Effect of Laser (670 nm) on Healing of Wounds
Covered with Occlusive Dressing: A Histologic and Biomechanical Analysis

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Abstract

Objectives: To analyze the effects of low-level laser therapy (LLLT), 670 nm, with doses of 4 and 7 J/cm², on the repair of surgical wounds covered by occlusive dressings. Background Data: The effect of LLLT on the healing process of covered wounds is not well defined. Materials and Methods: For the histologic analysis with HE staining, 50 male Wistar rats were submitted to surgical incisions and divided into 10 groups (n = 5): control; stimulated with 4 and 7 J/cm² daily, for 7 and 14 days, with or without occlusion. Reepithelization and the number of leukocytes, fibroblasts, and fibrocytes were obtained with an image processor. For the biomechanical analysis, 25 rats were submitted to a surgical incision and divided into five groups (n = 5): treated for 14 days with and without occlusive dressing, and the sham group. Samples of the lesions were collected and submitted to the tensile test. One-way analysis of variance was performed, followed by post hoc analysis. A Tukey test was used on the biomechanical data, and the Tamhane test on the histologic data. A significance level of 5% was chosen (p ≤ 0.05). Results: The 4 and 7 J/cm² laser with and without occlusive dressing did not alter significantly the reepithelization rate of the wounds. The 7 J/cm² laser reduced the number of leukocytes significantly. The number of fibroblasts was higher in the groups treated with laser for 7 days, and was significant in the covered 4 J/cm² laser group. Conclusions: Greater interference of the laser-treatment procedure was noted with 7 days of stimulation, and the occlusive dressing did not alter its biostimulatory effects.

Introduction

The cutaneous repair process is of great relevance in plastic surgery, in which the laser plays an important role in the scar quality. When a tissue trauma occurs, a complicated set of vascular, cellular, and biochemical events is unchained in an attempt to replace the dead or imperfect cells by healthy cells, the process of tissue repair. This process is not regenerative, as dermal integrity is established, but the result is the closure of the wound by scar tissue showing a disorganized pattern of collagen deposition and less tensile strength in relation to intact skin.

Low-level laser therapy (LLLT) is an important ally of health professionals for the treatment of a variety of skin injuries, such as exfoliations, burns, surgical incisions, and ulcerations, which are probably the most difficult to treat.

We conducted a meta-analysis to verify the efficiency of LLLT in tissue repair and pain relief and concluded that the therapeutic use of laser phototherapy is highly effective.

Tissue repair mediated by LLLT has received great deal of attention in research; however, it still has controversial and few reproducible results because of the lack of details on the physical parameters used in the experiments. Many authors agree that we need standard protocols and more controlled investigation of the alterations at the cell level, before it is disseminated for clinical use.

The occlusive dressing (O) has been widely used in the improvement of postoperative healing, as it reduces skin tension, and thus avoids the formation of hypertrophic scars. Nevertheless, according to a pilot study, the transmissivity of LLLT in occlusive dressing (3M hypoallergenic microporous surgical adhesive tape) is 36%. The reduction in transmissivity of the laser applied with interposed membranes was also observed by other authors.

Most studies on laser therapy are conducted with He-Ne and AsGa lasers. Very few studies that use the proposed 670-nm laser are found in the literature. Furthermore, the transmissivity of the laser used postoperatively may be blocked or

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attenuated by the occlusive dressing. The purpose of this study was to analyze the dose–effect relation of laser with and without occlusive dressing in tegumental regeneration. The hypothesis of the present study is that LLLT facilitates wound healing in wound dressings.

Materials and Methods

Seventy-five male Wistar rats, weighing between 200 and 250 g, were used in the study. The animals were kept in individual cages at controlled room temperature, with a 12-h light–dark cycle, and were given food as well as water ad libitum. During the experiments, the animals were cared for in compliance with the Statement of Principles adopted by the FASEB Board. They were subdivided into two groups, treated with an energy density of 4 J/cm² or 7 J/cm² with and without interposed material to verify the dose–effect relation and traction resistance.

Experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
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<tbody>
<tr>
<td>Control 7 days, C7</td>
<td>50 rats</td>
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<tr>
<td>Control 14 days, C14</td>
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</tr>
<tr>
<td>Treated with laser with 4 J/cm² for 7 days, L4J7</td>
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<tr>
<td>Treated with laser with 4 J/cm² for 14 days, L4J14</td>
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<tr>
<td>Treated with laser with an energy density of 4 J/cm² + dressing for 7 days, L4JO7</td>
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<tr>
<td>Treated with laser with an energy density of 4 J/cm² + dressing for 14 days, L4JO14</td>
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<tr>
<td>Treated with laser with an energy density of 7 J/cm² for 7 days, L7J7</td>
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<tr>
<td>Treated with laser with an energy density of 7 J/cm² for 14 days, L7J14</td>
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<tr>
<td>Treated with laser with an energy density of 7 J/cm² + dressing for 7 days, L7JO7</td>
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<tr>
<td>Treated with laser with an energy density of 7 J/cm² + dressing for 14 days, L7JO14</td>
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Each group consisted of five animals (n = 5), and all the animals were killed on postoperative days 8 and 15, respectively.

Tensile Test Group: 25 rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
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<tbody>
<tr>
<td>Control for tensile test for 14 days, CT</td>
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<tr>
<td>Submitted to laser with 4 J/cm² for 14 days, with tensile test, LT4</td>
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<tr>
<td>Submitted to laser with 7 J/cm² for 14 days, with tensile test, LT7</td>
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<tr>
<td>Submitted to laser with 4 J/cm² + dressing for 14 days, with tensile test, LTO4</td>
<td></td>
</tr>
<tr>
<td>Submitted to laser with 7 J/cm² + dressing for 14 days, with tensile test, LTO7</td>
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Each group consisted of five animals (n = 5), and all the animals were killed on postoperative day 15.

Surgical procedure

After anesthesia with sodium thiopental (Thionembutal) (50 mg/kg dose, 20 mg/mL concentration, intraperitoneally, and trichotomy of the dorsal region, 1 cm² of skin was removed, including the hypoderm, by using a millimetric hollow plastic template and a no. 11 scalpel blade. Soon after surgery, and at the place of the lesion, all the animals received a single application of polyvinyl pyrrolidone (active iodine), 10.0 g with 10% of stabilizer and emollient (PVPI).

Tensile test

The animals in this group were submitted to the same surgical procedures, the only difference being the 2-cm long and linear incision, perpendicular to the spinal column.

Equipment

The equipment used was the model Laserpulse from IBRAMED (Amparo, São Paulo, Brazil) with a visible beam, continuous pulse system, 30-mW power, 670-nm wavelength, and 0.06 cm² spot size. The equipment was calibrated before and after the experimental procedure.

Irradiation procedures

The lesions were stimulated daily, starting 24 h after surgery, and always in the morning for 7 and 14 consecutive days. The animals were immobilized by the examiner. The contact technique was used for GC and GL groups with perpendicular application divided into four points at the edges of the wound, and scanning on its bed with 4 J/cm² for 134 s, and 7 J/cm² for 234 s (Fig. 1A). The effectiveness of LLLT applied over the occlusive dressing, usually used in...
postsurgical procedures, was analyzed. Then the hypoalergenic microporous surgical adhesive tape (3M) was placed over the wound only at the time of the procedure of the respective groups: L7JP (2.52 J/cm²) and L4JP (1.44 J/cm²).

For the tensile-test group, the LLLT was applied daily at six points, with 4 J/cm² and 7 J/cm², divided bilateral to the incision (Fig. 1B). The time used for the biomechanical test was 14 days and not 7 days, as in the histologic study, because the collagen synthesis and maturation, which confer resistance to the tissue, do not occur during the acute healing phase.

**Sample processing**

**Histologic analysis.** After death, the lesions containing the intact skin around them were removed, fixed in a buffered solution of 10% formol for 48 h, and treated for inclusion in paraffin. Nonserial cuts of 7-μm thickness were stained with hematoxylin-eosin.

The linear measurements (in micrometers) of the re-epithelialization were calculated from the edge of the lesion to the extremity of the epithelium in regeneration along both edges. The values of the two edges in 20 nonserial cuts per animal were summed. These measurements were obtained with an image-analysis system (Image-ProPlus Software).

For the histometric analyses, three nonserial cuts were examined per animal in the same analysis system. Images of the three areas in each cut were captured, always with equal known dimensions (10.772,96 mm²): two areas close to the lateral borders and a central one, with a total of nine areas per animal, in which the numbers of fibroblasts, fibrocytes, and leukocytes were computed.

The morphologic criterion used to distinguish different cells was the cell morphology core. Fibroblasts have an oval and clear nucleus, and leukocytes have an irregular picnotic nucleus (dark) and are smaller. These cells are identified in Figs. 2 and 3. Leukocytes were identified in a single category of cells: the defense cells, cells characteristic of the inflammatory process. The cells were not distinguished from each other, as this was not the purpose of this study.

**Tensile test.** After the animals were killed, the large skin extension from the dorsal area of the animal was resected, including the surgical scar. The skin removed was submitted to a form of cut (Fig. 4) especially developed for the study. The cut had the shape of a test specimen, so that the scar was positioned in the center. The purpose was to standardize the samples.

To analyze the mechanical properties of the skin obtained by an axial tensile test, the Universal Mechanical Test Machine, model DL 2000 (Emic), equipped with a load cell capacity of 5 Kg (Emic Trd 24) was used. The calibration was conducted according to the Brazilian Standard Norms ABNT NBR NM-ISO 7500-1 (certificate N. 777/04), and carried out with the load cell connected to an extensometer bridge (Trd 15) for the reading of the applied loads.

For the mechanical tensile test, each extremity of the test specimen was fixed to the accessories made specifically to promote better stability of the skin, without any possibility of slipping. The extremities were fixed to the flanges of the Universal Test Machine by their ends, in a perpendicular position. Next, the skin was submitted to an axial traction force to increase its length during the test until the moment of rupture.

**Program Tec, version 1.10, was used to obtain graphically the deformation and force diagram.**

The constant speed established for the test was 5 mm/min, and the measurements were made every 0.5 mm. The tests were performed after the maximum limit, and the loads corresponding to each deformation measured were recorded. After the performance of each test, the place where the test specimen ruptured was inspected.

When the skin tensile test was being performed, the test equipment displayed the behavior of the material by means of a curve traced from the function force (Kgf) X deformation (mm), obtained for each test, in which data were supplied with reference to: maximum force (MF), rupture force (RF), and deformation (D).

**Statistical analysis**

Data were analyzed with SPSS 7.5 software. The Levene test was used to verify the homoscedasticity hypothesis. For the statistical comparison among the groups, one-way analysis of variance (ANOVA) and post hoc Tukey and Tamhane parametric tests were used. A significance level of 5% was selected (p ≤ 0.05).

Given the statistical significance among the different groups, a comparative analysis of the means was conducted by applying the Tukey HSD post hoc test to analyze the fibrocyte data, and the post hoc Tamhane Test to analyze the fibroblast and leukocyte data.
Results

The results showed statistically significant differences among groups ($p = 0.04$) for the leukocyte ($p < 0.001$) and fibroblast data ($p < 0.001$).

In Fig. 5, on day 7, a reduction was observed in the number of leukocytes of the groups treated with laser in comparison with the control group; however, this difference was not significant. All groups treated for 14 days showed no significant reduction in the number of leukocytes in comparison with those treated for 7 days. Among the groups treated for 14 days, a significantly higher reduction ($p < 0.01$) was found in the group treated with $7J/cm^2$ in comparison with the 14-day control group.

With regard to the number of fibroblasts, in Fig. 6, an increase can be observed in the treated groups on day 7, when compared with the control group. This difference is significant ($p = 0.032$) for the group treated with $4J/cm^2$ laser with occlusive dressing.

On day 14, the number of fibroblasts decreased in all groups, when compared with day 7.

With regard to the number of fibrocytes, in Fig. 7, a reduction was noticed in their number on day 7, when com-
pared with the control groups. Statistically significant differences were noted in the groups treated with 4 J/cm² and 7 J/cm² laser, with or without occlusive dressings. It was also noticed that the treatment effect is higher on day 7 than on day 14.

Statistical analysis of the tensile test showed no significant difference for the data with regard to deformation (D, Kgf) and maximum force (MF, Kgf); \( p > 0.05 \). The rupture force (RF, Kgf), data were statistically significant different for the control group in comparison with the group stimulated with 4 J \( (p < 0.05) \). The correlations between the group stimulated with 4 J and the one with 7 J, and between the 4-J group and the 4-J group associated with micropore \( (p < 0.05) \) can be observed in Fig. 8.

Epithelization data results did not show significant differences among the considered groups \( (p = 0.07) \).

Discussion

The results of the histologic and biomechanical analyses showed that the occlusive dressing did not alter the biostimulatory effects of 660-nm laser on the number of the analyzed cells, and the effects of irradiation are greater with 7 days of stimulation.

Occlusive dressings have been used to keep the wound moist and covered, as well as to induce tissue granulation, and to accelerate epithelization in acute wounds. They may also enhance healing in chronic wounds.10,11

Although it was observed in a pilot study that the occlusive dressing diminished the transmissivity of the laser by \( \sim 64\% \), the results of the present study (which used the same material) point to the fact that probably the radiation that was transmitted was sufficient to cause bioeffects on the lesions analyzed. Frequently, it is not possible to remove the occlusive dressing in all LLLT application sections, making us irradiate the lesion with the dressing interposed between the applicator and the target tissue with results.

LLLT is a widely used resource in clinical practice for the treatment of soft tissues to accelerate the tissue-repair process. However, scientifically, controversies exist with regard to its efficacy.12 This is probably because no standardized protocol exists for conducting research.

The 670-nm laser was selected for this study, as only a few scientific studies have been conducted with it, and it also presents some advantages in comparison with the HeNe laser.13

The mean evolution of epithelial tissue under wounds treated with laser in comparison with the controls, in the present study, was not significant. Many studies point out significant improvement in reepithelization of wounds treated with LLLT at doses that range from 1 to 4 J/cm².2,14 Perhaps the epithelization evolution was not significant in this study, as the animals disturbed the occlusive dressing when they moved, affecting the newly formed epithelium.

On day 7, the number of leukocytes diminished in groups treated with laser (4 J and 7 J), with or without occlusive dressing, although it was not statistically significant. This is probably due to the amplitude of the confidence interval, which demonstrates the variability of the samples.

Bisht et al. (1999)2 using HeNe laser at 4 J/ cm² in rat skin lesions, observed increased leukocytic infiltration and neovascularisation in lesions with 3 days of treatment. Similar results were found by Schindl et al. (1999)15 and Schindl et al. (2003)16 in human skin lesions.

After 14 days of treatment, a significant reduction \( (p < 0.01) \) was found in the group treated with 7 J/cm² in comparison with the control group. The results presented here corroborate those found in the literature.2,6,16 Thus, a reduction in leukocytes occurred because the inflammatory phase was accelerated by the treatment, with the disappearance of a large number of these cells.

On day 7 of treatment, a larger number of fibroblasts were observed in groups treated with laser. This difference was significant at the dose of 4 J/cm² with occlusive dressing, showing that laser (670 nm) can stimulate the proliferation of these cells and that the occlusive dressing does not alter this effect.

FIG. 8. Maximum force of rupture among control group \( (C) \) and groups treated with laser: with energy density of 4 J/cm² \( (L4J) \), with energy density of 7 J/cm² \( (L7J) \), and with occlusive dressing \( (LTO4 \text{ and } LTO7) \).

FIG. 7. Evolution of mean fibrocytes in the treated groups at 7 and 14 days. C, Control; L4J, treated with laser 4 J/cm²; L4PJ, treated with laser 4 J/cm² + dressing; L7J, treated with 7 J/cm²; and L7JO, treated with laser 7 J/cm² + dressing.
dressing, in comparison with the control group, suggesting higher cellular activity in these groups. As these cells represent inactive fibroblasts, one may infer that the treatment with laser stimulates the activities of these cells and that this stimulus is greater in the initial stage of the repair process (i.e., at the inflammatory stage). These findings may help to explain the effect of laser irradiation on rupture force, even though it was significant only in group LT4. Singer and Clark (1999) demonstrated fibroblastic activity induced by LLLT.

The clinical implications of this study suggest that the LLLT is an efficient therapeutic resource used for tissue repair, even with interposed occlusive dressing, allowing its early use, because significant results were observed on the first 7 days of application, although it is important to consider the energy loss due to the occlusive dressings.

Biomechanical tests are also used in studies as a variable for the study of regeneration. Although, according to some authors, the fibroblastic action conferred by LLLT may provide the injured tissue with more resistance, other studies did not find significant differences regarding the resistance of injured skin treated with LLLT and its respective controls, data that corroborate the results found in this study.

The results of this study show a beneficial effect of LLLT on the repair process of wounds covered with occlusive dressings. Further investigations are required with different occlusive dressings.

Conclusions

The results of the present study indicate that lasers with wavelength of 670 nm improve the repair process with or without occlusive dressings.

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Author Disclosure Statement

No competing financial interests exist.

References