

## First Experience with Photobiomodulation (PBM) in Post-Surgical Wound Healing In Dogs

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This prospective clinical study aimed to evaluate effectiveness and tolerability of PBM in canine post-surgical wound healing.

Seven female dogs with post-neutering surgical skin wounds of at least 3 cm in length were selected. One-half of the wound was treated with a portable soft GaAlAs-laser and the other left untreated thus all subjects included in the study were simultaneously “treated” and “control”. The treated and control areas were evaluated and allocated a clinical score on the first day (D0) and at the end of laser treatment (D4). The protocol was twice daily, 6 minute, laser treatments for 5 days. Paired and un-paired t-test were used to compare scores in treated and control areas and between treated and control areas at D0 and D4. Statistical significance was set at  $p < 0.05$ .

Almost all treated areas had greater visible clinical improvement compared to control areas. Nevertheless, statistical analysis showed that there was no significant difference between the total clinical score of treated areas and control areas at D0 ( $P=1.0000$ ) and no statistical significant difference between the two groups at D4 ( $P=0.2315$ ). There was a statistically significant decrease in exudate at D4 in treated areas compared to control areas ( $P=0.0300$ ). There was also a statistically significant difference between total score of the treated areas at D0 and at D4 ( $P=0.0167$ ) but also between D0 and D4 of the control areas ( $P=0.0223$ ). No adverse reactions were reported. PBM caused a visible clinical improvement of post-operative healing of surgical wounds, but this was not statistically significant; however there was a statistically significant decrease in exudate in treated areas. It would be interesting to extend the study to more extensive surgical wounds in more dogs.

**Abbreviation:** PBM: Photobiomodulation; NIR: near-infrared; GaAlAs: gallium aluminum arsenide; IM: intramuscular; IV: intravenously; SC: subcutaneous

### Introduction

Photobiomodulation (PBM), also known as “low-level laser therapy” or “cold laser therapy”, is a therapeutic modality that does not involve thermal effects, heat, sound or vibration, but rather acts through photochemical interactions that lead to a bio-cell stimulation [1]. Therapeutic laser use red and near-infrared (NIR) light with wavelengths between 300 and 10,600 nm and various substrates, including gallium aluminum arsenide (GaAlAs) [2].

The basic biological mechanisms responsible for the effects of PBM have not yet been fully elucidated [3]. Red or NIR light is absorbed by cytochrome C oxidase and resulting in activation of fibroblasts, keratinocytes, and endothelial cells and producing a reduction of inflammation, pain and edema [4]. Despite the encouraging in vitro studies, clinical studies in human dermatology have shown conflicting results; some studies have reported successes with PBM in the treatment of skin wounds [5], keloids [6] and ulcers [7] while other studies [8,9] have shown PBM to be ineffective in promoting the healing of skin. Animal model studies on experimentally induced wounds or burns in rats or mice show a significant acceleration of healing with PBM [10,11], but other studies in the same species and also in pigs [12] failed to show any positive effects.

There are a few clinical veterinary studies of the efficacy of PBM on the healing of skin wounds: in horses, with unsuccessful outcomes [13,14], in cattle [15] and only few studies in dogs, including an experimental study on five male beagle dogs, with no apparent beneficial effects [16] and a clinical study on an individual patient with a favorable outcome [17].

The main problem in human and veterinary medicine appears to be the absence of a standard protocol for wavelength, power, exposure time, type of low-intensity laser to be used for the different therapeutic treatments [3,5].

In view of the conflicting results highlighted by human literature, and given the scarcity of clinical data in veterinary medicine, the purpose of this preliminary clinical study was to evaluate the tolerability and the ability of PBM to reduce pain, swelling and inflammation and promote skin healing in the treatment of skin post-surgical wounds in dogs.

## Materials and Methods

Healthy female dogs of different ages and breeds that had undergone ovariectomy for elective sterilization in a private veterinary clinic were included. All dogs had a linear surgical wound with a minimum length of 3 cm.

All animals were client-owned dogs referred to the walk-in dermatology clinic at the Department of Veterinary Medicine, University of Milan on the day of surgery to receive the low-laser treatment. All owners gave informed consent for PBM, and the recording of measurements, photographs and data. The study was carried out in accordance with Italian law (DL 14 March 2014 n.26) and Europe Union legislation covering the use of animals for scientific purposes ("Animal Scientific Procedures Act" 63/2010/EU) and within the Institutional Ethical Guidelines.

Intradermal polydioxanone monofilament 2-0 or 3-0 (Surgicryl®) or braided and coated glycolic acid polymer 2-0 or 3-0 (Assufile®) continuous absorbable sutures were used for skin closure. Immediately after surgery rifaximin topical spray (Fatroximin®; Fatro Spa) was applied to the surgical wound.

All dogs were discharged from the clinic on the afternoon of the day of surgery, and immediately attended the Department for the first PBM therapy. They were then returned to their home environment wearing an Elizabethan collar until healing of the surgical wound was complete. They returned every day to the Department for PBM.

Post-operative analgesia was tramadol hydrochloride (Altradol®; Formevet Srl) 2 mg/kg per os for 3 days and/or carprofen (Rimadyl®; Pfizer Italia Srl Div.Vet) 2 mg/kg per os for 3-5 days. On the first day of the study (D0 – the day of surgery) signalment and history were recorded and a complete physical examination was performed. Each patient was classified as cooperative/non-cooperative according to whether they would remain for at least 10 min in lateral or dorsal recumbency on the examination table. Only cooperative dogs were included in the study. Dogs with a history of neoplastic disease were not included as recommended by the manufacturer of the laser device.

Each dog was both a "treated" and a "control", because only one half of the wound was treated with PBM with the remaining area acting as a control. The area of wound to be treated, was selected at random by an operator not involved in the study who did not see the dogs.

A clinical score was calculated for treated and control area (Table 1) at D0 and on the last day of treatment (D4) in a blind fashion by the operator not involved in the study. The scoring system used was derived (with appropriate changes and additions relating to the nature of the lesions in this study) from the Pressure Ulcer Scale for Healing developed by the National Pressure Ulcer Advisory Panel as a tool to monitor changes in pressure ulcers over time in human patients [18], the Bates-Jensen Wound Assessment Tool [19], and the wound bed scoring for chronic wounds [20].

Parameters	0	1	2	3	4
Skin color	Pink	Erythematous	Dark	-	-
Pain	None	Present	-	-	-
Granulation tissue	None	Present	-	-	-
Exudate (type)	None / transparent	Serous	Sero-hemorrhagic	Hemorrhagic	Purulent
Exudate (amount)	None	Light	Moderate	Heavy	-
Eschar	None	Present 0 - 25 % surface area	Present, > 25 % surface area	-	-

**Table 1:** Clinical score for objective assessment of wounds - derived and modified from PUSH (18), BWAT (19), and WBS (20) scales.

All lesions were photographed at D0 and at D4 and any side effects of treatment recorded. No medications other than analgesia were permitted during the study period.

The laser used was the B-808-CURE LLLT (Good-Energies, Haifa, Israel) - a portable laser for human use, lightweight (173 g), and equipped with rechargeable batteries. It is a diode laser having a GaAlAs solid medium, with a power of 250 mW (micro-pulsed to ensure greater effectiveness and penetration with laser pulse duration of 17 µs and a work rate of 25%), a pulse frequency of 15 kHz, laser beam dimensions of 45 mm length x 10 mm width and a wavelength of 808 nm (infrared). The energy density is 0.9 J/min/cm<sup>2</sup>, whereas the peak energy of 14.4 J/min is for the entire treated surface.

The following treatment protocol was used:

- D0: Initial application of PBM, maintaining the device as close as possible to the lesion without skin contact for 1.5 min (to record any side effects), followed by the second session of 6 min, after an interval of at least 30 min
- D1, D2, D3, and D4: twice daily treatment with a duration of 6 min, with sessions separated by at least 3 hours.

This protocol was selected partially following the therapeutic indications suggested by the manufacturer of the laser device in their guidelines for human cases of “fresh and old surgical wounds and scars.”

## Statistical analysis

Results were analysed using statistical software MedCalc (version 12.7.8.0). The total population of treated and controls was assessed by the Kolmogorov-Smirnov test which showed a normal distribution of available values. A paired t-test was used to compare scores in treated areas at D0 and D4. The same evaluation was performed in the control group areas. The un-paired t-test was used for comparison of the scores of the treated areas and the control areas at D0 and D4. Statistical significance was set at  $p < 0.05$ .

## Results

Ten dogs were enrolled in the study, but 3 dogs were lost due to non-compliance of the owner.

Seven dogs completed the study, all females, aged between 2 and 9 years old, of 5 different breeds (3 crossbreed, 1 Corso dog, 1 Springer Spaniel, 1 Chihuahua, 1 Dachshund). Table 2 shows the total score for each dog, for both control area and treated area, at D0 and D4.

Dog number	days	Total score	
		T (treated area)	C (control area)
1	D0	1	1
	D4	0	0
2	D0	1	1
	D4	0	1
3	D0	4	4
	D4	0	1
4	D0	2	2
	D4	0	0
5	D0	1	1
	D4	1	0
6	D0	1	1
	D4	0	1
7	D0	4	4
	D4	1	2

**Table 2:** Total clinical scores for each dog in treated area (T) and control area (C), on the first (D0) and the last day of treatment (D4)

There was no significant difference in the total clinical score of treated and control areas at D0 ( $P=1.0000$ ) and, despite an evident visual improvement in treated area compared to control area in the majority of subjects (Figure 1 and Figure 2), no statistically significant difference was found. No statistically significant difference was detected in the total clinical score between treated areas and control areas on D4 ( $P=0.2315$ ).

There was a statistically significant difference in the amount of exudate on D4 between treated and control areas ( $P=0.030$ ). There was also a statistically significant difference in the total score of treated area between D0 and D4 in the all subjects ( $P=0.0167$ ), as well as between D0 and D4 ( $P=0.0223$ ) of the control area.

All subjects included in this study were cooperative during the treatment. No adverse reactions or local or systemic side effects were reported during therapy.

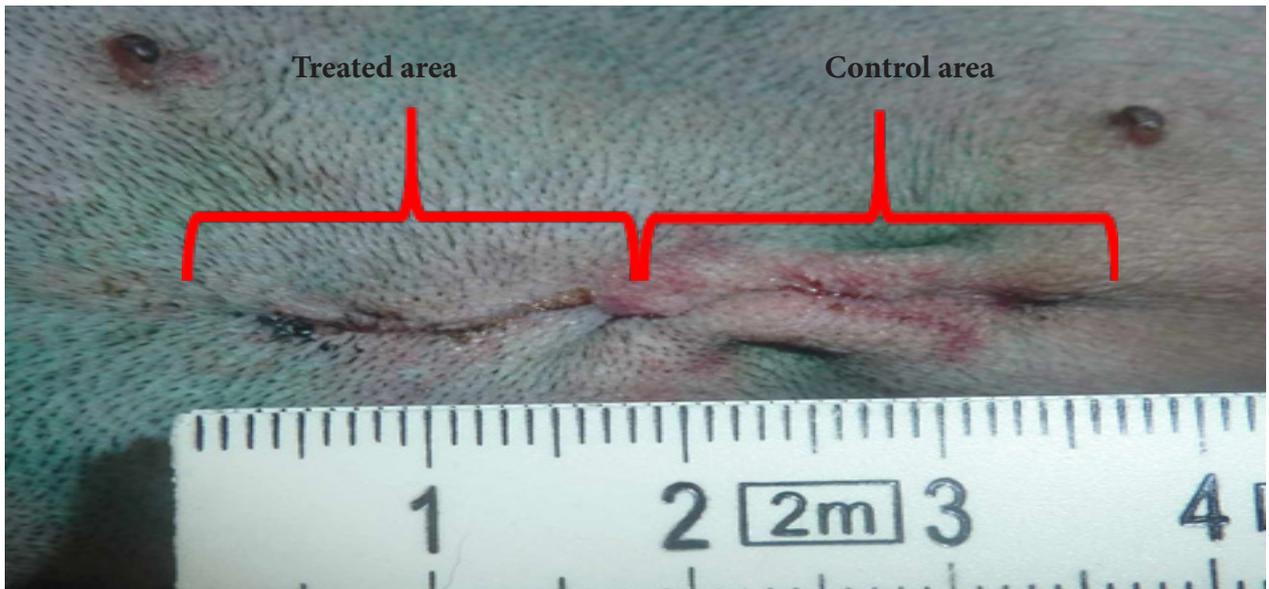
## Discussion

To the authors' knowledge, this is the first clinical study on the application of PBM in post-surgical wound healing in the dog and one of the first clinical studies on the use of PBM in canine dermatology. It is, therefore, very difficult to compare our data with those reported in the canine literature, which is extremely varied.

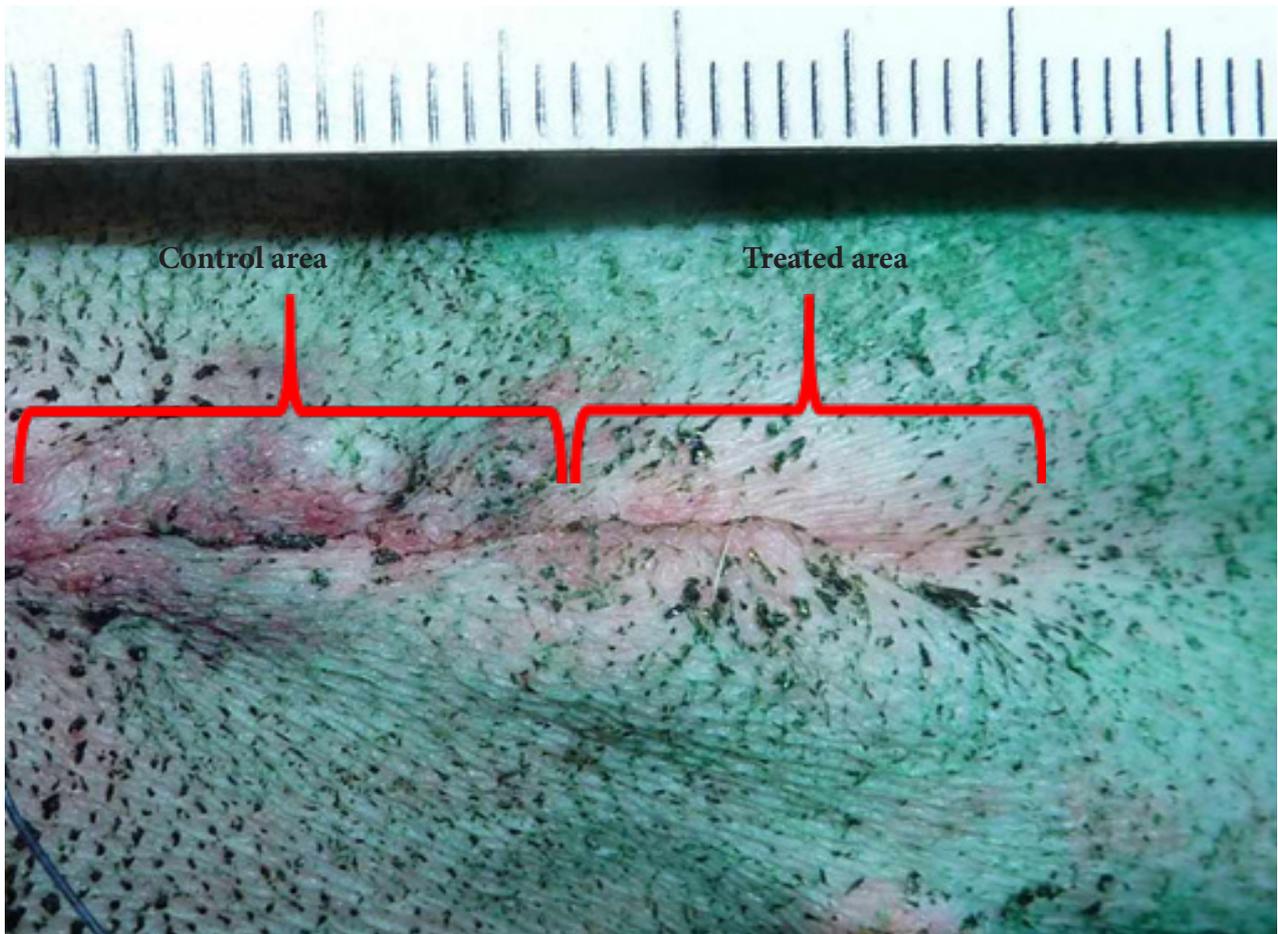
Lucroy et al. [17] reported laser treatment of a chronic non-surgical wound in a single dog and Olivieri et al. [21] described treatment of non-inflammatory alopecia in seven dogs; both reported a favourable outcome. Another study on the clinical efficacy of PBM on localized canine atopic dermatitis showed that the PBM was not an effective localized treatment for pedal pruritus [22], while a recent study on the clinical efficacy on canine sterile pyogranulomatous pododermatitis obtained positive results using the same PBM device [23].

In most literature studies, the effects of PBM in the healing of skin wounds have been studied on surgically induced lesions with significant tissue loss [11,13,17] where wound healing is by secondary intention.

In our study we treated sutured post-surgical wounds, where primary healing was occurring: following the application of PBM, we would expect a more rapid macroscopic improvement of the treated post-surgical wound area, with a reduction of the inflammation of the tissue surrounding the incision, a good reduction of skin margins and better epithelialization than in control sites. Although this was observed in many of the subjects results did not achieve statistical significance. The failure to reach statistical significance for this finding may have been due to the fact that wounds were evaluated by a clinical score only and no histologic evaluation of the wounds was performed. A study by Ghamsari et al. (1996) [15] evaluated the histopathologic effects of PBM on sutured wounds at the level of the nipples in a group of dairy cows, and showed that in the treated group there was better organization of collagen fibres alongside better clinical appearance of healing than in controls.



**Figure 1:** case n.7 at D4. Note the presence of small amount of transparent exudate on the wound and erythema in the control area but not in the treated area.



**Figure 2:** case n. 3 at D4. Note the presence of erythema and oedema in the control area but not in the treated area.

The bio-stimulatory effect of PBM was also confirmed histologically in a group of rats, in a study investigating the role of laser irradiation in the healing of burns: the PBM stimulated the local microcirculation increasing angiogenesis, proliferation of collagen fibres, deposition of new matrix and re-epithelialization of lesions undergoing treatment [10].

Not all studies on the use of PBM in skin repair have shown favourable results: Petersen et al. [13] applied laser treatment to surgically induced skin wounds in a group of horses, but found no statistically significant difference in epithelialization of the control areas and treated areas. Some studies in human medicine have investigated the effect of PBM on ulcerative lesions [9] pressure ulcers, venous ulcers and other chronic wounds [24], but were unable to demonstrate significant difference between the subjects undergoing PBM and the control group.

However despite the lack of statistical differences between the clinical scores of treated and control areas at the end of the therapy, the results of our study in 7 dogs seem to indicate a possible positive effect of PBM in the healing of surgical wounds. In fact, there was an evident clinical improvement of the treated area compared to control area in the majority of subjects.

The best improvements were seen in the reduction in exudate production, with a statistical difference between treated and control areas.

The reported reduction in exudate in this study is in line with what was observed in the study of Petersen et al. [13] in horses. A reduction of exudation was also demonstrated in a study carried out on a group of mice treated with PBM after the induction of pleurisy [25].

PBM may represent a non-invasive therapy that can improve healing of post-surgical wounds. Successful PBM treatment requires good owner compliance as they must be willing to present their dog for at least two sessions of laser therapy daily for 5 consecutive days. In the absence of standard published protocols for PBM treatment in the dog, it would be useful to test protocols with less frequent outpatient sessions and with increased irradiation time.

The cooperation of patients during the laser therapy sessions was important to the success of treatment. Dogs in which open ovarioectomy via ventral median celiotomy were chosen as the site of the wound (ventral abdomen) made patient restraint and handling for PBM treatment possible without the need for pharmacological sedation. All subjects included in this study were cooperative during the 6 min of treatment and this confirms the minimally invasive nature of the treatment.

This study was designed as a pilot study of a clinically innovative therapy on a small number of subjects following the literature on some similar studies on new treatments in dermatology [21,23,26,27]. We chose the "case-control" study design to reduce the variables of clinical response and avoid confounding factors such as age and breed. Having an untreated control area on each treated subject allowed us to assess the actual benefit of the PBM.

However, the lack of statistically significant difference between the treated portion and the control area, could be due to the fact that the laser irradiation affected not only the area directly treated, but also the surrounding tissues indirectly, as demonstrated by the study of Hopkins et al. [28]. This "spill over" effect could be an important confounding factor and a possible major limitation of our study design.

Among the limitations encountered in the clinical evaluation of the effectiveness of PBM one important factor is certainly the absence in the literature of standard treatment protocols.

In our study, there are many reasons for the absence of statistically significant differences between the scores of treated and control areas at the end of the therapy: the use of an inappropriate wavelength, an insufficient duration of therapy, or insufficient duration of the individual irradiations performed.

Finally, a further limitation of our study is that we did not follow up the patients until complete healing of the post-surgical wound and so have no data on time to complete healing, which may have been reduced by PBM.

In the light of the results obtained in this pilot study, it would be advisable to further investigate the clinical effects of PBM on post-surgical sutured wounds, in particular where the treated area and the control area are not part of the same wound. In addition, future studies could include further clinical evaluation of low-intensity laser irradiation of lesions with extensive loss of substance, where healing occurs by second intention. This would investigate the clinical effects of bio-stimulation of PBM, focusing on the contraction of margins of the lesion and the extent of epithelialization.

## Conclusion

We have demonstrated some positive effects of PBM on the healing of post-surgical wounds in neutered dogs. Given the results of this pilot study, it would be interesting to extend the study to confirm the validity of this type of therapy in a wider variety of surgical wound types in a greater number of dogs.

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