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Low-intensity laser therapy and led (light emitting diode) therapy in mechanical resistance of *Rattus norvegicus* chest inscision with implant of steel wire for sternal suture

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Abstract ***Introduction:*** *Low-intensity laser (LILT) and LED therapy are indicated in repairing surgical incisions, which occur in cardiac surgery. One major concern in this case is the presence of metallic wire used for sternal sutures, its temperature may rise while using a laser or LED. This study aimed to analyze the tensiometric properties of the skin tissue with longitudinal incision and metallic wire implant used for sternal suture irradiated with LILT and LED.* ***Methods:*** *Twenty-five subjects (Rattus norvegicus) were submitted to thoracic incision with implantation of metallic wire, and subsequently divided into five groups: GI (control group); GII (laser group 6J/cm^2); GIII (laser group 10J/cm^2); GIV (LED group 6 J/cm^2); and GV (LED 10J/cm^2). Photobiomodulation was performed on a daily basis for eight consecutive days and the local temperature was measured before and after phototherapy. After euthanasia the tissues were subjected to tensiometric and histological analysis.* ***Results:*** *All irradiated groups showed increase in break strength ($p = 0.009$), and decrease in tissue deformation strength compared with the control group ($p = 0.03$).* ***Conclusion:*** *Both LILT and LED can promote wound healing at the tested doses with increased tissue resistance, although its elasticity is reduced. The imprecision of the assessing instruments precluded an accurate measurement of a possible local tissue heating post irradiation and future studies are need to elucidate this effect.*

Keywords Low-intensity laser therapy, LED, Interaction laser biological tissue.

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Introduction

The low-intensity laser therapy (LILT) has been applied in tissue repairing and acceleration of the healing process, being a study object in numerous medical areas. The LED (light emitting diode) arises with the development of technological systems of light production and emission, being a hopeful alternative for tissue repair therapy (Meyer *et al*., 2010; Sousa *et al*., 2010; Trelles and Allones, 2006). Despite this fact, the number of researches about this technique comparing LILT to LED therapy is still insufficient.

The laser is characterized by the monochromaticity, collimation and consistency of the emission of its light beam. Its application has been widespread in medicine and physical therapy due to its therapeutic effects as analgesic, anti-inflammatory and tissue repairer (Brugnera, 2004). LILT stimulates release of growth factors by macrophages, increases keratinocytes proliferation and increases the number of mast cells (its granulation and even angiogenesis). The photons emitted by laser are converted into chemical and kinetic energy within the cell, which modifies the membrane permeability and increases the nitric oxide formation. The increase in oxidative metabolism may result in an increase of ATP synthesis (glycolysis and oxidative phosphorylation), which leads to cell function adjustment (Leal Junior *et al*., 2009). Calcium is released from mitochondria due to proton gradient increase within the cytoplasm, which generates regulation of cellular metabolism, repair, cell migration and proliferation, RNA, DNA and protein synthesis. There is an increase in immune response, with activation of T lymphocytes and macrophages (Giuliani *et al*., 2004), endorphin levels also increase and a parallel decrease of bradykinin resulting in pain relief (Lorenzini *et al*., 2010).

The high manufacture cost of laser emitting devices, besides the large number of parameters to be selected (such as fluency, power, treatment time and irradiance), encouraged researchers to develop alternative sources of phototherapy, such as light emitting diode (LED). Some studies show evidence that irradiation with non-coherent light proved to be more profitable and can be as efficient as laser radiation (Casalechi *et al*., 2009; Corazza *et al*., 2007; Dall-Agnol *et al*., 2009).

LED devices, according to Andrade *et al*. (2001), are systems that produce monochromatic and high cleanliness light when energized. Those systems work with semiconductors that present the characteristic of conducting electric current in one direction only. Although this type of light is present in our daily lives in many electronic components, its use as photobiomodulator is recent (Guaratini *et al*., 2007).

Other interesting features reported on LED show that its light emission is more selective when compared to regular bulbs, the device has more durability and need less electrical power for its operation. On the other hand, the device temperature increase during its operation is meaningless, thus not causing further damage to the unit even if it is used for prolonged periods (Kurachi *et al*., 2001). Those aspects associated to low cost and convenience, are leading LED devices to conquer the market, also they have been tested and studied increasingly in terms of their therapeutic properties. Minatel *et al*. (2009) reported in their study that LED therapy shows a wide variety of effects, as well as pain relief, tissue healing and anti-viral properties.

Among the biophysical effects of photobiomodulators, as low-intensity laser and LED therapy, is the heating caused by electromagnetic waves radiation in the infrared or red spectrum (Casalechi *et al*., 2009). In this manner, a better understanding of the healing process mediated with photobiomodulators and also skin heating processes using low-level laser and LED therapy are important in terms of providing more safety to the technique, obtaining the best results using recommended therapeutic doses without excessive tissue heating.

LED therapy and LILT have been used to irradiate surgical incisions in order to accelerate the postoperative recovery (Trelles and Allones, 2006). However, some specific surgeries require equally specific care. Cardiovascular surgery with sternotomy, for example, requires sternum suture with stainless steel wire, a heat sensitive material which may heat up when irradiated indirectly by LILT or LED therapy. This study aimed to analyze the tensiometric properties of the skin tissue with longitudinal incision and with a subcutaneous implant of a metallic wire used for sternal suture irradiated with LILT and LED.

Methods

This research was submitted to approval of the Ethics Committee from the Integral Diferencial University (FACID), being authorized by the protocol number 022/11. The procedures followed the recommendations of the Institute of Laboratory Animals Resources of the National Academy of Science Guide for the care and use of laboratory animals, Washington DC, and the Brazilian College of Animal Experimentation (COBEA) guideline.

Twenty-five male albinos Wistar rats (*Rattus norvegicus*) weighing 246.3 ± 50.7 g were

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studied. They were randomly organized in appropriate cages and subjected to a light/dark cycle of 12 hours each, starting the light phase at 7 a.m. with an environment temperature of 23 °C ± 1, with food and water *ad libitum*, at the bioterium from the University of Health, Human and Technological Sciences of Piauí – NOVAFAPI, in Teresina – PI.

The animals were anesthetized with a mixture of 10% ketamine and 2% xylazine, in the proportions 1:2, at doses of 0.10-0.15 mL per 100 g of body weight, intramuscularly. The upper sternum was shaved for hair removing. Then an incision, 4 cm length, was performed using a scalpel blade for a monofilament of Aciflex™ No. 3, which is a stainless austenitic steel 360L wire, (18% Cr 10% Ni 2% Mo), nonabsorbable, 480 J/(kg°C) specific heat and 14 W/(m°C) thermal conductivity at 20 °C. The monofilament was cut, 4 cm length, and longitudinally positioned on the sternum and then fixed with a monofilament of nylon synthetic nonabsorbable wire No. 3 at 0.5 cm from each edge of the steel wire, as observed in Figure 1. Finally, the skin wound was sutured with nylon wire (Figure 1). Upon surgery conclusion a single dose of Pencivet, an antibiotic 1 ml/kg was administered to all groups intramuscularly.

After surgery, the subjects were divided into five groups of five animals each, being four experimental groups and one control group, distributed as follows: GI (control group); GII (laser group 6 J/cm^2); GIII (laser group 10 J/cm^2); GIV (LED group 6 J/cm^2) and; GV (LED 10 J/cm^2). The first irradiation was performed two hours after surgery and then daily until the eighth postoperative day, in a total of eight sessions.

The equipment used for the LILT was a Laser Hand model (InGaAlP), MMOptics™. The device was adequately calibrated by the supplier. The LED therapy equipment was Starlaser model, Microdont™ (Table 1). The equipment was calibrated at the Laboratory of Tissue Biomodulation at the University of the Paraíba

Table 1. Parameters of the instruments used for phototherapy.

Parameters	Laser (660 nm)		LED (640 ± 20 nm)	
	GII	GIII	GIV	GV
ED (J/cm^2)	6	10	6	10
E (J)	2.4	4	10.1	18
P (W)	0.04	0.04	0.07	0.07
Ø (cm)	0.5	0.5	1.5	1.5
A (cm^2)	0.4	0.4	1.77	1.77
T (s)	60	100	152	253
PD (W/cm^2)	0.1	0.1	0.03	0.03

GII – group II; GIII – group III; GIV – group IV; GV – group V; ED – energy density; E – energy; P – power; Ø – diameter; A – beam area; t – time of irradiation; PD – potency density.

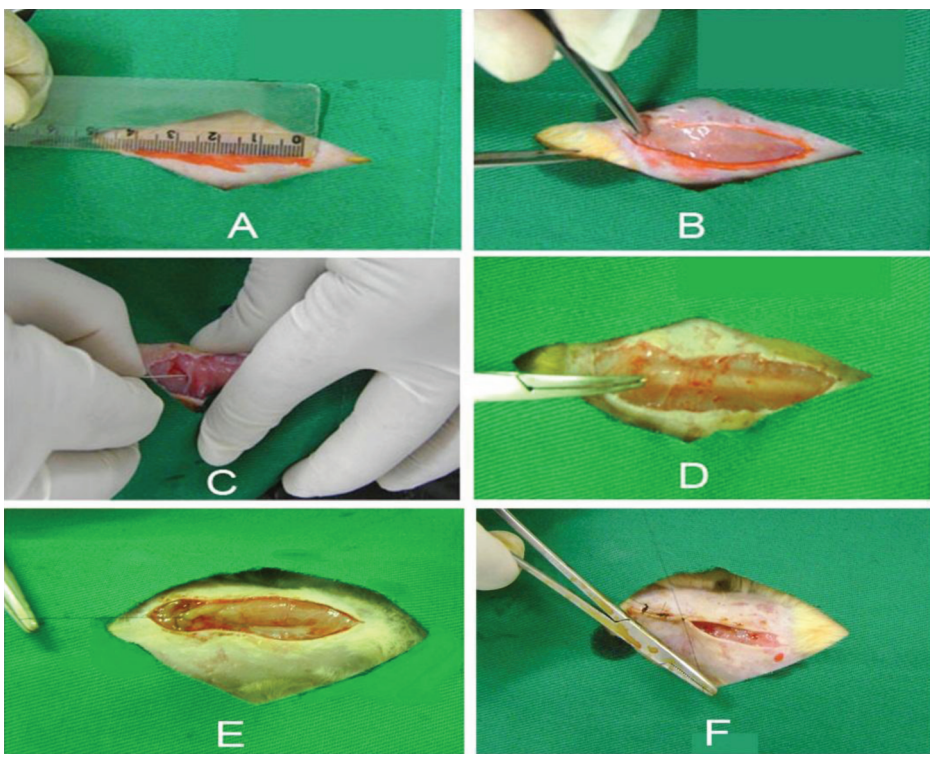

Figure 1. Surgical procedure: A) Incision area measurements; B) Surgical incision; C) Introduction of the wire; D) Positioning along the sternum; E) Fixing the wire into the surrounding tissue with nylon; F) Suture of the skin edges with nylon.

Valley (UNIVAP). The edge of the laser or LED emitter was wrapped with a translucent plastic film and then positioned 0.5 cm far from the irradiation area of the subject's chest. The irradiation was performed in two incision spots, 2 cm away from each other.

The studied variables were the local heating, the tissue maximal break strength and maximal deformation strength, and the presence of histological signs of tissue damage due to heating, such as blistering or necrosis.

The body temperature was assessed by a precision infrared thermometer, Testo™, 830 T1 model, ranging from –30 °C to +400 °C and 0.5 °C of resolution and 1.5% of m.v. The skin temperature was measured exactly at the LILT and LED irradiation spot, before and immediately after irradiation, maintaining a distance of approximately 2 cm far from the thermometer sensor and the animal skin, as represented in Figure 2. This procedure was repeated daily until the 8th day after surgery. In the case of the control group, the animals had their temperature measured every day until the 8th day after surgery at the corresponding skin spot of the other groups.

On the eighth day after surgery the animals were euthanatized using pentobarbital at a dose of 100 mg/kg via intraperitoneal route. A tissue sample, 3 cm long and 4 cm wide, was withdrawn of each animal by a scalpel blade, transversally to surgery incision scar, and submerged into a recipient with Ringer Lactate solution for transportation to the Mechanical Testing Laboratory of the National Service of Industrial Learning in Teresina (PI) for tensiometric tests performed with an equipment DL20000 Emic™. The travel time between the bioterium and the Mechanical Testing Laboratory consisted of less than 30 minutes, minimizing the possible mechanical changes of the tissue caused by the analysis delay. The grip pressure was manually adjusted and the data were electronically acquired by the Tesc™ software, version 1.01. The pressure claws were positioned 1.5 cm far from each other, fixing the tissue with the scar at an equal distance to the edges of the claws (Figure 3). The maximal break strength and maximal deformation strength of the tissue were measured at a speed of 50 mm/min.

A small piece of the same tissue was removed for microscopic analysis of the subcutaneous area surrounding the metallic implant. As this tissue was in close contact with the metallic wire, it would be more likely that it would suffer injury by the apparent heating of that material. The tissue was preserved into formaldehyde 10%, then kept with water softener for 2 days and, finally, sliced with a microtome in sections of 4 μm thickness and colored with eosin hematoxylin. The histological slides were analyzed by an optical microscope.

The maximal break strength and maximal deformation strength were statistically analyzed by Kruskal-Wallis test and Turkey HSD post-hoc test. The tissue heating was analyzed using the Wilcoxon sum runk test. Statistic analyses were performed using the software Statistica 7.0 StatSoft™. Confidence interval of 95% and $p \leq 0.05$ were considered as statistically significant.

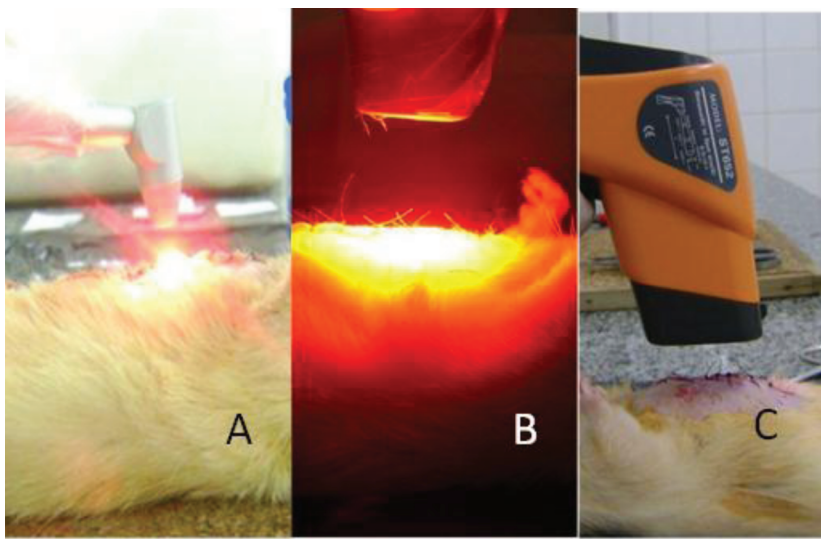

Figure 2. Incision temperature measurement: A) Laser irradiation; B) LED irradiations; C) Temperature measurement.

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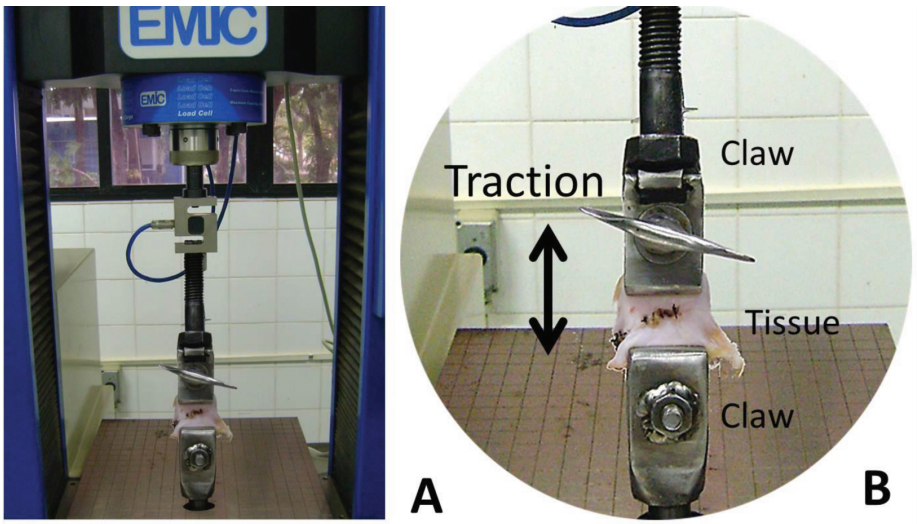

Figure 3. Tensiometric analysis: A) Equipment for tensiometric analysis at the Mechanical Testing Laboratory of the National Industrial Learning Service in Teresina (PI), model DL2000 (Emic ®); B) Positioning and stretching the tissue.

Results

Macroscopic aspects and microscopic analysis

In the macroscopic assess of the lesions, signs of infection were detected in tissue adjacent to the steel wire in two rats, one of GII and other of GIV. Suture dehiscence of the skin was also detected in other animal (GV). All of them were excluded from the study. The microscopic analysis showed no evidences of tissue overheating, such as swelling, blistering or tissue necrosis. Furthermore, all groups presented vascular increase (Table 2).

Local tissue temperature

All groups showed increase in temperature after irradiation, as seen in Table 2. Moreover, due to the minor temperature values recorded in the tissues and the accuracy limits of the instrument, this possible tissue heating cannot be statistically demonstrated or compared. Furthermore, according to results the average temperature increase in LED 6 J/cm^2 group was 1.07 ± 0.26 °C, in LED 10 J/cm^2 group was 1.58 ± 0.22 °C, in Laser 6 J/cm^2 group 0.37 ± 0.11 °C and Laser 10 J/cm^2 0.59 ± 0.25 °C, as indicated in Figure 4.

Tensiometric analysis

The maximal break strength (MBS) is the highest tension the tissue supports before rupturing, measured in gram × force (gf). The results showed that MBS was higher in LED 6 J/cm² group ($p = 0.04$), LED 10 J/cm² ($p = 0.02$) and laser 6 J/cm² ($p = 0.03$) when compared to the control group. Only 10 J/cm² laser group did not improve the MBS, according to Kruskal-Wallis test (Figure 5).

The maximal deformation strength (MDS) is the highest deformation the tissue supports before rupturing. The LED 6 J/cm² group showed less tissue deformation compared to LED 10 J/cm² group ($p = 0.03$) and control group ($p = 0.02$), as seen in Figure 6. The other groups showed no significant difference compared to the control group.

Discussion

Microscopic analysis

Sternal dehiscence and infection are serious surgical complications by high morbidity, mortality and also difficult to control, leading researchers to pursuit new diagnostic and therapeutic methods. According to the classification proposed by the Center for Disease Control and Prevention (CDC-USA), infection in the incision of sternotomy should be considered superficial if it affects only the skin and subcutaneous tissue, and deep when it extends to the sternum. The infection can involve other structures surrounding the sternum causing sternal osteomyelitis or mediastinitis. This study analyzed the incidence of superficial wound infection, which according to Brocki et al. (2010) affects between 1.6% and 6.4% individuals after surgery. In this study, 7.14% developed superficial infection.

The incidence of suture dehiscence was 3.57%. There is not a specific etiology for this incidence, but

Table 2. Microscopical analysis.

Group	Swelling	Blistering	Necrosis	Vascular increase
LED 6 J/cm²	-	-	-	++
LED 10 J/cm²	-	-	-	++
Laser 6 J/cm²	-	-	-	+
Laser 10 J/cm²	-	-	-	++

Absent (-); discrete (+); moderate (++); severe (+++).

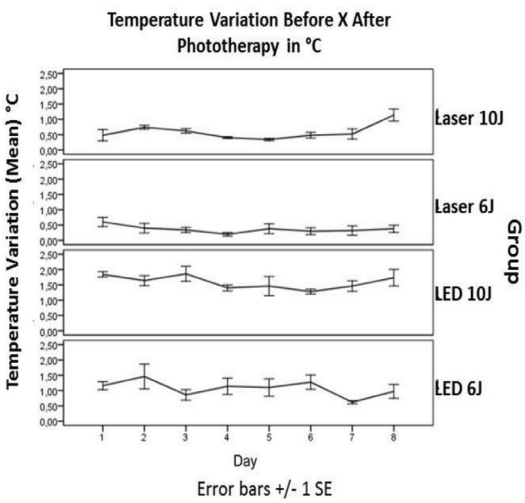

Figure 4. Temperature variation before X after phototherapy with LED and laser - Mean (°C) and standard error.

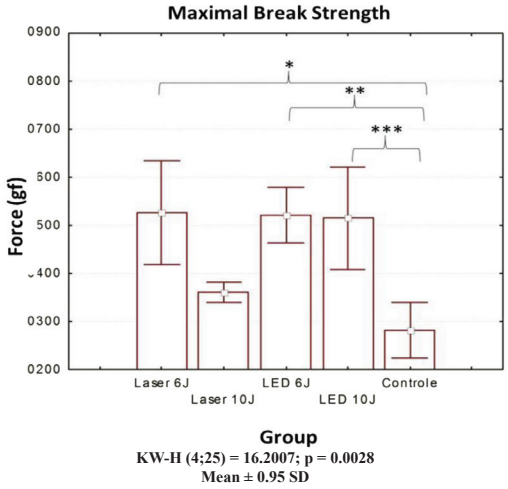

Figure 5. Median and standard error of the maximum force of rupture in terms of gram × force. Kruskal-Wallis Test (KW-H). Turkey HSD test showed significant differences between Laser 6 J x Control (*p = 0.03), LED 6J x Control (**p = 0.04) and LED 10 J x Control (***p = 0.02).

it is assumed that these results are due to the animal's own contact with the surgical wound.

There was no evidence of blisters and necrosis surrounding the tissue. This circumstance can be justified by the fact that the photobiomodulators intensities used in this research could not achieve

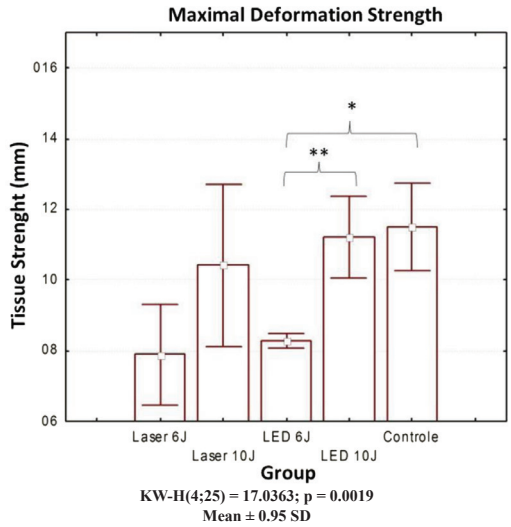

Figure 6. Graph of the mean and standard error of the tissue deformations in each group in millimeters. Kruskal-Wallis test (KW-H). Turkey HSD test showed significant differences between LED 6J x LED 10J (*p = 0.03) and LED 6J x Control (**p = 0.02).

a temperature level required to cause cell injury by heating. The biological effects of laser therapy are not thermal. Only the high-power lasers, operating with powers up to 1 W, whose effects are related to the high and rapid local heating of the tissue, can cause cellular damage due heating (Ignatieva *et al*., 2011).

Tissue heating

The groups irradiated with coherent light (laser) showed no significant temperature change comparing the measures taken before and after irradiation. This result is consistent with other reported studies. According to Capon and Mordon (2003), the use of doses smaller than 35 J/cm² are considered too low to make any detectable warming in irradiated tissues. Ignatieva *et al.* (2011) advised that the change in temperature due to LILT results in a maximum 1°C, which is sufficient to produce the photothermal effect. However the results showed an average increase of 0.59 ± 0.25 °C with laser 10J/cm² and 1.58 ± 0.22 °C with LED 10 J/cm².

Circeli *et al*. (2009) carried out a research on fibroblasts cells heating when irradiated with therapeutic LED and found that there was no significant

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increase in temperature, comparing the increase in time due to the cumulative effect of radiation. The temperature difference was 7 °C between groups irradiated with dose of 10.5 J/cm^2 and 37.5 J/cm^2, using two distinct intensities: 12.5 mW/cm^2 and 25 mW/cm^2. Nevertheless, few studies have been conducted on the thermal effects of LED therapy. The present study showed that there are evidences proposing that LED increases local temperature to a higher level when compared to LILT. It is important to note that this possible heating is unable to cause tissue damage independently, even in the presence of metal wire. These results show evidences that the LED therapeutic technique is safe when used to irradiate sternotomy sutured with steel wire.

Tensiometric analysis

The healing process is responsible for the skin integrity maintenance, resulting in higher tensile strength. Among the cellular response involved in repair process, we can mention the complex interaction of inflammatory cells such as neutrophils, lymphocytes, monocytes and macrophages (Uribe *et al*., 2009).

The fibroblasts collagen synthesis, induced by light stimulation, is the key to explain the increase in maximal break strength in tissues irradiated with laser or LED, which decreased in the control group.

There are several mechanisms by which photobiomodulators can induce mitotic activity of fibroblasts. Among them, the most relevant to this study is that they stimulate the basic fibroblast growth factor (FGF-b) production, which is a multifunctional polypeptide secreted by the fibroblasts, being capable to induce not only proliferation but also fibroblast differentiation (Circeli *et al*., 2009). Fushimi *et al*. (2012), in an *in vivo* study with skin wounds, showed that green LEDs promote wound healing by inducing migratory and proliferative mediators, which suggests that not only red LEDs but also green LEDs can be a new powerful therapeutic strategy for wound healing. They also showed that LILT and LED can stimulate immune cells to secret cytokines and other growth regulatory factors.

In vitro studies, using macrophage cell lineage, indicated that when these cells are submitted to LILT they release soluble factors promoting fibroblast proliferation. The fibroblasts growth and its movement through the matrix are also influenced by laser irradiation (De Araujo *et al*., 2007). Studies have also implied that LILT increases fibroblast proliferation regardless of the wavelength applied. These studies also showed that there is an increase in fibroblast proliferation in tissues irradiated with lower energy densities (4-6 J/cm^2) compared to those irradiated with higher doses. However, the limits of energy density to achieve a better response of fibroblasts are still not very clear.

According to Fushimi *et al*. (2012), coherent and non-coherent light (laser and LED) at the same parameters of wavelength, intensity and dose, provide the same biological response, which is confirmed by Sousa *et al*. (2010). Therefore, coherence was not essential in obtaining the same effects of laser using LED. Whelan *et al*. (2001) enhances this understanding when stating that coherence is lost at the first few layers of biological tissue.

In terms of Maximal Deformation Strength (MDS), the present study showed that there was a decrease in the elasticity of irradiated tissues, especially those irradiated with lower energy densities, when compared to control group. The differences where more significant between LED 6 J/cm^2 x LED 10 J/cm^2 groups ($p = 0.03$) and LED 6 J/cm^2 x Control group ($p = 0.02$), as showed in Figure 6. This result can be explained by the fact that the scar has only about 80% of the resistance compared to normal skin, however, their ability to resist deformation is decreased due to the collagen deposition and the fibrils rearrangement (Baum and Arpey, 2005; De Araujo *et al*., 2007; Reis *et al*., 2008).

Some studies indicate that energy densities over 12 J/cm² cause inhibitory effects, delaying tissue repair (Houreld and Abrahamse, 2007). Circeli *et al*. (2009) conducted LED therapy with different energy densities levels irradiated in fibroblast culture. The doses used were 10.5, 18, 25.5, 37.5 J/cm² in two different intensities (12.5 and 25 mW/cm^2). The results suggested that LED caused a statistically significant reduction in cellular metabolism of the irradiated groups when compared to the control group. This fact could also explain the decreased tissue ability of stretching after being irradiated with higher intensities of laser or LED.

Hawkins and Abrahamse (2005) irradiated human skin fibroblasts with LILT, using doses of 0.5, 2.5, 5.10 and 16 J/cm^2 for two consecutive days. The results showed that doses of 5 J/cm^2 stimulated mitochondrial activity, cell proliferation and fibroblasts migration. However, higher doses decreased cell viability and proliferation with damage to the cell membrane and DNA.

The results reported in this study emphasized the need for a better understanding about the optimum dose of LILT or LED therapy to obtain improved results in terms of mechanical resistance to deformation of the biological tissue.

Based on the parameters found by tensiometric analysis, the present study can conclude that

photobiomodulators irradiation using laser or LED, in doses of 6 J/cm² and 10 J/cm², increase the tissue resistance to rupture but decrease the strength capacity. The lack of deformation is probably due to an increased recruitment of fibroblast cells, which synthesize collagen, giving more resistance to the tissue but lower deformation capacity, also justifying the presence of increased tissue deformation in control and LED 10 J/cm² groups.

Tissue heating presented minor values which limited possible conclusions concerning this property, mainly due to accuracy limits of the instrument. Moreover, preliminary results advise that the increase in tissue temperature during LILT or LED irradiation is not sufficient to cause damage, as demonstrated by the microscopic analysis, even around metal suture.

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