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Effects of diode laser therapy on the acellular dermal matrix

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Abstract Acellular dermal matrix (ADM) was subcutaneously implanted into calvarian skin of male Wistar rats ($n = 40$). Low-level laser (λ 685 nm, 4 J/cm^2) was locally applied in experimental group ($n = 20$) above the skin flap. Grafts were harvested at 1, 3, 7 and 14 days after surgery and underwent histological analyses. In treated animals, the extent of edema and the number of inflammatory cells were reduced ($P < 0.05$). The amount of collagen in graft treated with low-level laser were significantly higher than those of controls ($P < 0.05$) and were statistically more prominent on the 14th day after surgery. The mean count of fibroblasts was significantly higher in the low-laser therapy group within the 3rd day, showing a marked influx of fibroblasts into area. In conclusion, wound healing of the ADM appear to be positively affected by laser therapy.

Keywords Xenograft · Biostimulation · Dermal implant · Low-level laser

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Introduction

Despite the biological advantages, the autogenous grafts also have inherent disadvantages, which include the need for graft harvesting, the potential for creation of donor scars, and the unpredictability of long-term volume retention. The search continues for a soft tissue graft, such as an off-the-shelf material, that does not require a concomitant donor site but that would biologically integrate into the implantation site without the risks associated with any known synthetic implant (Eppley 2000).

Currently, due to excellent achievement in a variety of different biomedical applications, the performance of acellular dermal matrices, which are derived from full-thickness skin treated to remove cells and cellular components but which retain the native dermal structure, has drawn the attention of researchers in many fields (Wainwright 1995; Smartt et al. 2008; Sclafani et al. 2000).

It has a potential for easy and relatively economic use to cover full-thickness skin defect wounds, serving as a dermal substitute. The collagen matrix of the allograft dermis is an excellent scaffold for fibrovascular ingrowth and native collagen replacement. Alloderm (LifeCell Corporation, Woodlands, TX) has been developed as a dermal/soft tissue replacement in accordance with this approach (Livesey et al. 1995). During the past 10 years, this material has been extensively evaluated in primary and secondary burn reconstruction, numerous facial soft

tissue argumentation procedures, and intraoral mucosal and gingival replacements (Sclafani et al. 2002; Wong et al. 2008; Bannasch et al. 2008).

Low-level laser therapy has been found to accelerate wound healing (Bisht et al. 1994) and reduce pain (Mazzetto et al. 2007; Ozcelik et al. 2008), possibly by stimulating oxidative phosphorylation (Karu 1987; Passarella et al. 1984) and reducing inflammatory responses, thus exhibiting several beneficial effects upon inflammation and healing.

No information is currently available on the fate of this dermal implant when subjected to postoperative low-level laser therapy. This animal study was conducted to determine the effect of low-level laser on parameters of graft viability and inflammatory response. This information is clinically relevant to know whether this type of graft material integrates biologically in a shorter period of time when associated with low-level laser therapy.

Materials and methods

Grafts

Grafts were obtained from Lifecell Corporation (Branchburg, NJ) a sealed foil bag that contains AlloDerm enclosed in an inner peel-pouch. The Freeze-dried and sterilely packaged sheet Alloderm was rehydrated in normal saline solution, and 5×5 mm^2 were cut and keep moist in normal saline solution.

Animals

All procedures were approved by Animal Care and Utilization Committee of Pontiff University Catholic of Rio Grande do Sul. Forty male Wistar rats (average weight 300 g) were randomly assigned to 1 of the 2 groups of 20 animals. The experimental animals were anesthetized with an intraperitoneal injection of mixture of 75 mg/kg ketamine and 10 mg/kg xylazine solution. After adequate anesthesia was confirmed, the animals were prepared for implantation of the graft, the heads were shaved and the area was cleaned with Betadine (povidone–iodine) solution.

Intradermal implantation of Alloderm

A 1 cm horizontal incision was made on calvarian area, parallel to the pupil line, and skin flaps were sharply elevated, exposing the underlying dermis. Before implantation, the Alloderm implant was measured with a handheld micrometer, and the graft thickness was recorded to the nearest 0.01 mm. The implant was placed between the dermis with care taken to orient the basement membrane surface towards the epidermis. The incision was closed with 2 interrupted 4.0 Mononaylon sutures. The 4 corners of the implant in each animal were palpated and marked the skin with India-ink tattoos. The animals were kept under daily observation throughout the experimental period. No clinical evidence of complications was observed during the period.

Low-level laser therapy

Right after the suture the wounds of the experimental group were irradiated in a contact mode with continuous wave (CW) 30 mW 685 nm diode laser (Ø $\sim$ 1 mm), with a total dose of 4 J/cm^2. The laser was applied transcutaneously, with the handpiece perpendicularly positioned above the wound (Kubota 2002). Irradiation was performed each 48 h, resulting in a total of 4 applications (16 J/cm^2). The applied doses were in accordance with previous studies, which varied from 1.8 to 5.4 J/cm^2 (Bisht et al. 1994).

Histological analysis

For histopathological studies wounds were biopsied after 1, 3, 7 and 14 of the surgical procedure. Five animals of each group were killed in each period of time and the implantation sites were removed in a full-thickness manner, placed in 10% formalin for 48 h, sequentially dehydrated, and then embedded in paraffin. Serial sections were then cut at 5 μm and colored with hematoxylin and eosin (H&E). An analysis of two different regions of each sections were made. The field near the basal lamina of the graft was region A and the opposite side was region B. Changes of collagen and inflammatory features were semi-quantitatively evaluated in coded slides and registered as absent (0), mild (1), moderate (2) and marked (3).

The number of fibroblasts was counted from photomicrographs of haematoxylin and eosin-stained specimens of five sites of the graft. The mean count was then reported.

Statistical analysis

Data were expressed as the mean ± SEM. *P* values of less than 0.05 were considered significant. Non-paired *t*-tests were used to compare control versus test group in the inflammatory response and wound healing rate data.

Results

During the post-surgery period, the animals remained healthy, with normal healing aspects on the operated site and without clinical evidence of infection. In the control group of animals within 1 and 3 days, an intense edema and polymorphonuclear inflammatory infiltrate was observed, although in the irradiated group the same features were seen the average was significatively smaller. The edema tended to be higher in the control group on the 1st, 3rd and 14th days with statistical difference from the irradiated group. There was no difference in mean polymorphonuclear inflammatory infiltrate scores between the control and the irradiated grafts at 7th and 14th days (Tables 1, 2).

Wounds histology showed a significant difference in leucocytic infiltration in the groups only in region B of wounds until day 3. This led to significantly more granulation tissue in the test wounds throughout the period of healing (Table 3).

The histological slides showed a significant expression of collagen fibers and an organized pattern in irradiated group since 3rd day of study (Fig. 1). It was verified that in the groups submitted to laser therapy, the intensity of stain was more evident, especially in region A (Table 4).

There was a marked influx of fibroblasts into the graft. They were identified based on their cell characteristics: up to 12 microns in length, spindle-shape form, round to ovoid nucleus. Care was taken to

Table 1 Distribution of edema in the control and laser treated groups at 1, 3, 7 and 14 days

Intervals	Group	Region A		Region B	
		Mean ± SD	*P*	Mean ± SD	*P*
1 day	Control	1.40 ± 0.54	0.0203*	2.20 ± 0.44	0.0125*
	Irradiated	0.40 ± 0.54		1.00 ± 0.70	
3 days	Control	0.40 ± 0.55	0.1411	1.00 ± 0.00	0.04*
	Irradiated	0.00 ± 0.00		0.40 ± 0.55	
7 days	Control	0.00 ± 0.00	1.00	0.20 ± 0.45	0.3466
	Irradiated	0.00 ± 0.00		0.00 ± 0.00	
14 days	Control	0.00 ± 0.00	1.00	0.80 ± 0.45	0.0039*
	Irradiated	0.00 ± 0.00		0.00 ± 0.00	

* The mean difference is significant at the 0.05 level

Table 2 Distribution of polymorphonuclear inflammatory cells in the control and laser treated groups at 1, 3, 7 and 14 days

Intervals	Group	Region A		Region B	
		Mean ± SD	*P*	Mean ± SD	*P*
1 day	Control	1.20 ± 0.44	0.0943	2.00 ± 0.70	0.172
	Irradiated	0.60 ± 0.54		1.40 ± 0.54	
3 days	Control	0.00 ± 0.00	1.00	0.20 ± 0.45	0.3466
	Irradiated	0.00 ± 0.00		0.00 ± 0.00	
7 days	Control	0.00 ± 0.00	1.00	0.00 ± 0.00	1.00
	Irradiated	0.00 ± 0.00		0.00 ± 0.00	
14 days	Control	0.00 ± 0.00	1.00	0.00 ± 0.00	1.00
	Irradiated	0.00 ± 0.00		0.00 ± 0.00	

Table 3 Distribution of leucocyte infiltration in the control and laser treated groups at 1, 3, 7 and 14 days

Intervals	Group	Region A		Region B	
		Mean ± SD	*P*	Mean ± SD	*P*
1 day	Control	0.00 ± 0.00	1.00	1.80 ± 0.44	0.0022*
	Irradiated	0.00 ± 0.00		0.40 ± 0.54	
3 days	Control	1.00 ± 1.00	0.4554	2.00 ± 0.00	0.0133*
	Irradiated	0.60 ± 0.54		1.00 ± 0.71	
7 days	Control	1.40 ± 0.54	0.0943	0.20 ± 0.45	0.3466
	Irradiated	0.80 ± 0.44		0.00 ± 0.00	
14 days	Control	1.20 ± 0.44	0.0943	1.80 ± 0.44	0.2415
	Irradiated	0.60 ± 0.54		1.40 ± 0.54	

* The mean difference is significant at the 0.05 level

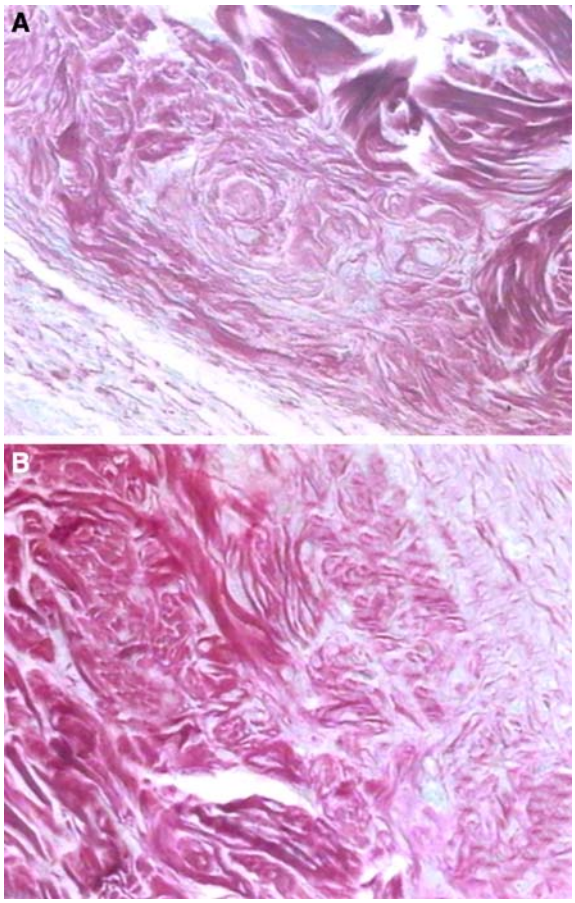

Fig. 1 **a** Collagen fibres of a control animal; 14 days (*Sirus red*, ×100). **b** Collagen fibres of a irradiated animal; 14 days (*Sirus red*, ×100)

exclude inflammatory cells and endothelial cells from the fibroblasts. The median fibroblast number in control and irradiated groups is shown in Table 5. The most significantly difference in fibroblast number also was established between day 3 and 7 ($P < 0.001$).

Discussion

Historically, collagen for human implantation was derived from xenogeneic sources, but this was never truly incorporated, being either resorbed, ultimately rejected as a result of foreign body-type reactions, or developed into stiff avascular geometry of fibrosis. Acellular human dermis is commercially available human collagen material in sheet form that offers the real possibility of collagen scaffold that can reliably be replaced by native collagen. Extensive experimental and clinical work in burn reconstruction attests to its ability to be incorporated; alloderm permits a skin graft to be either synchronously or metachronously applied with predictable postoperative "take" rates (Wong et al. 2008; Rennekampff et al. 1997).

It is known that the Low-level laser therapy has its better application in tissues under stress (Hawkins and Abrahamse 2006). Thus Low-level laser therapy is used to promote wound healing. Molecularly it is known to stimulate mitochondrial membrane potential, cytokine secretion, and cell proliferation. These changes reflect a biostimulative boost that causes a shift of the cell from a quiescent to an activated stage in the cell cycle heralding proliferation and suppression of inflammation (Gavish et al. 2004).

Although the clinical efficacy of the Alloderm has been proved through controlled studies (Livesey et al. 1995; Wong et al. 2008; Eppley 2000), nothing is known about the histological alterations that can occur due to its association to Low-level laser therapy. The

Table 4 Distribution of collagen fibers in the control and laser treated groups at 1, 3, 7 and 14 days

Intervals	Group	Region A		Region B	
		Mean ± SD	*P*	Mean ± SD	*P*
1 day	Control	0.00 ± 0.00	0.0003*	0.80 ± 0.45	0.3466
	Irradiated	1.20 ± 0.45		1.00 ± 0.00	
3 days	Control	1.00 ± 0.00	0.04*	0.80 ± 0.44	0.0353*
	Irradiated	1.60 ± 0.55		1.60 ± 0.54	
7 days	Control	1.00 ± 0.70	0.04*	0.40 ± 0.54	0.0140*
	Irradiated	2.20 ± 0.83		1.80 ± 0.83	
14 days	Control	1.40 ± 0.54	0.0085*	0.80 ± 0.44	0.0109*
	Irradiated	2.60 ± 0.54		2.20 ± 0.83	

* The mean difference is significant at the 0.05 level

Table 5 Fibroblast count per mm^2, in control and irradiated groups

Intervals	Group	Mean ± SD	*P*
1 day	Control	218.8 ± 67.9	0.0781
	Irradiated	308.2 ± 72.0	
3 days	Control	310.0 ± 34.1	0.001*
	Irradiated	482.0 ± 22.0	
7 days	Control	434.4 ± 33.8	0.001*
	Irradiated	583.4 ± 62.0	
14 days	Control	502.4 ± 76.3	0.0183*
	Irradiated	626.8 ± 55.2	

* The mean difference is significant at the 0.05 level

aim of this study was to evaluate the possibility of use Low-level laser therapy on acellular dermal implants postoperativerly to reduce the inflammatory reaction and improve its healing.

Due to the important circulation effects found as neoangiogenesis, the increasing of blood flow and fibroblast proliferation (Kami et al. 1985), the 685 nm diode laser was used in this research. The technique used was the punctual technique with contact, since this one is more efficient due to its small energy dispersion (Kubota and Oshiro 1996). It is generally accepted that the biological effect of Low-level laser therapy depends on three major parameters: Wavelength, dose, and power density. Because InGaAlP laser (685 nm) is commonly used for clinical practice, was decided to asses its effect in this experimental study.

The results of this study showed that the difference between the experimental group (irradiated) and the control group were statistically significant. Group irradiated was efficient for increasing the acellular implant healing, maybe due to the enhancement of vascular perfusion (Corazza et al. 2007). In the present study, in irradiated group, was found an accelerated process of inflammation and proliferation that is in agreement with previous histomorphological studies (Medrado et al. 2003).

The results of this study can be divided into 2 categories: (1) the effect of time on the implant, and (2) the effect of irradiation on the implant. The nonirradiated grafts showed a significant lower rate of healing based on collagen fibres, although the wound healing seem to be normal in all period of study.

Moreover, in this study, in treated sites an increase in the amount of collagen fibres and an more evident remodeling (maturation phase) of connective tissue were shown, and this corresponded with the results of experiments were radiation from InGaAl P laser (685 nm) was used for acceleration of healing (Silva et al. 2004).

The results from histopathological examination of the effect of Low-level laser therapy on acellular dermal implants show an accelerated process of reparation of the dermis and implant integration. These findings extend the theory of the positive influence of Low-level laser therapy on the healing of skin wounds to its use on acellular dermal implants sites.

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