Prevention of Abdominal Adhesions and Healing Skin After Peritoniectomy Using Low Level Laser

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Background: Adhesions commonly occur after abdominal surgery and can cause bowel obstruction, chronic abdominal pain, and infertility. Their prevention remains a challenge.
Objectives: To evaluate the effects of the application of low-level lasers on the prevention of adhesions and scarring of the skin after peritoniectomia.
Method: Twenty-four New Zealand breed male rabbits, approximately 2 months of age, were randomly divided into 3 groups ($n = 8$): GC—control group not subjected to laser, GL1—group with laser application at a dose of 0.2 J, and GL2—group with laser application at a dose of 3.6 J. All animals received a longitudinal midline incision and a bilateral resection of the peritoneal fragment, measuring $3 \times 1\,cm^2$. The animals received a laser treatment of one application every 24 hours, beginning at the time of surgery and lasting for a period of 4 days. After 14 days post-surgery, the animals were killed and adhesion formation was evaluated qualitatively and quantitatively by means of a laparotomy shaped inverted "U", which allowed for the verification of the broad wall of the abdominal cavity and organs. Differences were considered significant at $P < 0.05$.
Results: The adhesion formation was observed in 100% of the rabbits from groups GC and GL1, as compared to 37.5% of the rabbits from group GL2 ($P < 0.01$). The evaluation of the vascularization and tenacity of adhesions among the groups showed no significant difference. In groups CG and GL1, 72% and 83% of adhesions were verified between visceras, respectively whereas in GL2 occurred among abdominal wall. The tensile strength of the skin between the groups was not significant ($P = 0.3106$). The resistance of abdominal wall segments without skin he resistance of skin segments between groups GL2 and GC were higher than in GL1 ($P = 0.01$).
Conclusion: Low-level LASER is effective in preventing intra-abdominal adhesions in rabbits without compromising strength and healing of the abdominal wall. Lasers Surg. Med. 47:817–823, 2015. © 2015 Wiley Periodicals, Inc.

Key words: peritoneal adhesions; photobiostimulation laser; low-power laser irradiation; low-level laser; low-power laser therapy; laser therapy; healing; rabbit; tensile strength

INTRODUCTION

Adhesions are defined as abnormal connections of organs or body structures. These connections, or bridges, can form a thin layer of connective tissue, a slightly thicker tissue containing blood vessels and nerves, or can represent a direct contact between the surfaces of two bodies. Adhesions can be found in the peritoneal, pericardial, pleural, uterus, joints, or ocular chambers. Adhesions in the abdominal cavity are known as peritoneal adhesions, since the visceral or parietal peritoneum is always involved. Its incidence after surgery is high and can occur in up to 93% of all laparotomies [1].

Adhesions are associated with the risk of death, as they are related to serious complications, such as intestinal obstruction, which can also cause chronic abdominal pain and female infertility. These are the most prevalent causes of acute and recurrent obstruction of the small intestine and are associated with mortality rates of up to 15% [1,2].

Peritoneal trauma, foreign bodies, tissue ischemia, and infections of the abdominal viscera are the main factors associated with the formation of postoperative adhesions, but the specific pathophysiology of adhesions remains uncertain [1]. According to Henrrick et al. (2001), adhesion formation is a consequence of peritoneal injury with local ischemia and false regeneration [2].

This serosanguineous peritoneal injury can cause an inflammatory reaction that can lead to fibrin deposits. Well-oxygenated and intact mesothelial cells produce plasminogen activators, which smooth the fibrin clots formed after injury. The proliferation of mesodermal cells regenerates a single layer of mesothelium between three and five days after injury. In experimental wounds, fibrinolytic activity could be identified three days after the lesion and before the reconstruction of the integrity of the mesothelium. This process is rapid and generally reproduces the peritoneal wall with no formation of

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adhesion. Moreover, improper fibrinolysis allows the proliferation of fibroblasts to produce fibrous adhesions. With hypoxia, the fibrin matrix is invaded by fibroblasts, with the stimulation of angiogenesis and collagen synthesis. Fully developed fibrous adhesions begin to form after 10 days and reach their complete form between 2 and 3 weeks after injury [3].

Prophylactic measures have been taken to reduce adhesion formation, aimed at reducing the inflammatory response and coagulation and to prevent prolonged contact between surfaces connected by means of siliconized effects; however, to date, none have shown to be fully effective and safe in reducing adhesions [4,5].

The low intensity laser, also called low-power or biostimulant, has been used since the late 60's to heal wounds and ulcers. Although the laser is already used on a large scale, to facilitate the healing process for at least 50 years, studies evaluating the effect of this therapy on the formation of adhesions have yet to be published [6,7]. The aim of this study was to evaluate the effects of low-intensity lasers on the formation, morphology, and inflammatory aspects of abdominal adhesions and the scarring of the skin after peritoniectomy in rabbits.

METHOD

The present study was conducted at the Laboratory of Experimental Surgery, Federal University of Minas Gerais (UFMG), in accordance with recommendations set forth in the International Protection of Animals and the Brazilian Code of Animal Experimentation (1988), and was approved by the Ethics Committee on Animal Experimentation from UFMG.

Twenty-four adult male New Zealand rabbits (Oryctogalus cuniculus) were divided randomly into three groups. At the beginning of the experiment, all rabbits weighed, on average, 2.350 ± 240 g.

The animals were randomly divided into three groups:

- GC ($n = 8$) control group, without LASER;
- GL1 ($n = 8$) Group submitted to LASER: 0.2J of dose, fluence of 5J/cm^2 power of 0,005W, duration of 40 seconds per point, spot area, 0,04 cm^2;
- GL2 ($n = 8$) Group submitted to LASER: 3.6J of dose, fluence of 90J/cm^2 power of 0.04W, duration of 90 second per point, spot area, 0,04 cm^2.

The animals had their abdomens shaved and were then anesthetized with a deep intramuscular gluteus of Ketamine hydrochloride at 20 mg/kg, together with a 2% Xylazine at a dose of 6 mg/kg. Antibiotic prophylaxis with Cephalexin monohydrate was applied at a dose of 50mg/kg and was administered intramuscularly 30 minutes before carrying out the following procedures.

The surgery was performed according to the following steps (Fig. 1):

A. Median longitudinal laparotomy measuring 7 cm in length, 4 cm distal to the xiphoid process;
B. Resection of parietal peritoneum fragment of 1×3 cm^2, using a template to standardize the size of all excised fragments.
C. Aspect of the abdominal wall after resection.
D. Perioperative application of low-intensity lasers in Groups GL1 and GL2 at four points, located 1 cm from each other, two on the right side and two on the left side, in locations where the patches had been removed.
E. Continuous suture of the wound in layers (aponeurosis, parietal peritoneum, and skin) with 3–0 nylon thread.
F. Postoperative application of low-intensity lasers in Groups GL1 and GL2 at four points, located 1 cm from each other, two on the right side and two on the left side, in locations where the patches had been removed.

All animals were subjected to the same procedure as the GC group but without the application of low-intensity lasers.

The skin of the animals from the GL1 and GL2 groups received one daily application of low-intensity laser at the same dose used in the perioperative period, every 24 hours, beginning with the surgery and continuing for four consecutive days. Each application was performed at four points, two on the right side and two on the left side, spaced 1.5 cm from the midline, with a 1 cm distance between the points of application (Fig. 1F).

All animals were killed on the 14th post-operative day by applying an intramuscular anesthesia with ketamine hydrochloride at a dose of 50 mg/kg.

The abdominal cavity was examined after extensive longitudinal midline incision in the shape of an inverted "U" surrounding the surgical scar.

The qualification and quantification of adhesions were based on the study of MORENO-EGEA (1993), in a modified form [8].

The following parameters of adhesions were evaluated:

1. Animal-free grip: related to the number of animals where there was no formation of adhesion;
2. Count: related to the amount of adhesions found;
3. Area: measured using digital calipers, two measurements were conducted by adhesion, the first referring to the contact surface between the attached structures and the second referring to the thickness of the grip. The area of adhesion was calculated from these two dimensions;
4. Tenacity: adhesions were characterized as loose (that adherence would be peeled easily without requiring dissection) and firm (when there was need for dissection to be separated);
5. Vascularization: presence or absence of blood vessels in adhesion, without the use of a magnifying glass and by optical microscopy;
6. Location: classified as follows: adhesion between the parietal peritoneum and omentum, between the bowel and omentum, between other organs and the bowels, and between the parietal peritoneum and the bowels;

For the mechanical test of the tensile strength of the skin and the abdominal wall without the skin, a universal testing machine, Kratos® model DEK 200 kgf with a load cell of 20 kgf, was used.

The abdominal wall was removed, and a specimen was taken with a scalpel, using a plastic mold, aimed at

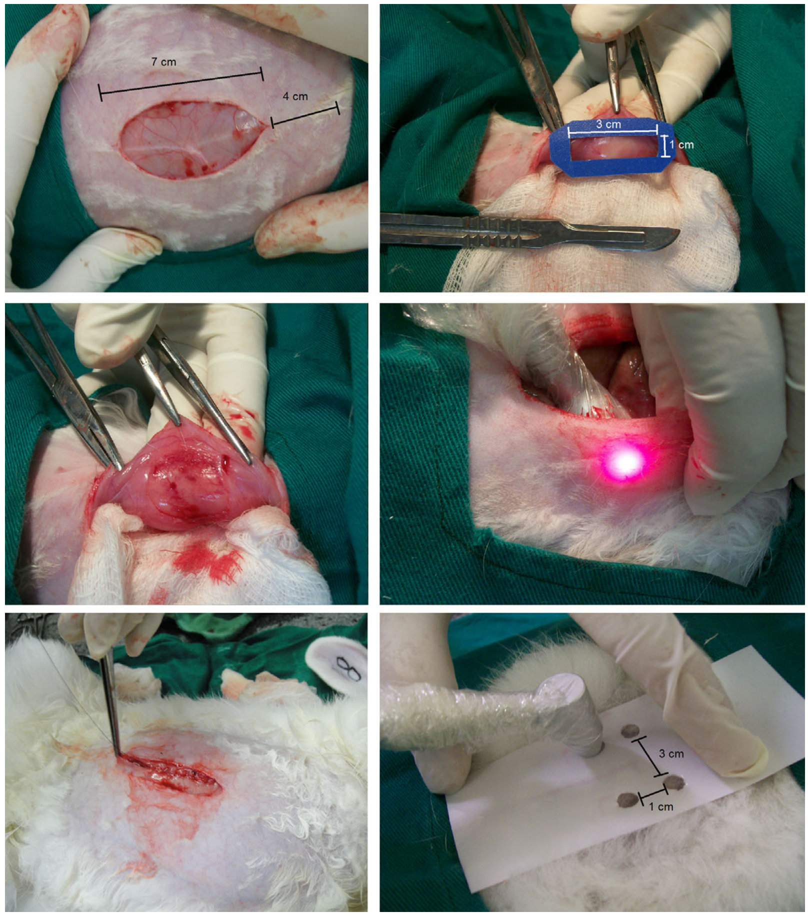

Fig. 1. Following the surgical procedure and LASER therapy: (**A**) Median longitudinal laparotomy measuring 7 cm long, 4 cm distal to the xiphoid process. (**B**) Resection of parietal peritoneum fragment using a template to standardize the size of every fragment excised. (**C**) Aspect of the abdominal wall after resection. (**D**) Perioperative application of low intensity LASER Groups GL1 and GL2 in four points, 1 cm distant from each other, two on the right side and two on the left side, in places where the patches were removed. (**E**) Continuous suture the wound in plans (aponeurosis and parietal peritoneum and skin) with nylon 3-0. (**F**) Postoperative application of low intensity LASER Groups GL1 and GL2 in four points, 1 cm distant from each other, two on the right side and two on the left side, in places where the patches were removed.

standardizing the specimens. The surgical scar was placed in the center of the mold.

The skin was separated from the rest of the abdominal wall using a scalpel. The fragments of the abdominal wall without the skin and the skin itself were placed in vials, individually identified, and moistened with gauze soaked in saline. Assays were performed in a maximum period of 3 hours after the preparation of the test specimens.

The information collected was entered into Excel spreadsheets. Analyses were performed using free calculators available at electronic addresses: http://faculty.vassar.edu/lowry/anova1u.html for the ANOVA and Tukey HSD Test and http://faculty.vassar.edu/lowry/fisher2×3.html for the Freeman-Halton test, an extension of the exact test.

The results were obtained using frequency and percentages for categorical variables and characteristics of measures of central tendency (mean and median) and dispersion (standard deviation) for quantitative variables.

Categorical variables were compared among the groups using the Freeman-Halton test, an extension of the exact test. Quantitative variables were compared among the groups using ANOVA. When a significant difference was identified, the Tukey HSD test was performed to verify which groups were different. Statistical analyzes were considered significant at $P < 0.05$.

RESULTS

One hundred percent of the animals in the GC and GL1 groups presented adhesion formation, whereas in the GL2 group, only 37.5% of the animals presented this type of formation. The number of adhesions found in the animals from the GC and GL1 groups was 3.12 ± 0.83 and 2.88 ± 1.96, respectively, while in the group GL2, this value was 0.38 ± 0.52. When the area was measured in the CG and GL1 groups, the values reached 1.17 ± 0.89 cm2 and $2.00 \pm 0.93\,cm^2$, respectively, whereas in the GL2 group, the average area reached $0.50 \pm 0.76\,cm^2$. Regarding toughness (fine or strong) and vascularization (vascular or avascular), no differences could be found among the groups. When the location of adhesions in the GC and GL1 groups was assessed, these formations could be identified in the intestines (72% and 83%, respectively), whereas in the GL2 group, the intestines showed no sign of these formations in 100% of the cases (Table 1).

The evaluation of the skin's and the abdominal wall's resistance had the objective of determining whether or not irradiation by low-intensity lasers would have any effect on this resistance. Upon analyzing the limit of tensile strength of the skin, no difference among the three groups could be observed. When analyzing the limit of tensile strength of the abdominal wall (not including the skin), no difference could be identified between the GC and GL2 groups. However, this value did prove to be higher than that found in the GL1 group (Table 2).

DISCUSSION

Abdominal adhesions remain a challenge for health care professionals, especially for surgeons. These formations are commonly related to hospital readmission after abdominal and pelvic surgery, bowel obstruction, as well as female infertility and pain [9]. Furthermore, they represent a considerable socio-economic burden on the health system in many countries [10,11].

Adhesiolysis (lysis of adhesions) is the only known form of treatment currently used to combat abdominal adhesions. However, this procedure carries a greater risk of perioperative complications and a high recurrence rate. Moreover, it is unable to prevent the recurrence of adhesions that could lead to future intestinal obstruction [9].

Fevang et al. (2004) illustrated that approximately 30% of all patients undergoing adhesiolysis due to an obstruction of the small intestine required a second operation to remove recurring adhesions [12].

TABLE 1. Characteristics of Peritoneal Adhesions in Different Groups

Groups	GC	GL1	GL2	*P*-value
Number	25	23	3	
				0.000[a]
				GL2 < GC = GL1[b]
Presence of adhesions				
Presence	8 (100%)	8 (100%)	3 (37.5%)	
Absent	—	—	5 (62.5%)	
				0.003[c]
Area (cm^2)	1.75 ± 0.89	2.00 ± 0.93	0.50 ± 0.76	
				0.006[a]
				GL2 < GC = GL1[b]
Sites				
Wall and omentum	7 (28%)	4 (17%)	3 (100%)	
Omentum and intestines	9 (36%)	—	—	
Other organs and intestines	1 (4%)	5 (22%)	—	
Wall and intestines	8 (32%)	14 (61%)	—	
Intestinal involvement	8 (100%)	8 (100%)	0 (0.0%)	
Vascularization (valued without magnifying glass)				
Vascularizada	20 (80%)	20 (86,96%)	3 (100%)	
Avascularizada	5 (20%)	3 (13.04%)	—	
				0.8240[c]
Vascularization (by microscopy)				
Vascularized	25 (100%)	23 (100%)	3 (100%)	
No vascularized	—	—	—	
Tenacity				
Firm	9 (36%)	15 (65%)	3 (100%)	
Loose	16 (64%)	8 (35%)	—	
				0.710[c]

CG, Control Group; GL1, Low Dose Group LASER, GL2, High Dose Group LASER. The number values, presence of adhesion sites, vascularization and tenacity are absolute.
The area values were calculated per animal by total.
[a]Anova.
[b]Tukey test for multiple comparison tests.
[c]Freeman-Halton test, extension of Fisher's exact test.

TABLE 2. Limit Tensile Strength of Skin and Wall Without Skin of the Three Groups on the 14th Postoperative Day

Groups	GC	GL1	GL2	*P*-value
Limit of tensile strength of skin (kgf/mm^2)	4.23 ± 1.20	3.10 ± 1.79	3.42 ± 1.25	0.3106[a]
Limit of tensile strength of wall without skin (kgf/mm^2)	3.70 ± 0.89	2.79 ± 0.74	4.88 ± 1.21	0.001[a] GC=GL1, GC=GL2 GL2>GL1[b]

CG, Control Group; GL1, Low Dose Group LASER, GL2, high Dose Group LASER.
The values expressed as mean ± standard deviation.
[a]Anova.
[b]Tukey test for multiple comparison analysis.

These data also suggest that surgery performed to lyse the adhesion is almost as adhesion-inducing as the original transaction. Two thirds of all bowel obstructions occurred within the first five years, nearly one fourth in the 10 subsequent years, and in many patients the risk was still present even 20 years later. This clearly places adhesions in the category of long-term complications [12].

The prevention of the formation of adhesions should therefore be the focus of attention. Peritoneal trauma represents the basis for the formation of adhesions after abdominal surgery [9]. According to Arung et al. (2011), only meticulous surgical techniques can be recommended to reduce the formation of adhesions, as well as reduce the morbidity and mortality rates stemming from these [9].

Several agents and their abilities to prevent the formation of postoperative adhesions were investigated. These agents worked to change the activation of fibrinolysis, hinder coagulation, decrease inflammatory response, inhibit collagen synthesis, or create a physical barrier between adjacent surfaces of wounds. The results, performed in animal models are encouraging, but most are contradictory [13–16].

In the present work, rabbits were chosen as an experimental model. In the literature, the rabbit has been used in approximately 35% of the complex musculoskeletal studies in biomedical sciences. Most studies present the advantage of size, ease of handling, and the fact that they reach skeletal maturity, after sexual maturity, at around six months of age. Moreover, rabbit skin is also quite similar to human skin.

The peritoniectomy model is devoted to the experimental induction of adhesion formation [3,16]. In the present study, such an intervention proved to be effective, since all animals in the control group (CG) presented adhesions. This finding is consistent with the literature, which identifies the peritoneum's aggression as a cause of adhesions [17]. The etiology of these would be related to failures in the peritoneal repair mechanism, in turn causing scarring that develops at trauma sites after surgery [10,18].

The use of low-level lasers with the aim of aiding in tissue repair has been widely researched since 1963. Six review articles, published between 2006 and 2010, and a meta-analysis [19], published in 2009, evaluated these lasers' effects on wound healing and pain relief. These investigations have shown that low-intensity lasers play an important role in wound healing, in activating the photobiostimulation of injured tissues, in accelerating tissue repair, in modulating the inflammatory process, as well as in reducing acute pain due to the inflammatory process. Several previous studies have shown that lasers regulate the release of cytokines responsible for fibroblast proliferation and collagen synthesis, thus, improving collagen deposition, and further resulting in the improved organization of architectural collagen fibers [20]. A study conducted in burned showed increased deposition of type III collagen in the laser irradiated animals when compared with control, non-irradiated, after 16 days of lesion, indicating that the laser treatment is able to accelerate the proliferative phase of healing [21].

Considerable variation in the research project, methodology, and irradiation parameters used limited the comparison of results among studies [22–24].

To define the parameters of laser application, it is necessary to consider a wide range of variables. What determines whether the light is photobiostimulating or is detrimental to the tissue is the intensity (I), also called irradiance or power density. Intensity is defined as the useful laser power, expressed in watts (W), divided by the irradiated area expressed in square centimeters (cm^2). The fluence (F), also called energy density or energy dose (ED), is the term used to describe the rate of energy applied to the biological tissue and is determined by multiplying the intensity (expressed in Watts per square centimeter or W/cm^2) by the exposure time (in seconds), which is expressed in Joules per square centimeter (J/cm^2).

Also present is the physical quantity of energy (E) that, in the application of laser light, represents the amount of laser being deposited upon the tissue and is calculated by multiplying the optical power of the appliance (expressed in watts) by the exposure time (expressed in seconds). The result is represented as the unit joule (J) [25].

Most works describe the dose fluence (J/cm^2), others in Joules (J), but most fail to inform other parameters, such as wavelength, energy deposited in the tissue density energy beam area, time of application, peak power (continuous), medium power (pulsed mode), and power density. The lack of comprehensive data makes it difficult to reproduce and compare results [19,25].

According to the World Association for Laser Therapy (WALT), the application of 1–4 J Final energy is required to achieve photobiostimulating effects caused by low-intensity lasers at wavelengths ranging from 780 to 904 nm, considering that no reference table exists for the wavelength of 660 nm. In this study, two laser doses were used, one outside the therapeutic window (5J/cm^2 for 40 second = 0.2 J) and one near the bottom of the window (90J/cm^2 for 90 s = 3.6 J) to determine if the therapeutic window, according to that proposed by WALT would also apply to the laser wavelength of 660 nm [26].

There is great confusion in relation to units of measurement for laser application. Some authors report that parameter in fluence (J/cm^2) and other in energy (J). These measurement units differ widely. The original work of Mester (1985) used a dose of 4 J and many authors use values close to 4J/cm^2 believing to be using the value proposed by Mester [6]. In fact, they are quite different. We used in Group 1 dose as close to 4 J/cm^2 (not therapeutic dose, but very used in papers) and in G2 the value closer to 4J (therapeutic dose) in order to demonstrate that the therapeutic response is totally different if we vary the deposited energy this way.

One of the difficulties in conducting research on adhesion formation has been the lack of uniformity in the classifications and interpretations of adhesions in the literature [4,5]. Most methods, using various parameters, consist of indexes and fail to accurately report the evaluations of each. The use of subjective terms, like "small number","generalized adhesions", "limited vascularization", "moderate adhesions", and "minimal adhesions" also make it difficult to compare results [5].

In assessing the presence or absence of adhesions, the low-intensity lasers, at a dose of 3.6 J (within the therapeutic window) in the GL2 group, were able to significantly reduce these formations. The application at a dose of 0.2 J (outside the therapeutic window) in the GL1 group, presented results that were similar to the CG group. The index of the animals from the GL2 group that were free of adhesions (62.5%) was superior to that achieved by Seprafilm® resorbable membrane (51%), currently considered one of the most effective methods of reducing the formation of adhesions. However, the use of Seprafilm® significantly increases the incidence of abdominal abscesses and fistulas, as well as dehiscences of anastomoses, which can potentially contaminate surgery [27].

At a dose of 3.6 J (within the therapeutic window), the application of low-intensity lasers proved effective in reducing the area of adhesions that had developed. Unfortunately, measuring the area of adhesion formation is quite unusual in the findings from prior medical literature, making it difficult to compare the data from this experiment [28]. The difficulty of measuring this parameter can also be a limiting factor, since adhesions are not presented uniformly. In this paper, the method used is original and sought data that referred to the contact between the adhered surfaces. Further research should be conducted to clarify the pathophysiological mechanisms of this reduction.

The lack of difference between the tenacity of adhesions among the groups suggests that the application of low-intensity lasers did not affect the quality of the treated tissue. Although there are no similar studies, some investigations have shown that the application of low-intensity lasers does not alter the quality of the formed scar tissue and only accelerates the healing process, which could explain, in theory, the lack of difference among groups [19,22–24].

In the present work, a total of 53 adhesions formed: 45 (84.90%) could be observed when viewed by the naked eye; when the evaluation was performed by light microscopy, 100% could be viewed. This finding is in agreement with the literature which describes adhesions as highly vascularized tissue containing well-developed arterioles, capillaries, and venules [16].

The greater involvement of small and large intestine adhesion is similar to findings from other reports, (1,4). However, at a dose of 3.6 J (within the therapeutic window), the application of low-intensity lasers was able to minimize this involvement. All adhesions developed in the GL2 group (3.6 J) involved only the abdominal wall and the omentum. According to the literature, the low-intensity lasers accelerate the healing process. It is possible to conclude that a faster healing process also presents the severity of the adhesions. Hence, bowel adhesions are less likely to become symptomatic and cause acute abdomen [4].

The aim of biomechanical studies is to determine the mechanical properties of a material, which, depending on its physical characteristics, can be submitted to tensile strength, torsion, compression, and bending [29]. The present study was carried out to test the tensile strength in an attempt to identify whether or not the application of low-intensity lasers are able alter this limit of scar tissue on the skin and on the abdominal wall, not including the skin. This study sought to identify the elastic limit, i.e., the point at which the fabric undergoes no permanent deformation. By halting the test before this point and removing the traction force, the specimen returned to its original shape, like a rubber band.

Laser application, either within (3.6 J-GL2) or outside the therapeutic window (0.2 J-GL1), did not alter the limit of the skin's tensile strength when compared to the CG (no treatment). This finding is consistent with the literature, confirming that the application of low-intensity lasers does not alter the quality of the scar tissue, which could explain the lack of difference among groups [23,24].

As there is no bone support on the wall of the abdominal cavity, its strength is derived entirely from the shape of its soft tissue structures. The strength of the abdominal wall is of great importance because its weakening is primarily responsible for the formation of hernias and eviscerations. Laser application in (3.6 J-GL2) or outside the therapeutic window (0.2 J-GL1) did not alter the tensile strength limit of the abdominal wall when compared to the CG (no treatment), showing that laser applications cause no weakening of the abdominal wall and are safe for use in abdominal surgery, especially as regards the formation of hernias and eviscerations.

Further studies using different dosages and other laser wavelengths are warranted to better comprehend the mechanism of action of low-intensity lasers.

CONCLUSIONS

The application of low-intensity lasers at a dose of 3.6 J, a fluence of 90 J/cm^2 of 40 mW power, and a time of 90 seconds is effective in reducing the formation, number, and area of abdominal adhesions in rabbits, without modifying the characteristics (tenacity and vascularization), nor compromising the postoperative strength and healing of the abdominal wall.

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