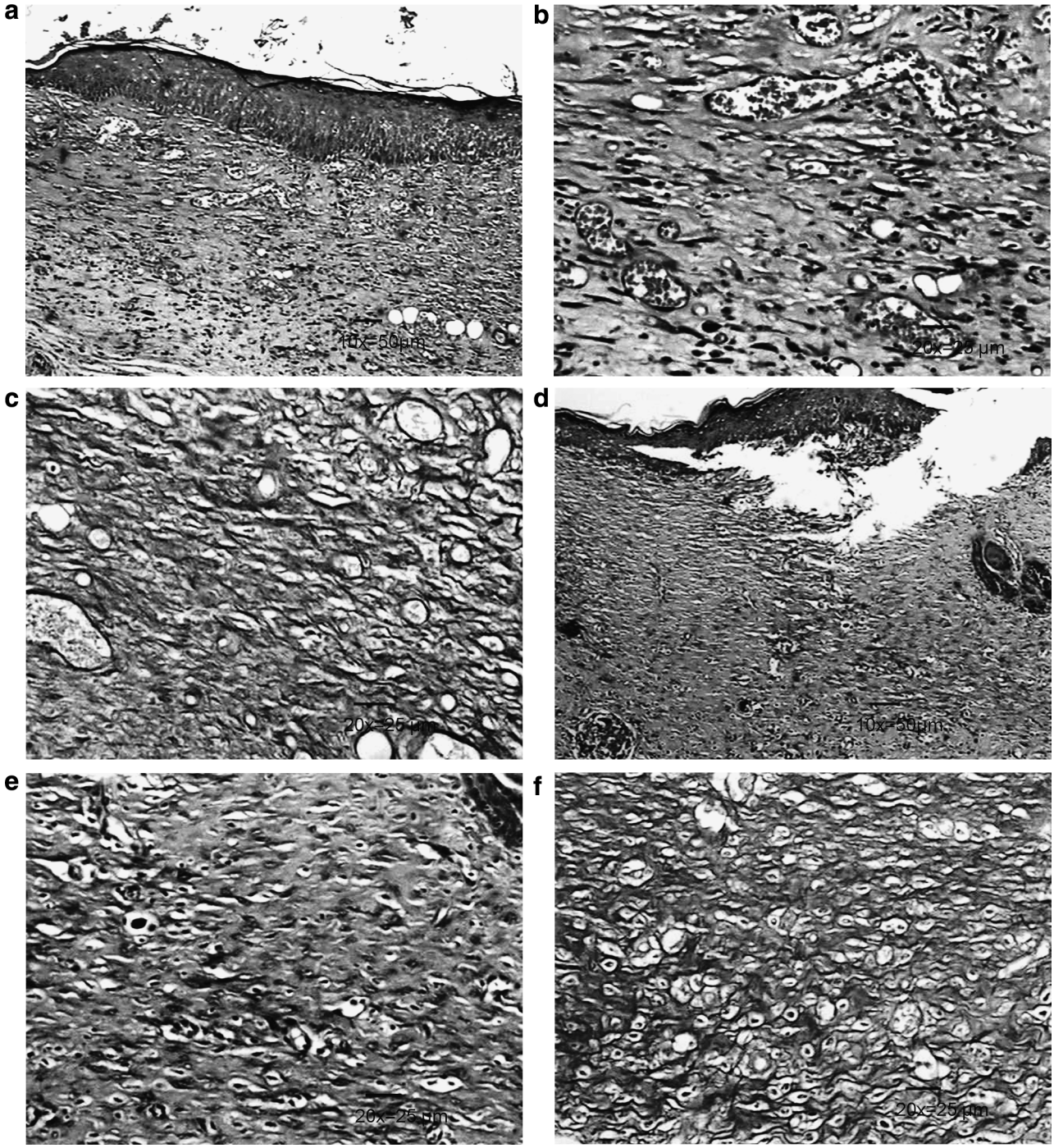

FIG. 3. (**a, b**) Photomicrography of 660 nm laser-light-treated specimen 7 d after surgery, showing epithelial pavementing covering an intense chronic inflammatory reaction. HE, approximately×40,×100. (**c**) Young fibroblasts, discrete collagen deposition, and congested blood vessels were seen at this stage. Sirius red, approximately×40. (**d, e**) On day 14, crusting covering an intense inflammatory reaction and fibroblasts were also seen. HE, approximately×40,×100. (**f**) Intense neoangiogenesis and collagen deposition was seen at the end of the experimental period. Sirius red, approximately×100.

before the animal was killed. Laser light was applied transcutaneously at four equidistant points on the wound margin. The dose per point was 5 J/cm^2 and the dose per session was 20 J/cm^2.

According to the subgroup, each animal was killed by an overdose of general anesthetic. Specimens were taken, coded, and kept in 10% formalin for 24 h. The coded specimens were routinely cut and processed to wax, and stained with both HE and Sirius red stains.

Light microscopy was performed (Axion Vision, Zeiss, Berlin, Germany) by the capitation of four random fields on each slide (X400, Axiostar Plus Microscope, Zeiss, Berlin, Germany). The images were coded accordingly and were analyzed by an experienced oral pathologist, who was not

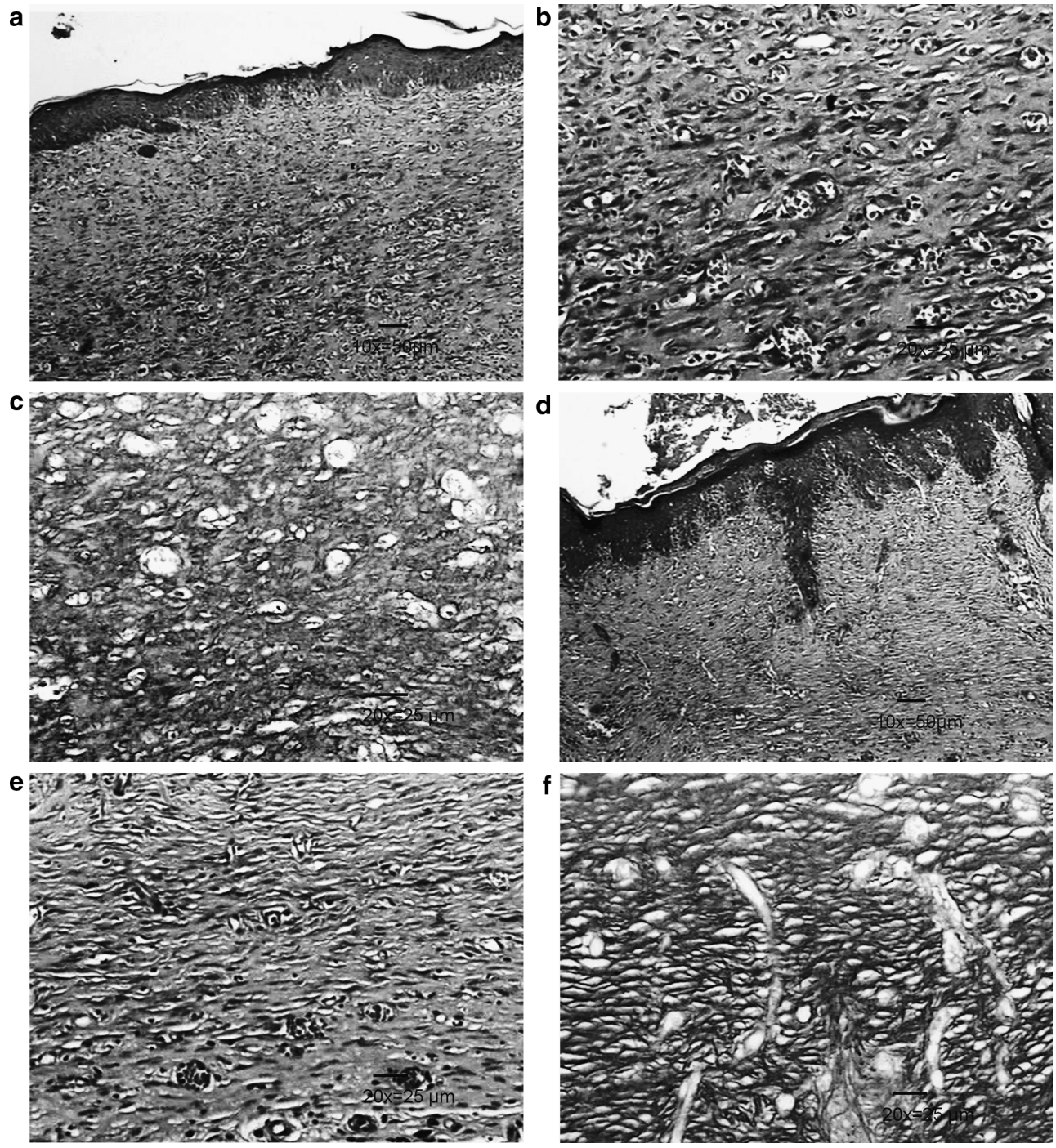

FIG. 4. (**a, b**) Photomicrography of 790 nm laser-light-treated specimen 7 d after surgery, showing granulation tissue and chronic inflammation and epithelial pavementing. HE, approximately×40,×100. (**c**) The collagen matrix was not well organized at this stage. Sirius red, approximately×100. (**d, e**) 14 days after surgery epithelial pavementing, a moderate chronic inflammatory reaction, and fibroblasts were seen. HE×40,×100. (**f**) Intense collagen deposition was seen at the end of the experimental period. Sirius red, approximately×100.

aware of either the coding or experimental procedure carried out in the study, using the criteria outlined in Table 2.

For the criterion *inflammatory reaction*, the number of inflammatory cells was counted on 10 fields in each slide (6×10), giving 60 counts for each group. The deposition of collagen was scored in six areas of each slide (6×6), resulting in 36 readings. The score was set as: 1 = presence of a collagen matrix ranging from 0 to 25% of the wound area; 2 = presence of a collagen matrix ranging from 25 to 50% of the wound area; 3 = presence of a collagen matrix ranging from 50 to 75% of the wound area; and 4 = presence of a collagen matrix in more than 75% of the wound area. Statistical analysis was performed using the Minitab 12® statistical software (Minitab, Belo Horizonte, Brazil).

Results

Control

On day 7, the wounds were covered with queratinized epithelium without skin appendices (Fig. 1a). Leukocytic exocitocis and vacuolization was evident (Fig. 1b). Fusiform fibroblasts were seen, organized in a parallel manner to the surface; blood vessels were mostly not congested, and a discrete chronic inflammatory infiltrate was seen at this stage. Sirius red staining evidenced the presence of delicate and fragmented collagen fibers in a moderate manner (Fig. 1c). On day 14, the wounds were covered with queratinized epithelium without skin appendices with a plain interface (Fig. 1d). The dermis showed the presence of fusiform young fibroblasts, blood vessels, and rare lymphocytes (Fig. 1e). Sirius red staining showed the cells disperse among thick and mature collagen bundles organized in a parallel manner similar to the adjacent dermis (Fig. 1f).

Mitomycin C

On day 7, the wounds were ulcerated and covered with a crust (Fig. 2a). Below the surface, granulation tissue rich in newly formed blood vessels, areas of hemorrhage, and interstitial edema were observed. Chronic inflammation was intense at this stage (Fig. 2b). A variable amount of fusiform and triangular fibroblasts were seen. Sirius red staining showed an immature and disorganized collagen matrix at this stage (Fig. 2c). On day 14, the specimens showed complete and atrophic epithelial pavementing (Fig. 2d). The dermis showed moderate hyperemia and interstitial edema. The inflammatory reaction was chronic and mostly moderate. A large number of fusiform and triangular fibroblasts were seen (Fig. 2e). However, Sirius red staining showed a mild to moderate amount of disorganized and immature collagen matrix (Fig. 2e).

Mitomycin C + λ660 nm

On day 7, the wounds showed epithelial pavementing covering a deep area of ulceration (Fig. 3a) characterized by a hyperemic granulation tissue, interstitial edema, mostly congested newly formed blood vessels, and an intense inflammatory reaction (Fig. 3b). Fibroblasts were also seen on this area. Sirius red staining showed a discrete amount of a disorganized collagen matrix (Fig. 3c). On day 14, no crusting was observed, and the epithelium was atrophic (Fig. 3d). The dermis showed a moderate to intense chronic inflammatory infiltrate, intense hyperemia, interstitial edema, and multiform fibroblasts (Fig. 3e). Sirius red staining showed intense deposition of a more organized collagen matrix (Fig. 3f).

Mitomycin C + λ790 nm

On day 7, epithelial pavementing could be seen covering a large amount of granulation tissue, rich in newly formed blood vessels, and a discrete inflammatory reaction (Fig. 4a). Hyperemia and interstitial edema were intense at this stage. Some fibroblasts were also seen (Fig. 4b). A mild deposition of a collagen matrix was observed with Sirius red staining (Fig. 4c). On day 14, the specimens showed complete epithelium pavementing (Fig. 4d). The granulation tissue showed a chronic aspect. Inflammation was moderate. Hyperemia, interstitial edema, and fibroblasts were also seen at this stage (Fig. 4e). An intense deposition of collagen matrix could be seen (Fig. 4f).

A summary of the light microscopic study can be seen in Tables 3 and 4.

Statistical analysis

Statistical analysis was carried out using Ficher's Test. Results showed statistically significant differences between groups G1a and G2a, G3a, and G4a ($p = 0.019$) on day 7,

Table 3. Summary of the Computerized Hystomorphometric Analysis on Day 7

	Group			
Criterion	*Control*	*Mytomicin C*	*λ660 nm*	*λ790 nm*
Edema	83.4% Absent 16.6% Present	100% Present	100% Present	100% Present
Hyperemia	50% Discrete 15% Moderate 25% Intense	66.7% Moderate 33.3% Discrete	33.4% Moderate 33.4% Absent 16.6% Discrete 16.6% Intense	50% Absent 50% Discrete
Fibrinous exsudate	33% Absent 33% Discrete 33% Moderate	66.7% Discrete 33.7% Absent	83.4% Absent 17.6% Discrete	83.4% Absent 16.6% Intense
Crust formation	100% Absent	83.4% Present 16.6 Absent	83.4% Present 16.6% Absent	83.4% Present 16.6% Absent
Neoangiogenesis	100% Present	100% Present	100% Present	100% Present
Epithelial pavementing	100% Complete	83.4 Partial 16.6% Complete	83.4% Partial 16.6% Complete	100% Partial
Inflammatory infiltrate	83.4% Discrete 16.6% Moderate	83.4% Intense 16.6% Moderate	83.4% Intense 16.6% Moderate	66.6% Discrete 16.6% Moderate 16.6% Intense
Fibroblastic proliferation	33% Discrete 66% Moderate	83.4% Discrete 16.6% Moderate	16.6% Discrete 50% Moderate 33.3% Intense	83.4% Discrete 16.6% Moderate

Table 4. Summary of the Light Microscopy Study on Day 14

Criterion	Group			
	Control	*Mytomicin C*	*λ660 nm*	*λ790 nm*
Edema	83.4% Absent 16.6% Present	100% Present	100% Present	100% Present
Hyperemia	66.7% Discrete 33.3% Intense	66.6% Moderate 33.4% Intense	50% Intense 33.3% Absent 17.7% Discrete	66.6% Discrete 16.6% Absent 16.6% Moderate
Fibrinous exsudate	83.4% Discrete 16.6% Absent	33.4% Absent 33.3% Discrete 33.3% Moderate	50% Intense 25% Discrete 25% Intense	83.4% Absent 16.6% Intense
Crust formation	100% Absent	100% Absent	100% Absent	100% Absent
Neoangiogenesis	100% Present	100% Present	100% Present	100% Present
Epithelial pavementing	100% Complete	100% Complete	83.4% Complete 17.6% Partial	100% Complete
Inflammatory infiltrate	50% Discrete 25% Moderate 25% Intense	83.4% Moderate 17.6% Intense	50% Intense 25% Discrete 25% Moderate	66.8% Moderate 16.6% Discrete 16.6% Intense
Fibroblastic proliferation	83.4% Moderate 16.6% Intense	66% Discrete 33% Moderate	16.6% Moderate 83.4% Intense	16.6% Moderate 83.4% Intense

with regards to edema, epithelial pavementing, crust formation, and fibroblast proliferation ($p < 0.001$). The results were smaller for the experimental groups for all criteria except fibroblastic proliferation where the opposite was observed.

There was a significant difference in the fibroblastic proliferation between groups G1a and G2a ($p < 0.001$), with a reduction in the number of fibroblasts for G2a. There were also significant differences between groups G1a and G4a ($p < 0.001$), with a reduction in the proliferation for the experimental group. Further, there were significant differences between groups G3a and G2a/G4a ($p < 0.001$), with an increase in fibroblast numbers for G3a.

On day 14, there was a significant difference between groups G1b and G2b ($p = 0.05$) with regards to hyperemia, with an increase in the response for G2b. Significant differences in fibrinous exudate were seen between groups G1b and G3b ($p = 0.019$), as a result of the decreased reaction in the experimental group. There were significant differences between groups G1b, G2b/G3b and G4b ($p = 0.0019$), with a decrease in the number of fibroblasts for G2b and an increase in the other groups. Significant differences were seen between groups G3b/G4b and G2b ($p < 0.001$), with a reduction in the proliferation for G2b.

Table 5. Mean Count and Standard Deviation of Inflammatory Cells During the Experimental Period

Group	*Mean ± SD*
Control 7	21.10 ± 8.92
Control 14	19.05 ± 5.79
MitoC 7	28.60 ± 6.09
MitoC 14	18.63 ± 4.62
MitoC 660 nm 7	22.03 ± 6.49
MitoC 660 nm 14	17.28 ± 4.63
MitoC 790 nm 7	16.93 ± 9.26
MitoC 790 nm 14	14.70 ± 4.27

The influence of time was also assessed, and significant differences were found in crust formation and epithelial pavementing. Crust formation decreased for groups G2b, G3b, and G4b ($p = 0.019$). Epithelial pavementing increased for groups G2b and G4b ($p = 0.019$). Time also significantly influenced the fibroblastic proliferation between the control and experimental groups ($p = 0.04$). There was an increase in the fibroblastic differentiation for group G3b ($p < 0.001$).

The mean values and standard deviation for the count of inflammatory cells are shown in Table 5. The data was normally distributed and analyzed using ANOVA and *t* test. The statistical analysis showed significant differences between groups at both experimental times (ANOVA, $p < 0.001$). There were significant differences between groups G2a and G2b (*t* test, $p < 0.001$); G3a and G3b (*t* test, $p < 0.001$); G1a and G2a (*t* test, $p < 0.001$); G1a and G4a (*t* test, $p < 0.001$); G2a and G3a (*t* test, $p < 0.001$); G2a and G4a (*t* test, $p < 0.001$); G3a and G4a (*t* test, $p < 0.001$); G1b and G3b (*t* test, $p = 0.03$); G1b and G4b (*t* test, $p < 0.001$); G2b and G4b (*t* test, $p < 0.001$); and G3b and G4b (*t* test, $p = 0.001$) (Figs. 5 and 6).

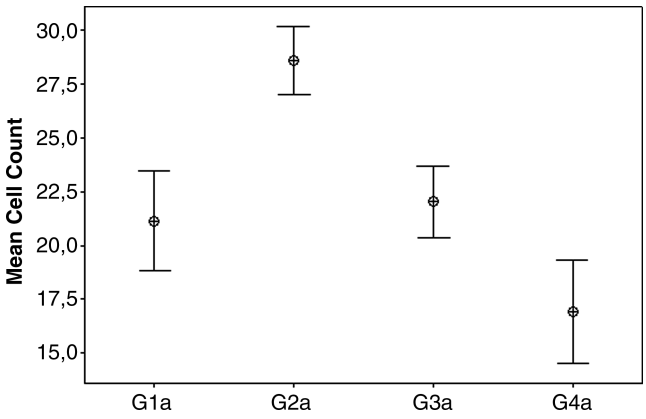

FIG. 5. Mean count and standard deviation of inflammatory cells on day 7 of the experiment.

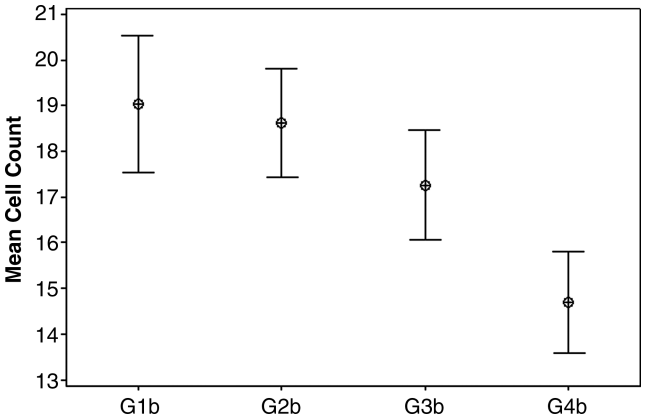

FIG. 6. Mean count and standard deviation of inflammatory cells on day 14 of the experiment.

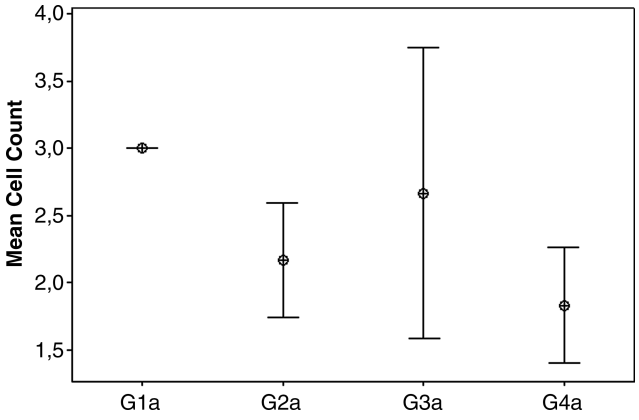

FIG. 7. Mean count and standard deviation of levels of collagen deposition on day 7 of the experiment.

The mean values and standard deviation for the scoring of collagen deposition are shown in Table 6. The data were normally distributed and analyzed using ANOVA and *t* test. The statistical analysis showed significant differences between groups on day 7 (ANOVA, $p = 0.01$) but not on day 14. Significant differences were observed between groups G1a and G2a (*t* test, $p = 0.004$) and between groups G2a and G4a (*t* test, $p = 0.001$) (Figs. 7 and 8).

Discussion

The healing of cutaneous wounds is accomplished through a complex process involving several types of tissues and cellular lineages as well as biochemical and metabolic reactions.[12]

Wound healing occurs in several phases and the behavior of the cellular component, the presence and effects of growth factors, and the characteristics and composition of the matrix are not fully understood yet, as many local and/or systemic factors may significantly affect the healing process.[14]

Some factors can therefore interfere with wound healing. These include: the patient's age;[15] nutritional status;[13] systemic diseases such as diabetes,[4] cardiovascular and clotting disorders, atherosclerosis, renal dysfunction,[1] and infections;[16] and the use of some drugs.[7]

Among the drugs that have an inhibitory effect on the healing process is MMC. There are several reports showing that the topical use of this drug inhibits fibroblast proliferation.[7–11] The concentration used in this study has been shown to be effective and does not result in any additional problems on the application site.[9–11]

On the other hand, there are treatments that may improve the healing process either by quickening the process or improving the quality of the newly formed tissue, or both. Among those therapies is LPBM.[12]

Most studies on the effects of phototherapies on the healing process have attributed the observed effects to several treatment parameters and the properties of the light source used.[13,17] One of the most frequently reported effects of LPBM is proliferation[10] and increased collagen deposition.[18,19]

In this study, we aimed to observe if the use of LPBM would have a significant effect when healing was impaired.

Previous reports have indicated that the chronic inflammatory reaction starts around day 7 after wounding.[9,18] In the present study, we found an intense inflammatory reaction in groups G2a and G3a when compared to the control (G1a) subjects in whom the inflammation varied from discrete to moderate. This result indicates that the onset of the inflammatory reaction occurred earlier in laser-irradiated subjects.

In addition, the groups showing a more prominent inflammatory reaction on day 7 were not treated with laser

Table 6. Mean Score and Standard Deviation of Collagen Deposition During the Experimental Period

Group	*Mean ± SD*
Control 7	3.0 ± 0.00
Control 14	3.3 ± 0.51
MitoC 7	2.2 ± 0.40
MitoC 14	2.8 ± 0.40
MitoC 660 nm 7	2.7 ± 1.03
MitoC 660 nm 14	3.5 ± 0.83
MitoC 790 nm 7	1.8 ± 0.40
MitoC 790 nm 14	3.5 ± 0.83

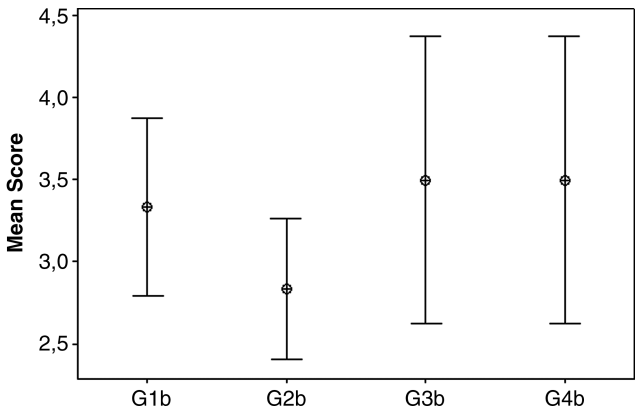

FIG. 8. Mean count and standard deviation of levels of collagen deposition on day 14 of the experiment.

light (G1a and G2a). This aspect may be suggestive of the anti-inflammatory action of the laser light. [9,12,17]

In the present study, we found intense neoangiogenesis and fibroblastic proliferation on irradiated subjects, a similar result to that found in another report.[19] However, the number of fibroblasts was smaller in the control group.[20]

The collagen matrix on MMC-treated subjects varied from discrete to moderate. The fibers were not well organized and not completely mature. This was also described previously,[7,9] when it was found that the use of topical MMC resulted in a decrease in the collagen matrix.

The optimal therapeutic dose, different wavelengths, power densities, timing of the sessions, and different protocols may account for some of the conflicting results found in the literature. The findings of the present study are indicative that LPBM results in reduced inflammation and an increase in both fibroblast proliferation and collagen deposition in subjects where wound healing was impaired by MMC.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Prof. Antonio Luiz Barbosa Pinheiro, Ph.D., MDentSci
Laser Center
Faculdade de Odontologia
Universidade Federal da Bahia
Av. Araújo Pinho, 62, Canela
Salvador, BA, CEP 40140-110
Brazil

E-mail: albp@ufba.br