The effects of different doses of 670 nm diode laser on skin flap survival in rats[1]

O efeito de diferentes doses de laser de diodo 670 nm na viabilidade de retalho cutâneo randômico em ratos

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ABSTRACT

PURPOSE: To investigate the effects of different low-level laser therapy (LLLT) doses on random skin flap rats.

METHODS: Forty Wistar rats were randomly divided in four groups. The control group (CG) was not irradiated. The experimental groups were irradiated with a diode laser 670 nm with different energies per point: group 2 (G2) with 0.06 J; group 3 (G3) 0.15 J and group 4 (G4) 0.57 J. The three groups were irradiated in 12 equally distributed points in the cranial skin flap portion. They were submitted to the irradiation during the immediate, first and second postoperative days. The necrosis area was evaluated in the seventh postoperative day.

RESULTS: The CG shows 49.35% of necrosis area in the skin flap; G2, 39.14%; G3, 47.01% and G4, 29.17% respectively. There was a significantly difference when G4 was compared with CG`s skin flap necrosis area.

CONCLUSION: The low-level laser therapy diode 670 nm with 0.57 J energy per point increases the survival in randomic skin flap rats.

Key words: Surgical Flaps. Laser Therapy, Low-Level. Lasers, Semiconductor. Rats.

RESUMO

OBJETIVO: Investigar os efeitos de diferentes doses de laserterapia de baixa intensidade na viabilidade de retalhos cutâneos randômicos em ratos.

MÉTODOS: Quarenta ratos Wistar foram randomizadamente distribuídos em quatro grupos. O grupo controle (GC) não foi irradiado. Os animais dos grupos experimentais foram irradiados por laser de diodo (670 nm) com as seguintes energias ofertadas por ponto: grupo 2 (G2) 0,06J; grupo 3 (G3) 0,15 J e grupo 4 (G4) 0,57 J. Os três grupos foram irradiados em 12 pontos igualmente distribuídos na porção cranial do retalho cutâneo. Todos os animais dos grupos 2, 3 e 4 foram submetidos ao protocolo de irradiação por três dias consecutivos, iniciando no pós-operatório imediato. A área necrótica foi avaliada no sétimo dia pós-operatório.

RESULTADOS: A porcentagem de área necrótica apresentou-se da seguinte forma, nos diferentes grupos: GC= 49,35%, G2= 39,14%, G3= 47,01 e G4= 29,17%. Foi encontrada diferença estatisticamente significante quando se compararam os dados entre GC e G4.

CONCLUSÃO: A irradiação com laser de diodo (670 nm) de baixa intensidade, com oferta de 0,57 J de energia por ponto influenciou positivamente a viabilidade de retalhos cutâneos randômicos em ratos.

Descritores: Retalhos Cirúrgicos. Terapia a Laser de Baixa Intensidade. Lasers Semicondutores. Ratos.

Introduction

In plastic and reconstructive surgery, skin flap is the most frequently used method for repairing tissue loss following trauma or surgical procedures[1,2].

Low-level laser irradiation may increase flap survival, provided that an appropriate amount of energy is delivered. Laser treatment has been reported to give good results when applied to skin flaps[3-8], except for Smith[9], who did not find any significant effect in the use of this therapy.

The state of the art in all areas of laser therapy indicates the necessity of parameterization studies in order to determine the effectiveness of therapeutic applications.

The current challenge for researchers in laser therapy is to find the appropriate parameters (wavelength, output power, emission mode, spot size and shape of the laser beam, length of irradiation, and applied technique) for the therapeutic application to be effective, whether at high or low power, or in photodynamic therapy[10-14].

To the best of our knowledge, the evaluation of the viability of random-pattern skin flaps in the rat after 670 nm laser treatment at different therapeutic doses has not been carried out by any other researcher, and this provided the motivation for this study.

The purpose of this study was to investigate the effects of different irradiation doses of a 670 nm low-power diode laser on the viability of random-pattern skin flaps in the rat.

Methods

This study was submitted to Research Ethics Committee of the Paulista University and approved certification is register by protocol 008/2011.

The use of laboratory animals followed the ethical code for animal experimentation of the Council for International Organization of Medical Sciences, the norms of Brazilian Society of Science at Laboratory's Animal (SBCAL-COBEA) and the current national legislation about procedures to use animals experimentation (Federal Law 11,794 on October 9th, 2008). Forty adult male Wistar rats (*Rattus norvegicus albinus*), weighing between 251 and 358g, were used in the study.

In the present study, a low-power diode laser model Physiolux Dual (BIOSET Indústria de Tecnologia Eletrônica Ltda, Rio Claro, SP, Brazil) was used, emitting red light at 670 nm, with an output power of 30 mW and laser beam diameter of 0.028 cm^2. Supplies included tracing paper, felt-tip pen, medical-surgical materials and a template made to ensure uniformity in the application of low-level laser.

All 40 rats were housed in individual cages in a temperature-controlled environment (21°C), on a 12:12 hour light-dark cycle, and fed standard rat chow and water *ad libitum*. The rats were randomized into 4 groups of 10 animals each, and the animals in the groups were subjected to the following procedures:

Group 1 (G1) – A random-pattern skin flap was created and exposed to sham irradiation (control group);

Group 2 (G2) - A random-pattern skin flap was created and exposed to 670 nm laser irradiation at an energy density of 2.14 J/cm^2 for 2s, delivering 0.06 J per point;

Group 3 (G3) – A random-pattern skin flap was created and exposed to 670 nm laser irradiation at an energy density of 5.36 J/cm^2 for 5s, delivering 0.15 J per point;

Group 2 (G4) - A random-pattern skin flap was created and exposed to 670 nm laser irradiation at an energy density of 20.36 J/cm^2 for 19 s, delivering 0.57 J per point.

Surgical procedure

The animals were anesthetized with tiletamine hydrochloride and zolazepam hydrochloride (25 mg/kg, intraperitoneally). Following anesthesia, the rats were placed on a flat surface with legs extended and their backs were shaved. The skin flap was outlined with a surgical skin marker considering the inferior angles of the scapula and the bones of the pelvic girdle as anatomical limits (Figure 1).

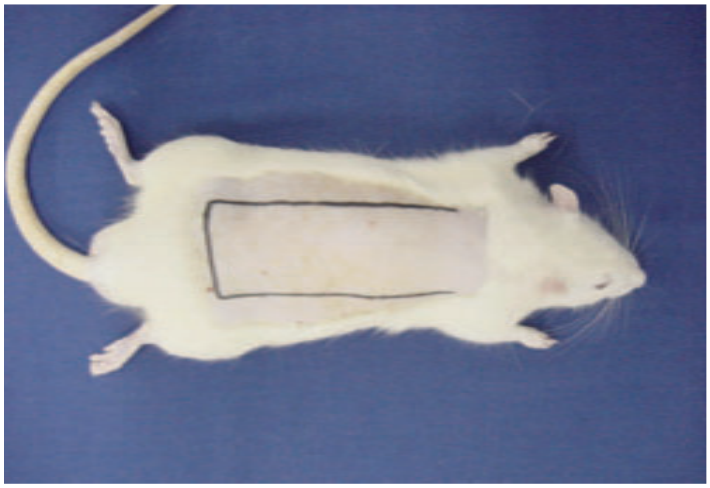

FIGURE 1 - Dorsal view of the animal after shaving, showing the flap outline.

After, a 10 x 4-cm, cranially-based, random-pattern, dorsal skin flap was elevated in all groups according to the procedures described by McFarlane[15] (Figure 2).

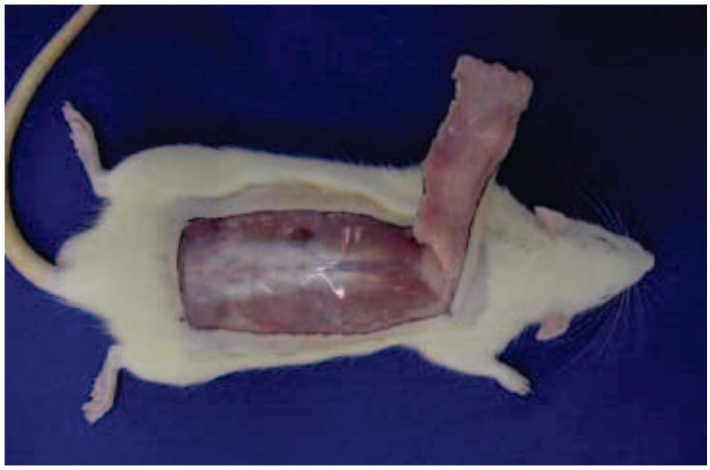

FIGURE 2 - Skin flap elevation and positioning of plastic barrier F1.

The flap consisted of superficial fascia, panniculus carnosus, subcutaneous tissue and skin. Once the flap was elevated, a plastic barrier (film F1) was cut to shape and placed between the superficial fascia and the superficial muscle layer to prevent revascularization of the flap from the recipient bed. The flap was returned to its original position and sutured in place with 25 simple interrupted 4-0 monofilament nylon sutures[15] (Figure 3).

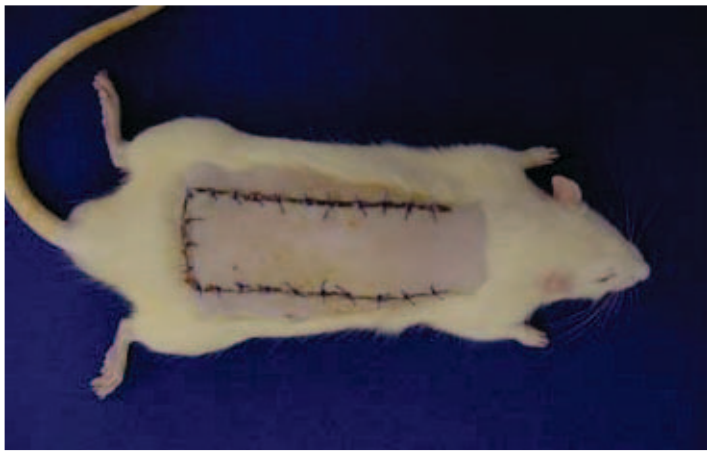

FIGURE 3 - Skin flap sutured in place with 4-0 monofilament nylon.

A template was made of black Bristol board, having the dimensions of the skin flap and containing guide holes, which corresponded to the location of the treatment points where the tip of the probe was inserted. The template enables interference-free beam transport and ensures uniform positioning of the probe, so that when the cranial side of the template contacts the inferior angle of the scapula, its lateral edges are positioned exactly over the margins of the flap, ensuring that the laser beam is applied to the same locations on the flap of the study animals. The treatment points were placed 1 cm from the margin of the flap and spaced at 1-cm intervals, so that 12 guide holes were made in the cranial half of the template.

After this procedure, the animals in the G1 group were anesthetized for 20 min, the laser probe was positioned and no irradiation was applied. The G2 group was anesthetized for 20 min and exposed to 670 nm laser irradiation at an energy density of 2.14 J/cm^2 for 2 s (0.06 J per point). The G3 group underwent the same procedure as the G2 group, but was exposed to laser irradiation at an energy density of 5.36 J/cm^2 for 5 s (0.15 J per point). The G4 group underwent the same procedure as the other groups, but with laser irradiation at an energy density of 20.36 J/cm^2 for 19 s (0.57 J per point) (Figure 4).

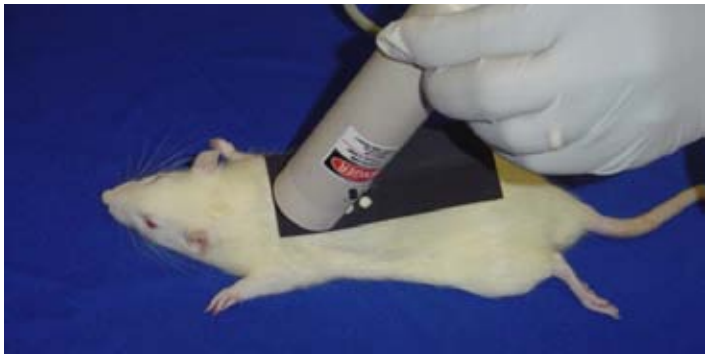

FIGURE 4 - Positioning of the animal, template and probe for laser irradiation.

Animals in the G2, G3 and G4 groups were irradiated immediately after the surgical procedure and on postoperative days 1 and 2. In all irradiations, the laser probe was placed in direct contact with the skin of the animals.

Method to estimate the percentage of flap necrosis

The percentage of skin flap necrosis was measured on postoperative day seven, using the paper template method described by Sasaki and Pang[16]. Viable tissue was characterized by warm, pink, soft, hair-bearing skin, while necrotic tissue was characterized by cold, dark, dry, hard, hairless skin. An individual template was made for each animal and the area of necrotic skin was traced. The tracing was cut out and weighed to the nearest 0.001 g on an electronic analytical balance. The percentage of necrotic skin was estimated using the formula:

$$\text{percentage of skin flap necrosis} = \frac{\text{weight of paper area equivalent to the area of necrotic skin}}{\text{total weight of the flap template}} \times 100\%$$

All sheets of tracing paper used in this study were produced by the same manufacturer and the cut paper pieces were weighted on the same electronic analytical balance.

Statistical analysis

The difference in percentage of skin flap necrosis and the homogeneity of the groups were analyzed using the non-parametric tests Kruskal-Wallis and Dunn's Multiple Comparison for k independent samples.

The significance level for rejection of the null hypothesis was set at $p \leq 0.05$ (5%) for all tests.

Results

The results from the statistical analysis are listed in Table 1.

TABLE 1 – Percentage of skin flap necrosis in all groups – Postoperative day 7

Animal	G1 (%)	G2 (%)	G3 (%)	G4 (%)
1	45.01	32.82	53.85	36.61
2	50.8	21.17	43.54	41.57
3	46.93	32.29	61.97	25.95
4	58.54	62.95	57.14	14.8
5	55.8	37.5	42.4	32.02
6	55.29	58.26	47.27	15.04
7	39.43	54.32	47.6	39.39
8	45.6	27.95	38.42	32.82
9	45.07	24.22	38.46	21.17
10	51.06	39.92	39.47	32.29
Mean	**49.35**	**39.14**	**47.01**	**29.17**

G4 < G2 = G3 = G1 ($p < 0.001$, Dunn's Multiple Comparison Test)

G1= control group, sham irradiation
G2= 2.14 J/cm^2
G3= 5.36 J/cm^2
G4= 20.36 J/cm^2

FIGURE 5 - Distribution of the percentage of skin flap necrosis in rats.

According to the results, the percentage of skin flap necrosis in the G1 group varied from 39.43% to 58.54% (mean, 49.35%); in the G2 group, from 21.17% to 62.95% (mean, 39.14%); in the G3 group, from 38.42% to 61.97% (mean, 47.01%); and in the G4 group, from 14.80% to 41.57% (mean, 29.17%) (Figure 6).

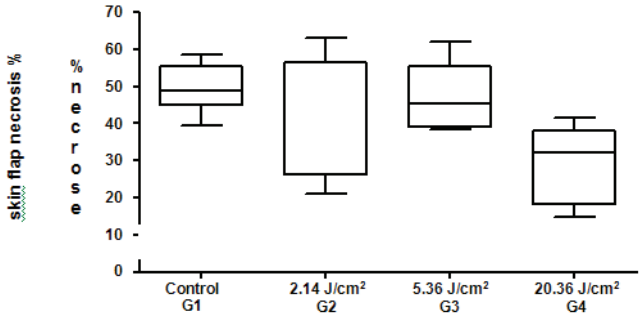

FIGURE 6 - Mean area of necrosis in skin flaps of animals in the G1 group on postoperative day 7.

TABLE 2 – Two-by-two comparisons performed after Dunn's Multiple Comparison test.

Dunn's Multiple Comparison Test	p-Value	Significance
G1 x G2	$p > 0.05$	ns
G1 x G3	$p > 0.05$	ns
G1 x G4	$p < 0.001$	hs

ns = not significant; hs = highly significant.

Comparison of results between the groups G1-G2, G1-G3 and G1-G4 was carried out, and a statistically significant difference was found between the G1 and G4 groups using the Kruskal-Wallis test ($p=0.0007$) and Dunn's Multiple Comparison test ($p<0.001$), while, for the other comparisons, no significant difference was found ($p>0.05$) (Table 2).

Discussion

In the present study, a rat model of skin flap necrosis was used. Although it is known that pig skin is more similar to human skin, a rat model was chosen because rats are readily available, relatively simple to maintain, inexpensive, and there are a large number of studies that have used this model.

A 10 x 4 cm, cranially-based, dorsal skin flap was created in the rat according to the procedure described by McFarlane *et al.*[15] which has been used as an experimental model for the study of necrosis and its prevention. Skin flap necrosis represented between 25% and 50% of the total area of the flap and was located

at its distal portion; in 5.7% of the flaps no significant necrosis was observed[17]. In order to obtain uniform conditions for the necrosis in the model, a plastic film F1, having the dimensions of the skin flap, was placed between the flap and its recipient bed to prevent revascularization, resulting in significant necrosis. In this study, a mean area of necrotic skin of 49.35% was found for the control group (no laser irradiation).

Kami *et al*.[3] elevated 9 x 3-cm, caudally-based, dorsal skin flaps in rats and reported a mean skin flap necrosis of 53.81%. In a later study, Smith[9] elevated 7 x 2-cm, caudally-based, dorsal skin flaps in rats, and also 7 x 2-cm dorsal skin flaps, perpendicular to the vertebral column, with a medial base, and spaced at 4-cm intervals in pigs; mean skin flap necrosis of 53.35% and 32.35% were reported for rats and pigs, respectively. Amir *et al*.[5] elevated 8 x 2.5-cm, cranially-based, dorsal skin flaps in rats and reported a mean skin flap necrosis of 51.5%. These random-pattern skin flap models resulted in skin flap necrosis similar to that observed in this study. However, in none of these studies a plastic barrier was placed between the flap and its vascular base.

The paper template method described by Sasaki and Pang[16] was used to evaluate the percentage of flap necrosis on postoperative day 7. This method was chosen due to its accuracy (error of less than 5%), ease of application, and because it is widely used in the literature, which facilitates comparison of results across studies. The evaluation of flap necrosis was performed by an observer unaware of the treatment the rats had received to avoid bias. Other investigators[3,17,18] have also evaluated flap necrosis on postoperative day 7, since this is the period of time needed for clear delineation of the necrotic area.

The paper template method[16] is used by the majority of researchers[2-8,17,19-22], its application facilitates the comparison of results across studies.

The experimental protocol is easily applicable and reproducible and did not lead to the death of the animals. No animals died during the experiments prior to euthanasia.

Several studies[10-12,23-24] have used low-level laser therapy as a means to reduce wound healing time, and to improve the condition of the tissue during the healing process. Other studies have also been carried out with the purpose to understand what laser-tissue interaction mechanisms are involved in the repair process[25-28].

Laser irradiation parameters, such as wavelength, pulse frequency, energy density, power output, irradiance, total energy delibered, and application technique can affect cellular activities and, consequently, the obtained results.

The studies found in the literature dealing with this topic used lasers operating at wavelengths of 632.8[5,7,9], 660[19] and 830 nm[3,4,6,8], and emitting continuous light (Table 3). In the present study, the increase in survival observed in irradiated skin flaps agrees with previous studies[19-22], except for Smith,[9] who did not find any significant effect in the use of laser therapy.

A large discrepancy is observed among energy density values reported by various authors, varying from 0.19 J/cm^2 [9] to 288 J/cm^2 [6]. According to the Arndt-Schultz law, low doses of irradiation accelerate cell activities while high doses of irradiation inhibit cell activities. In this study, the animals were randomized into 4 groups (G1 to G4), where G1 was not irradiated (control group) and the other groups were exposed to laser irradiation at energy densities of 2.14, 5.36 and 20.36 J/cm^2, respectively. Only in the G4 group (20.36 J/cm^2), a statistically significant increase in flap survival was observed. However, our results and those given by Smith[9] and Kubota[6] apparently did not follow the Arndt-Schultz law since irradiations at energy densities of 0.19, 2.14 and 5.36 J/cm^2 did not ameliorate ischemic conditions of the skin flap, while an energy density of 288 J/cm^2 led to a significant increase in flap survival (Table 3).

Therefore, it is important to consider other parameters that may affect cellular responses to laser irradiation in order to better understand the results.

In the studies where an increase in flap survival was observed after low-level laser irradiation[3-6], the energy per point varied from 0.18 to 8.64 J, which agrees with the values found in the present study for the G4 group (0.56 J). In the only study in which the results were not favorable to laser stimulation[9], the energy per point was 0.0825 J, a lower value than those mentioned above. In the present study, the energy per point for the G2 and G3 groups was of 0.06 and 0.15 J, respectively, and no beneficial effect due to laser irradiation was observed, suggesting that the tissue requires a minimum energy in order to be stimulated. It is possible that the energy per point was the key factor in explaining the discrepancy of the results, and confirms that laser irradiation affects biological activities of cells as described by the Arndt-Schultz law.

Some authors[25-27] using low-power diode lasers emitting at 630, 780 and 904 nm have reported that laser irradiation increases blood flow in the microcirculation by inducing the release of nitric oxide (NO), a strong vasodilator, in the irradiated area. These authors have also reported that laser irradiation does not significantly increase tissue temperature, which makes it a useful treatment tool even during an acute inflammatory condition.

TABLE 3 - Laser parameters used in various studies on irradiation of random-pattern skin flaps in the rat.

Authors	Wavelength λ (nm)	Power Output (mW)	Spot Size (cm^2)	Irradiance (W/cm^2)	L_I (s)	Energy Density (J/cm^2)	E_D (J)	Energy per point (J)
Kami *et al.*[3]	830	15	0.02	0.75	20	15	0.3	0.3
Smith[9]	632.8	2.75	0.43*	0.01	30	0.19	0.0825	0.0825
Kubota & Oshiro[4]	830	60	0.1	0.60	60	36	3.6	3.6
Amir *et al.*[5]	632.8	6	0.62	0.01	300 / 600	2.9	1.8 / 3.6	1.8 / 3.6*
Kubota[6]	830	100	0.0054	18.52	10	185	1	1
Pinfildi *et al.*[7]	632.8	10	0.1	0.10	30	3	8.1	0.3
Prado *et al.*[8]	830	30	0.07	0.43	84	36	41.85	2.79
Bossini *et al.*[19]	670	30	0.06	0.50	6 / 12 / 24 / 48	3 / 6 / 12 / 24	4.32 / 8.64 / 17.28 / 34.56	0.18 / 0.36 / 0.72 / 1.44
Pinfildi *et al.*[20]	670	30	0.06	0.50	144	6 / 12 / 144 / 288	0.18 / 0.36 / 4.32 / 8.64	0.18 / 0.36 / 4.32 / 8.64
Prado *et al.*[21]	830	30	0.06	0.50	72	36	2.16 / 4.32 / 6.48	2.16 / 4.32 / 6.48
Costa *et al.*[22]	660	30 / 60	0.028	1.07 / 2.14	48 / 24	3	1.44	1.44
Present study - (G2)	670	30	0.028	1.07	2	2.14	0.72	0.06
Present study - (G3)	670	30	0.028	1.07	5	5.36	1.8	0.15
Present study - (G4)	670	30	0.028	1.07	19	20.36	6.84	0.57

Notes:
Data provided by the authors
Data not provided by the authors, but computed in this study
Studies where laser irradiation increased flap survival
Studies where no significant difference in flap survival was found after laser treatment
* Data provided by the equipment manufacturer
□= wavelength
L_I = length of irradiation per point
E_D = total energy delivered to the tissue

The literature indicates that photobiomodulation accelerates wound healing and promotes angiogenesis[25,26], which can be used to justify the results reported in this paper.

Some authors have suggested that free radicals are important mediators in ischemia and can lead to tissue destruction. Free radicals can participate in chain-reactions, and cause peroxidation of cell membranes and damage to intracellular proteins, resulting in irreversible cellular injury. According to Stadler *et al.*[28], low-level laser induce release of superoxide dismutase (SOD), which inhibits the action of free radicals. Therefore, these factors may explain some of the results obtained in the present study.

More studies on this topic are needed since clinical results depend on a large number of variables, such as laser wavelength, energy density, pulse duration and frequency, length of treatment and a combination of these factors.

Furthermore, the use of "energy delivered to the tissue" instead of "energy density" as a parameter for analysis may be necessary since many studies[3-8] show apparent contradictions in the results when the parameter energy density is evaluated. The World Association for Laser Therapy (WALT)[29] recommends on their website the use of energy dose delivered to the tissue as a dosimetric parameter in the treatment of pain.

In this website the standardization of laser treatment protocol is also recommended, as it can facilitate the comparison of results and the definition of a therapeutic window for the various clinical situations where the therapeutic laser can be used.

Conclusion

The 670 nm low-level laser was effective in improving the viability of randomized skin flaps in rats, when the irradiation

energy was 0.57 J per point (energy density of 20.36 J/cm^2).

References

1. Kerrigan CL. Skin flap failure: pathophysiology. Plast Reconstr Surg. 1983;79:766-73.
2. Esteves Júnior I, Tacani PM, Liggieri VC, Ruggi BG, Ferreira LM, Liebano RE. Histamine iontophoresis on the viability of random skin flap in rats. Acta Cir Bras. 2009;24(1):48-51.
3. Kami T, Yoshimura Y, Nakajima T, Oshiro T, Fujino T. Effects of low powered diode lasers in flap survival. Ann Plastic Surg. 1985;14:278-83.
4. Kubota J, Oshiro T. Effects of diode laser therapy LLLT on flap survival: measurement of flap microcirculation with laser speckle flowmetry. Laser Ther. 1996;8:241-6.
5. Amir A, Solomon AS, Giler S, Cordoba M, Hauben DJ. The influence of helium-neon laser irradiation on the viability of skin flaps in the rat. Br J Plast Surg. 2000;53:58-62.
6. Kubota J. Effects of diode laser therapy on blood flow in axial patterns flaps in the rat model. Lasers Med Sci. 2002;17:146-53.
7. Pinfildi CE, Liebano RE, Hochman BE, Ferreira LM. Helium-neon laser in viability of random skin flap in rats. Lasers Surg Med. 2005;37(1):74-7.
8. Prado RP, Liebano RE, Hochman BE, Pinfildi CE, Ferreira LM. Experimental model for low level laser therapy on ischemic random skin flap in rats. Acta Cir Bras. 2006;21(4):258-62.
9. Smith RJ. The effect of low-energy laser on skin-flap survival in the rat and porcine animal models. Plast Reconstr Surg. 1992;89:306-10.
10. Al-Watban FAH, Delgado GD. Burn Healing with Diode Laser: 670 nm at different doses as compared to a placebo group. Photomed Laser Surg. 2005;23(3):245-50.
11. Al-Watban FAH, Zhang XY. The comparison of effects between pulsed and CW lasers on wound healing. J Clin Laser Med Surg. 2004:22(1):15-8.
12. Mendez TM, Pinheiro AL, Pacheco MT, Nascimento PM, Ramalho LM. Dose and wavelength of laser light have influence on the repair of cutaneous wounds. J Clin Laser Med Surg. 2004;22(1):19-25.
13. Bjordal JM, Couppe C, Chow RT, Tuner J, Ljunggren EA. A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders. Aust J Physiother. 2003;49(2):107-16.
14. Gur A, Cosut A, Sarac AJ, Cevik R, Nas K, Uyar A. Efficacy of different therapy regimes of low-power laser in painful osteoarthritis of the knee: a double-blind and randomized-controlled trial. Lasers Surg Med. 2003;33(5):330-8.
15. McFarlane RM, DeYoung G, Henry RA. The design of a pedicle flap in the rat to study necrosis and its prevention. Plast Reconstr Surg. 1965;35:177-82.
16. Sasaki GH, Pang CY. Hemodynamics and viability of acute neurovascular island skin flap in rats. Plast Reconstr Surg. 1980;65:152-8.
17. Esteves Junior I, Masson IB, Ferreira LM, Liebano RE, Baldan C, Gomes AC. Administração tópica de cloridrato de hidralazina na viabilidade de retalho cutâneo randômico em ratos. Acta Cir Bras. 2005;20(2):164-7.
18. Atalay C, Koçkaya EA, Çetin B, Kismet K, Akay MT. Efficacy of topical nitroglycerin and transcutaneous electrical nerve stimulation on survival of random-pattern skin flaps in rats. Scand J Plast Reconstr Hand Surg. 2003;37:10-3.
19. Bossini PS, Fangel R, Habenschus RM, Renno AC, Benze B, Zuanon JA, Neto CB, Parizotto NA. Low-level laser therapy (670 nm) on viability of random skin flap in rats. Lasers Med Sci. 2009;24(2):209-13.
20. Pinfildi CE, Liebano RE, Hochman BS, Enokihara MM, Lippert R, Gobbato RC, Ferreira LM. Effect of low-level laser therapy on mast cells in viability of the transverse rectus abdominis musculocutaneous flap. Photomed Laser Surg. 2009;27(2):337-43.
21. Prado RP, Pinfildi CE, Liebano RE, Hochman BS, Ferreira LM. Effect of application site of low-level laser therapy in random cutaneous flap viability in rats. Photomed Laser Surg. 2009;27(3):411-6.
22. Costa MS, Pinfildi CE, Gomes HC, Liebano RE, Arias VE, Silveira TS, Ferreira LM. Effect of low-level laser therapy with output power of 30 mW and 60 mW in the viability of a random skin flap. Photomed Laser Surg. 2010;28(1):57-61.
23. Benedicenti A, Verrando M, Cherlone F, Brunetti O. Effect of 904 nm laser on microcirculation and arteriovenous circulation as evaluated using telethermography imaging. Parodontol Stomatol. 1984;23(2):167-78.
24. Wilden L, Karthein R. Import of radiation phenomena of electrons and therapeutic low-level laser in regard to the mitochondrial energy transfer. J Clin Laser Med Surg. 1998;16:159-65.
25. Schaffer M, Bonel H, Sroka R, Schaffer PM, Busch M, Reiser M, Dühmke E. Effects of 780 nm diode laser irradiation on blood microcirculation: preliminary findings on time-dependent T1-weighted contrast-enhanced magnetic resonance imaging (MRI). J Photochem Photobiol B Biol. 2000;54:55-60.
26. Schindl A, Schindl M, Schindl L, Jurecka W, Honigsmann H, Breier F. Increased dermal angiogenesis after low-intensity laser therapy for a chronic radiation ulcer determined by a video measuring system. J Am Acad Dermatol. 1999;40:481-4.
27. Yu W, Naim JO, Lanzafame RJ. Effects of photostimulation on wound healing in diabetic mice. Lasers Surg Med. 1997;20:56-63.
28. Stadler I, Evans R, Kolb B, Naim JO, Narayan V, Buehner N, Lanzafame RJ. In vitro effects of low-level laser irradiation at 660 nm on peripheral blood lymphocytes. Lasers Surg Med. 2000;27(3):255-61.
29. World Association for Laser Therapy [Internet]. Recommended treatment doses for Low Level Laser Therapy; c2010 [Revised April 2010]. Available from: http://www.walt.nu/images/stories/files/Dose_table_904nm_for_Low_Level_Laser_Therapy_WALT-2010.pdf

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