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CLINIC AND SURGERY

Comparison between continuous and pulsed low-intensity laser on the healing of skin grafts applied to recently created wounds in rabbits (*Oryctolagus cuniculus*)

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ABSTRACT: Skin grafting is a simple and very useful surgical technique for wound repair, especially where there is difficulty in performing direct synthesis or other reconstructive techniques. low-intensity laser (LIL) has already been used successfully in cases where the graft was applied to wounds shortly after its creation. However, LIL still lacks standardization due to conflicting studies on its results. Thus, this study compared the effectiveness of continuous wave LIL with pulsed wave LIL in stimulating the healing of skin grafts, applied to newly created wounds in rabbits. For this purpose, 31 rabbits were distributed into: control group (CG, n = 10), continuous wave laser group (GLC; n = 10) and pulsed wave laser group (GLP; n = 11). Postoperative management was similar between all groups, with the exception of laser application, as indicated by the group. The GLC and GLP groups showed similar evolution, with a satisfactory healing process of the graft, unlike the GC group. These results were maintained in the microscopic evaluation, as the groups treated with laser showed less inflammation, better integration with the receptor area, greater re-epithelialization and collagenization. There was no difference in vascular density between the treatment groups on immunohistochemical analysis. The newly created wound was not able to provide nutrition for the skin graft in rabbits, but LIL is an efficient adjuvant therapy in stimulating healing between the recipient area and the graft, promoting full integration. However; although, statistically there was no difference in the form of light emission, pulsed or continuous, patients who received continuous wave LIL showed superior microscopic evolution.
Key words: cicatrization, exergy, laser therapy, rabbit.

Comparação entre as ondas contínua e pulsada do laser de baixa intensidade na cicatrização de enxertos cutâneos aplicados em feridas recém-criadas de coelhos (*Oryctolagus cuniculus*)

RESUMO: A enxertia cutânea é uma técnica cirúrgica simples e bastante útil para o reparo de feridas, principalmente, quando há dificuldade em realizar a síntese direta ou outras técnicas reconstrutivas. O laser de baixa intensidade (LBI) já foi utilizado com sucesso em casos em que o enxerto foi aplicado em feridas logo após a sua criação. No entanto, a LBI ainda carece de padronizações, mediante estudos conflitantes sobre seus resultados. Desta forma, este trabalho objetivou comparar a eficácia da LBI de onda contínua com onda pulsada na estimulação da cicatrização de enxertos cutâneos, aplicados em feridas recém-criadas em coelhos. Para tanto, foram utilizados 31 coelhos distribuídos em: grupo controle (GC, n = 10), grupo laser onda contínua (GLC; n = 10) e grupo laser onda pulsada (GLP; n = 11). O manejo pós operatório foi semelhante entre todos os grupos, com exceção da aplicação do laser, conforme o grupo indicado. Os grupos GLC e GLP apresentaram evolução semelhante, com processo cicatricial satisfatório do enxerto, ao contrário do grupo GC. Tais resultados se mantiveram na avaliação microscópica, pois os grupos tratados com laser apresentaram menor inflamação, melhor integração à área receptora, maior reepitelização e colagenização. Não houve diferença na densidade vascular entre os grupos de tratamento na análise imuno-histoquímica. A ferida recém-criada não é capaz de fornecer nutrição para o enxerto cutâneo nos coelhos, mas a LBI é uma terapia adjuvante eficiente em estimular a cicatrização entre a área receptora e o enxerto, promovendo integração total. Entretanto, embora não houve diferença estatística na forma de emissão de luz, pulsada ou contínua, os pacientes que receberam LBI em onda contínua apresentam evolução microscópica superior.
Palavras-chave: cicatrização, enxertia, laserterapia, coelho.

INTRODUCTION

Skin grafting is based on filling a wound defect with a segment of the epidermis and dermis that is completely removed from the donor area and transferred to the receptor bed (SCHARF, 2017; KOHLHAUSER, 2021). The benefits of its use are incontestable in regions where there is insufficient adjacent skin for primary coverage. The technique is especially important in veterinary medicine due to the high occurrence of skin loss as a result of injuries or removal of tumors, especially in the limbs, in which the scarcity and immobility of skin prevent the formation of local skin flaps (TONG & SIMPSON, 2012; SCHARF, 2017).

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Skin grafting has limitations for application to domestic animals, mainly because of the need for healthy granulation tissue and absence of necrotic tissue, infection, and foreign bodies (FOWLER, 2006). Usually, it is necessary to prepare the wound to induce secondary healing before application of the technique, requiring large availability of donor tissue and special care of the animal's owner (TONG & SIMPSON, 2012; KOHLHAUSER, 2021). Therefore, some tools have been studied for potential stimulation of healing of grafts applied to recently created sounds. Options such as platelet-rich plasma, vacuum-assisted and low-intensity laser (LIL) therapies have been found effective (STANLEY et al., 2013; REIS FILHO et al., 2017; PAZZINI et al., 2018). Clinically, LIL has effects such as reducing pain, decreasing edema and inflammation, and improving healing due to improved tissue oxygenation.

LIL therapy has various potential clinical benefits, including stimulation of healing of open wounds, consolidation of fractures, support in the repair of tendon and ligament injuries and alleviation of pain (GODINE, 2014). Nevertheless, despite promising results, some studies, such as GAMMEL et al. (2018), have found no evidence of the benefit of using LIL. Often the explanation for the conflicting results is the absence of standardization of the variables involved in the technique. Although, there is some degree of consensus regarding the best wavelength and acceptable doses, there is no agreement regarding whether emission of continuous wavelength (CW) or pulsed wavelength (PW) is best for spot applications (HASHMI et al., 2010).

Considering this situation, this study compared the efficacy between LIL with continuous and pulsed wavelength on the stimulation of healing of skin grafts applied to recently created wounds in rabbits (*Oryctolagus cuniculus*), so as to avoid the need to apply the graft in the presence of granulation tissue.

MATERIALS AND METHODS

The research used adult male rabbits (*Oryctolagus cuniculus*) of the white New Zealand breed. The animals were obtained from the central animal house of Paulista State University, Botucatu Campus, and were kept during the entire experiment in individual cages with feed and water available ad libitum. The animals had a weight ranging from 2.0 to 2.5 kg. There were originally 36 rabbits, distributed in three groups of 12 each: control group (CG), continuous laser group (CLG) and pulsed laser group (PLG).

The anesthetic protocol consisted of pre-anesthetic intramuscular administration of chlorpromazine at a dose of 0.5 mg/kg and morphine at a dose of 0.5 mg/kg. Twenty minutes later, anesthetic induction was performed with isoflurane, administered with a face mask, followed by maintenance anesthesia with 3% isoflurane diluted in 100% oxygen.

After anesthetic induction, the animals were prepared with broad trichotomy in the donor area (AD), corresponding to the third distal region of the lateral wall of the right thorax, in the position of the costochondral joint, and in the receptor area (AR), located on the lateral face of the middle third of the radius and ulna of the right thoracic member (Figure 1). Then previous and definitive antisepsis was carried out, both with the use of 2% chlorhexidine degermante and 70% alcohol.

A wound was created in the AR including the skin and subcutaneous layer, with a #15 scalpel, with base in the form of a plastic mold measuring 2,0 cm x 2,0 cm (Figure 1). The subcutaneous layer was divulged until the entire fragment was removed, and then discarded. The same procedure was repeated in the DA, except the fragment was used as the graft (Figure 1). The entire subcutaneous portion of the graft was removed with scissors until only the dermis and epidermis remained (Figure 1). Then small randomly shaped fenestrae were made, crossing the graft completely, in the dorso-ventral or cranial-caudal direction, according to the hair growth on the graft, using a #11 scalpel (Figure 1).

The graft was then positioned in the wound of the AR and sutured at the borders using separate single stitches and nylon 3-0 sutures. The AD was sutured in a continuous single pattern using the same 3-0 nylon sutures for approximation of the incised borders (Figure 1). The surgical wounds of both the AR and AD were covered with bandages composed of sterile cotton gauze moistened with a 0.9% saline solution and secured with medical tape. All surgical procedures were performed by the same surgeon.

For the control group (CG), the randomly chosen animals were submitted to the same surgical procedure described above, with application of the graft immediately after creation of the wound, without the presence of granulation tissue and without application of any product or process that could stimulate the healing process.

For the continuous laser group (CLG), the animals were submitted to LIL immediately after the surgical procedure. The wound was cleaned with 0.9% saline solution and covered with gauze. Nine

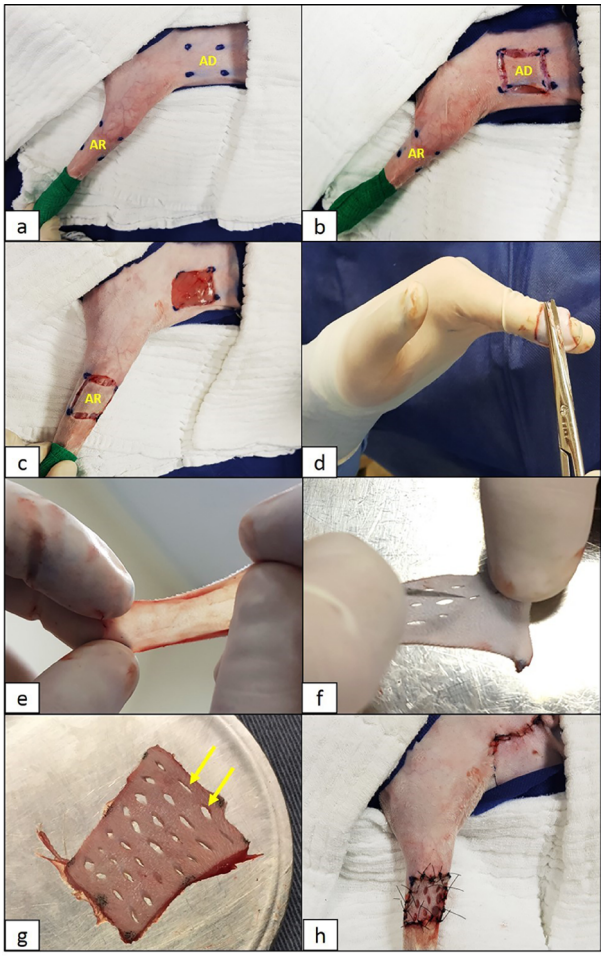

Figure 1 - Surgical procedure for skin graft in rabbits: a) Demarcation of the donor area (AD) and receptor area (AR). b) Incision of the AD utilized subsequently as graft. c) Incision in the skin for creation of the cutaneous defect (AR). d) Complete removal of the subcutaneous tissue of the graft. e) Demonstration of the graft prepared for implantation. f and g) Fenestration of the graft (yellow arrows). h) Attachment of the graft in the AR with interrupted single stitches and suture of the AD with continuous single stitches.

predetermined points were submitted to application of the laser (Figure 2), using a Laserpulse model L42 device (Ibramed®, Amparo, SP, Brazil), with 660 nanometer wavelength transducer (nm) and power of 30 mW (milliwatts). The laser dose was 4 J/cm^2 in continuous wavelength for eight seconds at each predetermined point. The same laser procedure was applied to the animals on the third and seventh postoperative days, during the change of dressing after cleaning the wound.

For the pulsed laser group (PLG), the same method was employed as for the CLG, except the laser dose was 4 J/cm^2 at pulsed wavelength of 150 Hz for 16 seconds at each predetermined point (Figure 2). In the postoperative period, the animals received enrofloxacin (5 mg/kg every 12 hours subcutaneously) and tramadol chlorhydrate (3 mg/kg every 12 hours subcutaneously), in both cases for five consecutive days, along with meloxicam (0.1 mg/kg subcutaneously) every four hours on the first three days.

The dressings were changed on the third and seventh days after surgery in all the groups. After removal of the bandage, the wound was cleaned with sterile gauze. Then a fresh bandage was applied according to the same procedure in the immediate postoperative period. In the animals of

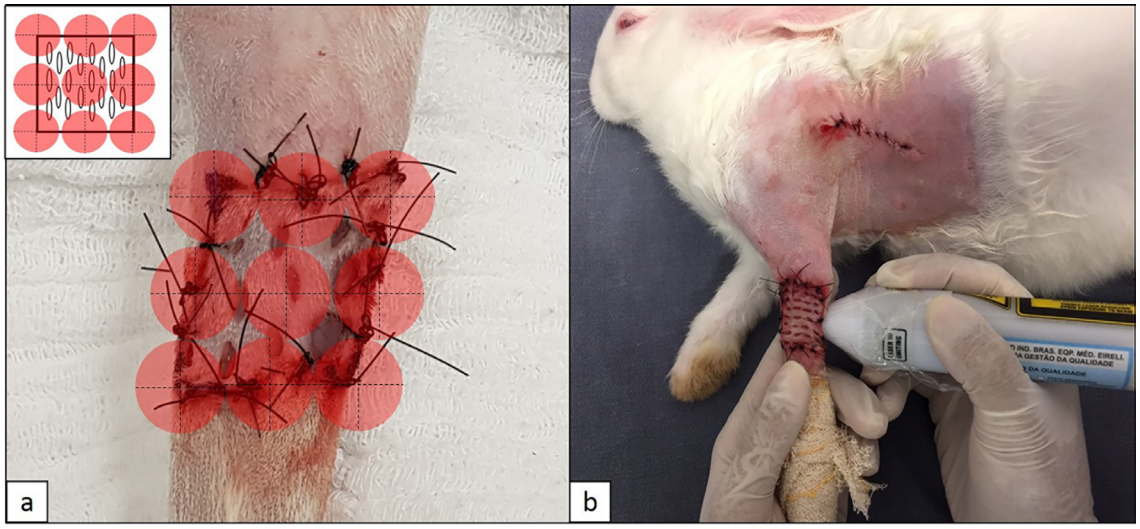

Figure 2 - Low-intensity laser technique (LIL) applied to the skin graft of continuous laser group (CLG) and pulsed laser group (PLG). a) Representation of the predetermined areas to application of the LIL (red circles). b) Application of the LIL.

groups CLG and PLG, the LIL was applied after wound cleaning but before applying fresh bandages. The postoperative management, laser application and changing of bandages was performed by the same veterinarian, taking care not to damage the graft. During the postoperative management, the coloration, presence of necrosis, presence and characterization of secretions were evaluated.

Animals were excluded from the study in the postoperative period in case of occurrences related to the dressing, such as detachment of the bandage or its removal by the animal itself. Euthanasia was performed on the 15th postoperative day in all the animals, according to the procedure proposed by CONCEA and CEUA.

Immediately after the euthanasia, the cutaneous portion of the graft was removed from the AR in a block, including a small portion of the skin of the receptor bed, to allow analysis of the transition area between skin and graft. These samples were placed in 10% formol, in proportion of 1:9, for 48 hours and then transferred to pure 70% alchhol5 until the moment of processing. The samples were cleaved and imbedded in paraffin. Transversal sections were cut with thickness of 4 µm (micrometers) in a microtome. After preparation, the slides were stained with hematoxylin and eosin (HE) or Masson's trichrome (MT).

The parameters inflammation, reepithelialization, skin-graft integration and necrosis were analyzed in the slides stained with HE. The readings were performed sequentially by a single evaluator without identification of the group, using a light microscope with 4x, 20x and 40x objectives, as described by REIS FILHO et al. (2017). The formation of collagen and granulation tissue in the samples was evaluated by staining with Masson's trichrome, and the area of collagen deposition in the graft site was measured in percent, as described by REIS FILHO et al. (2017). The parameters inflammation, reepithelialization and necrosis were classified as absent, slight, moderate or accentuated. The parameter graft x skin relation was classified as integral or degenerate, and the formation of collagen was classified as slight, moderate or accentuated. The used criteria are described in table 1.

For immunohistochemical analysis, the sections were sent for processing with CD31 antibodies diluted 1:50, followed by enzyme recovery for 45 minutes, using a detection system for reading. The angiogenic index obtained after marking with CD31 was determined by the microvascular counting technique (MVC), as described by MAEDA et al. (1995). The vessels were counted in five fields, selected previously, with high vascular density, at 400x magnification using an optical light microscope. After adding the number of vessels, the final count was expressed as the average number of vessels in each group.

The results obtained were submitted to multiple correspondence analysis and were processed with the Statistical software, version 7,

Table 1 - Microscopic parameters adopted to evaluate the epithelialization of skin grafts in rabbits (*Oryctolagus cuniculus*), in stains Hematoxylin and Eosin and Masson's trichrome, used in all the experimental groups. Adapted from REIS FILHO et al., 2017.

PARAMETER	--CLASSIFICATION--	----------INDICATORS----------
INFLAMMATION	ABSENT (A)	Inflammatory cells, such as heterophils and lymphocytes, visualized in the optical field.
	SLIGHT (S)	Inflammatory cells evidenced in isolation, making it possible to distinguish areas free of infiltrate inflammatory.
	MODERATE (M)	Inflammatory cells appeared more frequently, constituting dense aggregates, but allowing visualize infiltrate-free areas.
	ACCENTUATED (AC)	Cells were seen with great frequency, constituting dense and juxtaposed aggregates, without areas free of infiltrators.
GRAFT X SKIN RELATION	INTEGRAL (I)	Graft fully integrated and connected to the wound.
	DEGENERATED (D)	Most of the graft detached from the superficial dermis.
GRAFT REEPITHELIZATION	ABSENT (A)	Absence of epithelium in the optical field.
	SLIGHT (S)	Incomplete epithelialization, with a predominance of the area not re-epithelialized.
	MODERATE (M)	Incomplete epithelialization, with predominance of the area re-epithelialized.
	ACCENTUATED (AC)	Complete re-epithelialization over the connective tissue.
PRESENCE OF NECROSIS NO GRAFT	ABSENT (A)	Graft cells, in their entirety, did not show signs such as nuclear pyknosis, karyolysis and eosinophilia of the cytoplasm.
	SLIGHT (S)	Isolated cell necrosis signs.
	MODERATE (M)	Signs of multifocal cell necrosis.
	ACCENTUATED (AC)	Signs of cellular necrosis along the entire length of the graft.
COLLAGENIZATION	SLIGHT (S)	0 to 33% area of collagen and bone tissue formation granulation.
	MODERATE (M)	34 to 66% area of collagen and bone tissue formation granulation.
	ACCENTUATED (AC)	67 to 100% area of collagen and bone tissue formation granulation.

employing the Burt X'X table, where X is the original matrix of absence-presence values for each group. The correspondences obtained were referenced to assess the effects of the treatments of the different groups. Besides this, the results of the microscopic evaluation were used to calculate descriptive statistics.

The data on the means of the microvascular counts were submitted to the Tukey test using statistical version 7 (STATSOFT, 2004), for comparison of the means of the treatment groups.

RESULTS

Of the 36 rabbits originally included, five were excluded due to occurrences related to the dressing. Therefore, there were 31 animals in the study, distributed as follows: 10 in the control group (CG); 10 in the continuous laser group (CLG); and 11 in the pulsed laser group (PLG).

The macroscopic clinical evaluation revealed that the evolution of the grafts was similar between the CLG and PLG groups, which were different than the CG. In both groups treated with LIL, the grafts presented pink color on the day of first dressing change (third postoperative day), which remained the same on the seventh day (Figure 3). In contrast, in the CG, pallor was often observed on the third postoperative day, and on the seventh day it was possible to note blackened areas on the surface of the graft (Figure 3). On the 15th postoperative day, the grafted areas of the majority of the animals in the CLG and PLG had similar color as normal tissue, along with complete incorporation with the AR (Figure 3). In the CG animals, there was frequent presence of degeneration of grafts on the 15th postoperative day, with blackened color and some exudation, signs indicating failure of the surgical technique (Figure 3).

In the microscopic evaluation, some relevant findings were detected. Most of the animals presented signs of slight inflammation. The animals of the CLG had the least indications of inflammation (Figure 4). With respect to the graft x skin relation, the CG had the largest percentage of cases where degeneration was observed, even though most of the animals presented good integration (Figure 4). The reepithelialization was classified as accentuated in all the animals of the CLG and PLG, and in 60% (n = 6) of those in the CG (Figure 4). Furthermore, the

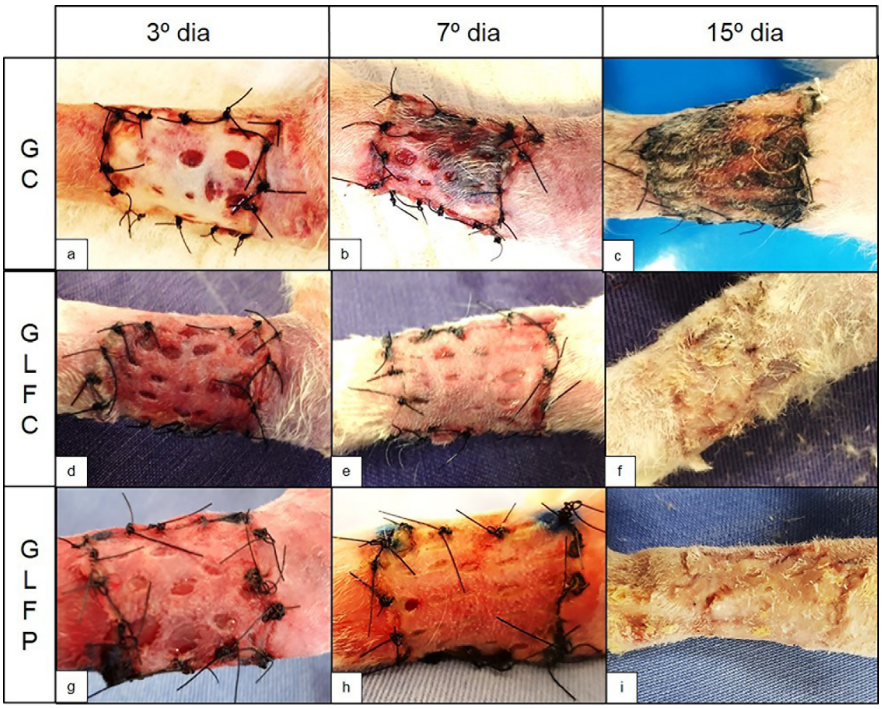

Figure 3 - Skin grafts in rabbits in the different treatment groups during the three moments of changing the dressing. a) Macroscopic appearance of the graft on the 3rd postoperative day (PO) of a rabbit of the control group (CG). b) Macroscopic appearance of the graft on the 7th PO day of a rabbit of the CG; note the start of degeneration. c) Macroscopic appearance of the graft on the 15th PO day of a rabbit of the CG; observe the extensive necrosis. d) 3rd PO day of the continuous laser group (CLG); note the pinkish color already in the initial moments. e) 7th PO day of the CLG. f) 15th PO day of the CLG; note the satisfactory integration of the graft. g) 3rd PO day of the pulsed laser group (PLG). h) 7th PO day of the PLG. i) 15th PO day of the PLG; note the total integration of the graft.

CG had the highest percentage of animals classified as having accentuated necrosis (Figure 4). Finally, the collagenization and formation of granulation tissue varied from moderate to accentuated in the majority of the rabbits, with the PLG standing out, in which 63.6% (n = 7) of the animals presented accentuated collagenization (Figure 4).

After application of multiple correspondence analysis of the results obtained for the different treatments, it was possible to identify two specific groups of categories. The first was denoted by absence of re-epithelialization (REEA), slight re-epithelialization (REES), degenerate graft x skin relation (GSRD), accentuated necrosis (NAC) and accentuated inflammation (IAC) in the animals of the CG, statistically significant at $P < 0.05$ (Table 2). The second group of categories identified a specific correspondence to the PLG regarding the presence of necrosis slight (NS), statistically significant at $P < 0.10$ (Table 2).

Two other groups of categories were identified, but independently of the treatment group. The NAC characteristic presented high correspondence with IAC, GSRD, REEA, REES ($P < 0.05$; Table 2), and GSRD with REEA ($P < 0.05$; Table 2). Although, these findings are not directly related to any group, they demonstrate that the microscopic parameters indicating graft failure were highly correlated, reinforcing the reliability of the method used in this study.

When analyzing the formation of new vessels beyond the 15th postoperative day by the immunohistochemical method, it was possible to note greater vascular density in the animals of the PLG in comparison with the other two groups. Therefore, the animals of the PLG ($\bar{x}$ 30.32) presented a slightly higher average compared to the animals of the CG ($\bar{x}$ 24.06) and CLG ($\bar{x}$ 25.76), but without statistical significance ($P > 0.05$) (Figure 5).

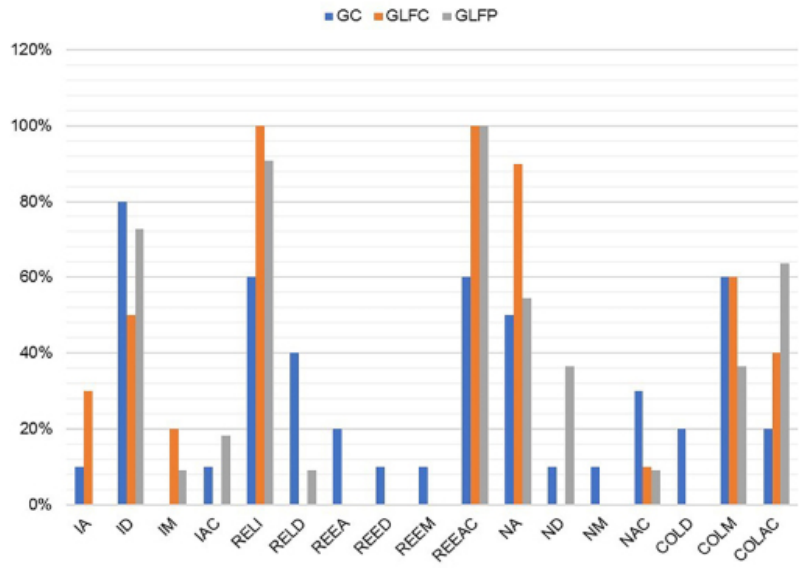

Figure 4 - Graph illustrating average percent of the parameters from the microscopic evaluation of each treatment group of rabbits submitted to skin graft. Legend: inflammation absent (IA); inflammation slight (IS); inflammation moderate (IM); inflammation accentuated (IAC); graft x skin relation integral (GSRI); graft x skin relation degenerate (GSRD); reepithelialization absent (REEA); reepithelialization slight (REES); reepithelialization moderate (REEM); reepithelialization accentuated (REEAC); necrosis absent (NA); necrosis slight (NS); necrosis moderate (NM); necrosis accentuated (NAC); formation of collagen slight (COLS); formation of collagen moderate (COLM); formation of collagen accentuated (COLAC); control group (CG); continuous laser group (CLG); pulsed laser group (PLG).

DISCUSSION

Based on the results observed from the macroscopic evaluation in the first days after the skin graft, the diagnosis of necrosis by means of the pallid color might have been imprecise. It should be recalled that during this period the viability of the graft is maintained by plasma imbibition, and only on the sixth day of healing is the blood flow totally reestablished (SCHARF, 2017). Hence, the pallid color in this period does not necessarily indicate degeneration. However, the observation of a pinkish color three days after the surgery of the animals treated with LIL can indicate an advanced healing process, probably by early angiogenic stimulus cause by the laser. The confirmation of this hypothesis only would have been possible if the samples for microscopic analysis had been collected on the third postoperative day, which did not occur in this study, to avoid repetitive surgical procedures on the experimental animals.

Although, visibly distinct, the macroscopic variables corresponding to the clinical evolution of the skin grafts were not quantified or statistically analyzed in this study. The subjectivity and high degree of observational interference in this type of analysis make microscopic evaluation more relevant, as demonstrated in other studies (STANLEY et al., 2013; PAZZINI et al., 2016; REIS FILHO et al., 2017). However, we believe it is relevant to report these observations here, because they are parameters found by lab assistants while changing the dressings of the skin grafts.

With respect to the microscopic analysis, based on previous findings of the healing process of grafts, the presence of inflammation 15 days after the surgical procedure is not expected, but it is expected at the initial moments (BOHLING & SWAIM, 2018). It is not possible to attribute anti-inflammatory potential directly to LIL application only based on this observation, but it is possible to infer that

Table 2 - Correspondence matrix between the categories of the microscopic findings among all the groups of rabbits receiving skin grafts. The values in red denote significance at P < 0.05, and blue denote P < 0.10.

	CG	CLG	PLG	IA	IS	IM	IAC	GSRI	GSRD	REEA	REES	REEM	REEAC	NA	ND	NM
CG																
CLG	-1.796															
PLG	-1.884	-1,884														
IA	-0.256	1.505	-1.191													
IS	0.471	-0.682	0.201	-1.646												
IM	-0.984	1.049	-0.063	-0.622	-1.426											
IAC	0.033	-0.984	0.907	-0.622	-1.426	-0.539										
GSRI	-0.824	0.557	0.255	0.352	0.092	0.305	-0.956									
GSRD	1.880	-1.270	-0.581	-0.803	-0.210	-0.696	2.180	-2.048								
REEA	1.687	-0.803	-0.842	-0.508	-0.305	-0.440	1.833	-1.295	2.953							
REES	1.193	-0.568	-0.596	-0.359	0.392	-0.311	-0.311	-0.916	2.088	-0.254						
REEM	1.193	-0.568	-0.596	-0.359	0.392	-0.311	-0.311	0.176	-0.402	-0.254	-0.180					
REEAC	-0.918	0.437	0.459	0.277	-0.068	0.239	-0.379	0.495	-1.128	-1.320	-0.933	-0.933				
NA	-0.572	1.003	-0.412	0.884	-0.149	0.046	-0.672	0.543	-1.239	-1.136	-0.803	-0.803	0.6183			
NS	-0.483	-1.270	1.671	-0.803	0.333	0.742	-0.696	0.394	-0.898	-0.568	-0.402	2.088	-0.170	-1.796		
NM	1.193	-0.568	-0.596	-0.359	0.392	-0.311	-0.311	0.176	0.402	-0.254	-0.180	-0.18	0.138	-0.803	-0.402	
NAC	1.092	-0.483	-0.581	-0.803	-0.210	-0.696	2.180	1.559	3.556	2.953	2.088	-0.402	-1.128	-1.796	-0.898	-0.402

Legend: inflammation absent (IA); inflammation slight (IS); inflammation moderate (IM); inflammation accentuated (IAC); graft x skin relation integral (GSRI); graft x skin relation degenerate (GSRD); reepithelialization absent (REEA); reepithelialization slight (REES); reepithelialization moderate (REEM); reepithelialization accentuated (REEAC); necrosis absent (NA); necrosis slight (NS); necrosis moderate (NM); necrosis accentuated (NAC); control group (CG); continuous laser group (CLG) and pulsed laser group (PLG).

the receptor area without granulation tissue did not provide a suitable bed for healing of the skin graft in the animals of the CG.

The data obtained reflect differences in the healing evolution among the groups, mainly between the animals of the CG on the one hand and the CLG and PLG on the other. The best results can be attributed to the use of LIL, but it must be noted that despite the slower healing in the CG, some animals in that group presented good integration, reepithelialization and collagenization. That fact can be explained because despite their degeneration, in many cases skin grafts can serve as temporary biological dressings, favoring reepithelialization of the wound below the grafted fragment (FOWLER, 2006).

It is widely accepted that the receptor area should be free of necrotic material, foreign bodies and infection, so as to maintain the viability of the graft (SCHARF, 2017; BOHLING & SWAIM, 2018). However, the presence or absence of granulation tissue is a source of controversy. ERWIN et al. (2016) studied the ideal period for application of grafts in cats and found better results when the wounds already had granulation tissue. Conversely, RIGGS et al. (2015) attributed the presence of granulation tissue to a low success rate in a retrospective study of grafts in dogs. The parameters found in our study, attributed to the CG, are associated with the failure of the graft, so we can state that the recently created wound, without granulation, did not provide an ideal environment for survival of the graft. The divergence of results in the literature is probably due to marked methodological differences among the studies, which increases the importance of our results.

GAMMEL et al. (2018) did not find relevant differences in dogs where LIL was applied to incision wounds, although the greater extent of necrosis in animals not receiving laser treatment was statistically significant, agreeing with our results. Likewise, POSTEN et al. (2005) stressed the lack of confirmation or discordance about the action of LIL in wound healing processes. On the other hand, some studies have demonstrated its efficacy in ischemic lesions (PRADO et al., 2006; PRADO et al., 2012; MOREIRA, 2020). Therefore, some specific action of LIL probably exists in tissues that are degenerating, as naturally occurs in grafts, promoting more positive results compared with its application in incision wounds or after debridement.

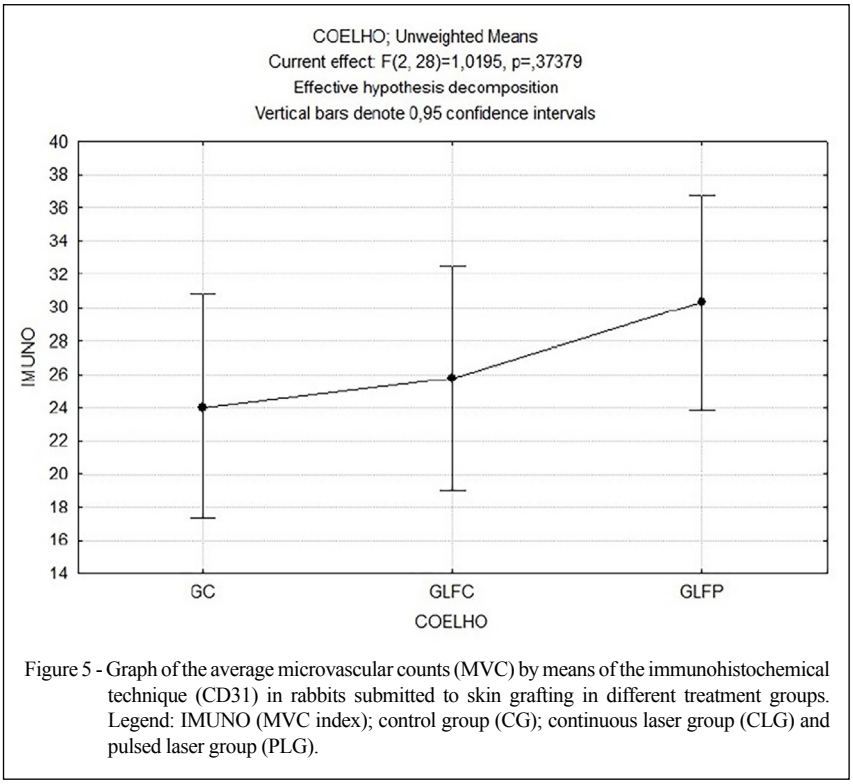

Figure 5 - Graph of the average microvascular counts (MVC) by means of the immunohistochemical technique (CD31) in rabbits submitted to skin grafting in different treatment groups. Legend: IMUNO (MVC index); control group (CG); continuous laser group (CLG) and pulsed laser group (PLG).

In pulsed LIL, the brief interruptions in the light emission provide a greater ability to penetrate the tissues without causing thermal damage due to temperature increase (HASHMI et al. 2010). These characteristics are very attractive for application in skin grafts, because these are delicate skin portions that suffer damage by temperature change, and need the action of the laser in the receptor area, below the graft. Although, we expected better evolution in the animals of the PLG, we did not observe relevant differences in relation to the CLG. Probably the LIL dose used in this study was high enough to cause some damage when emitted in a continuous wave. According to ILIC et al. (2006), thermal lesions are only observed at very high LIL doses around 750 mW/cm^2.

We can infer that in this study the LIL contributed to the viability of the skin grafts, but probably due to other benefits, such as the increased cell metabolism, instead of by angiogenic stimulus. This is perhaps explained by the dose utilized, since CURY et al. (2013) demonstrated the angiogenesis potential of LIL, but stressed its dose-dependent effect.

CONCLUSION

In conclusion, the use of the skin grafts immediately after creation of the defect in rabbits did not promote satisfactory results, because the receptor area is not able to nourish the graft effectively until vascularization is reestablished. The application of LIL is an effective adjuvant therapy to stimulate healing, contributing to the integration of the graft with the skin. However, at the dose of 4 J/cm^2, there was no difference between the continuous and pulsed laser emission.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest of any kind.

AUTHORS' CONTRIBUTIONS

NPRF, ABN, PCM contributed to the conception and design of the work. NPRF, MGPAF, ALCRP contributed in the acquisition of data. NPRF, JMP, ASF contributed in the analysis and interpretation of data. NPRF, FNP, BSF contributed to the conception and drafting the work. All authors contributions in critically revising and gave the final approval of the version to be published and agreement to be accountable for all aspects of the work.

ETHICAL STATEMENT

The design of this study respected the Ethical Principles of Animal Experimentation adopted by Brazil's National Council for Control of Animal Experimentation (CONCEA), and was approved by the Animal Use Ethics Committee (CEUA) of the institution of origin, under protocol number 03286/18.

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