Introducing

3.5 mm LCP® Distal Femoral Osteotomy System

For use in medium to large breed dogs

Features:

- Plates are pre-contoured to match the anatomic surface of the femur
- Distal locking screw trajectories are targeted to avoid the intercondylar notch and stifle joint
- Plate tapered design minimizes soft tissue interference
- Plates are offered in shorter and longer lengths to address patient variability
- Plates are compatible with both DePuy Synthes locking and cortex screws

LEARN MORE AT
www.jjmdanimalhealth.com

DePuy Synthes
VET
PART OF THE Johnson & Johnson FAMILY OF COMPANIES

Received: 17 April 2018 | Revised: 27 January 2019 | Accepted: 21 April 2019

DOI: 10.1111/vsu.13415

WILEY

ORIGINAL ARTICLE - CLINICAL

Effect of the topical Klox fluorescence biomodulation system on the healing of canine surgical wounds

Alberto Salvaggio DVM, PhD | Gian Enrico Magi DVM, PhD, DECAAH | Giacomo Rossi DVM, PhD, DECZM | Adolfo Maria Tambella DVM, MSc | Cecilia Vullo DVM, PhD | Andrea Marchegiani DVM, PhD | Riccardo Botto DVM | Angela Palumbo Piccionello DVM, PhD

School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy

Correspondence
Angela Palumbo Piccionello, Veterinary Teaching Hospital Surgery Division, University of Camerino School of Biosciences and Veterinary Medicine, Via Circonvallazione, 93/95 - 62024 Matelica, MC, Italy.
Email: angela.palumbo@unicam.it

Abstract

Objective: To determine the effect of the Klox fluorescence biomodulation system (Phovia) on the healing of surgical wounds.

Study design: Prospective, blinded, controlled clinical trial.

Sample population: Healthy dogs undergoing orthopedic surgery (n = 10).

Methods: Half of the length of each surgical wound was treated with Phovia, and the remaining 50% was treated with saline solution on the first day after surgery and every 3 days until day 13. Wound healing of treated and control areas within each wound was evaluated via macroscopic assessment and histological and immunohistochemical analysis of treated and control wounds.

Results: The areas treated with Phovia achieved lower histology scores ($P = .001$), consistent with complete re-epithelialization, less inflammation of the dermal layer, and greater and more regular deposition of collagen. According to immunohistochemistry, expression of factor VIII, epidural growth factor, decorin, collagen III, and Ki67 was increased in treated compared with untreated tissues.

Conclusion: Phovia therapy improved re-epithelialization, decreased dermal inflammation, and improved matrix formation in uncomplicated cutaneous incisional wounds by regulating the expression of key biological mediators.

Clinical significance: Phovia may be a beneficial adjunct to promote the healing of incisional wounds.

1 | INTRODUCTION

Wound healing starts immediately after injury and requires a complex balance between matrix elements and growth factors. Multiple factors influence this process, including blood supply, defect size, tension, mobility, susceptibility to infection, type, and condition of the underlying tissue.[1] Excessive, continuous, and chronic inflammation may affect healing and prevent morphofunctional normality, resulting instead in the formation of disoriented connective tissue. This abnormal architecture reduces mechanical strength of tissues and leads to scar formation.[2,3] In recent years, many products and innovative techniques for increasing the quality and speed of wound healing have been investigated. Topical

Results of this research were presented at the 2018 European College of Vetrinary Surgeons Congress; July 5-7, 2018; Athens, Greece.

treatments include hyaluronic acid, growth factors and blood components, stem cells, and adipose-derived stromal vascular fraction as well as negative-pressure wound therapy, ozone therapy, electrical stimulation, ultrasound, and phototherapy.[1-8] The variety of tested therapies highlights the level of interest in improving the healing of cutaneous lesions because of their frequency and potential challenging consequences.

One of the trends in wound therapy focuses on biofunctional materials that can interact with light to promote tissue regeneration. In this context, photobiomodulation (PBM) has been generating substantial interest. This cell stimulation technique relies on a light (light emitting diode [LED]) that does not emit heat but can induce transduction pathways; these pathways can modulate biological processes through the activation of photoacceptors that are present in several cell and tissue types. Fluorescence biomodulation (FB), a type of PBM, leverages the unique ability of light absorbing molecules to convert light emitted by diodes to broader wavelengths and lower energy (fluorescence) that can penetrate skin and stimulate healing. Phovia (Klox Technologies Limited, Dublin, Ireland) is a novel FB product comprising two components, a blue LED lamp and a carbopol-based amorphous hydrogel (the gel) acting as a topical photoconverter. When they are activated by the LED lamp, the chromophores contained in the gel release photons at different wavelengths within the spectrum of visible light in the form of fluorescence. These low-energy photons have been found to improve wound healing in human patients,[9,10] and the excellent safety profile and efficacy of this technology to treat conditions such as pyoderma and otitis has been documented in dogs.[11,12]

The objective of the present study was to determine the effect of Phovia therapy on the healing of cutaneous incisional wounds as assessed via macroscopic evaluation and histological and immunohistochemical assessment of the treated and control areas. We hypothesized that Phovia would accelerate wound healing by day 13 after surgery by improving matric formation and epithelialization while reducing dermal inflammation.

2 | MATERIALS AND METHODS

Ten healthy client-owned dogs undergoing orthopedic surgery at the Veterinary University Teaching Hospital L'Ospedale Veterinario Universitario Didattico (OVUD) were prospectively recruited. Owners provided informed consent for the procedure, and the protocol was reviewed and accepted by the OVUD ethics committee (Prot N1; February 10, 2017). Dogs were enrolled if they were undergoing surgery for nontraumatic orthopedic diseases, in general good health, and classified according to the American Society of Anesthesiologists physical status classification system as ASA I on the basis of physical examination and laboratory blood test (at day 0) results. They were excluded if they had skin changes or other dermatological/metabolic diseases that could confound data interpretation. Dogs that had received steroid therapy in the previous 6 weeks or photosensitizing molecules were also excluded.

2.1 | Treatment protocol

All surgical procedures, including suture closure of the skin, were performed by the same orthopedic surgeon (A.P.P.). All dogs received 20 mg/kg cefazoline (Teva Italia Srl, Milano, Italy) IV 20 minutes before surgery. All wounds were closed with a monofilament nonabsorbable suture (USP 3/0 Ethilon; Ethicon Somerville, New Jersey) in a simple interrupted pattern. Postoperatively, dogs received 20 mg/kg cefadroxil (Intervet Italia Srl, Peschiera Borromeo, Italy) orally every 12 hours for 5 days and 3 mg/kg carprofen (Zoetis Italia Srl, Roma, Italy) orally every 24 hours for 7 days. Dogs remained in the hospital or were discharged to the owners' care as appropriate and according to OVUD standard protocols. All nonhospitalized dogs received standard postoperative instructions for the type of surgery they had. On the first day after orthopedic surgery (T0) and every 3 days until day 13 (T4), 50% of the length of the surgical wound was cleaned with sterile saline solution and treated with Phovia for 2 minutes, while 50% was only cleaned with sterile saline solution so that dogs could serve as their own controls. All dogs wore Elizabethan collars for 18 days.

The treatment (T) and control (C) portions of each surgical wound were randomly assigned by using sealed, opaque, sequentially numbered envelopes created by the two investigators (A.S. and A.M.T.) performing the Phovia procedure prior to study initiation. The surgeon and the two pathologists (G.E.M and G.R.) were unaware of the treatment assignments.

A layer of photoconverter gel measuring about 2 mm in thickness was applied to each portion of wound assigned to treatment. The treated wound was illuminated with an LED lamp (KT-V lamp; Klox Technologies, Laval, Quebec, Canada) at a distance of approximately 5 cm for 2 minutes; this device delivers blue light with peak wavelengths between 440 and 460 nm and a power density of between 55 and 129 mW/cm^2. During illumination with the LED lamp, the control part of the surgical wounds was covered with a surgical drape to prevent exposure to the LED lamp illumination. After illumination was completed, any gel residue was carefully removed by using gauze dipped in sterile saline solution. This procedure was repeated five times (from T0 to T4) until the removal of sutures on day 13. After each procedure had been completed, the surgeon was

invited back into the room to evaluate and score the two portions of the surgical wounds.

With previous agreement from the owners, sutures were removed on day 13, and two biopsies (2 mm in diameter and 3-5 mm deep) were obtained from the median portions of the treated and control portions of the wound in a position that avoided suture tracts. Biopsies were performed under local anesthesia by injecting the biopsy site with 0.5 to 1 mL 2% lidocaine (Zoetis Manufacturing & Research, Girona, Spain) with a 25-gauge needle and a syringe after trichotomy. Each biopsy was obtained with a sterile 2-mm biopsy punch (Kai Medical, Seki, Japan). No sutures were required at the biopsy sites, and dogs resumed the postoperative protocol based on the surgical procedure performed.

2.2 | Measures of outcome

2.2.1 | Macroscopic evaluation

Wounds were visually evaluated with a semiquantitative scoring system by taking into consideration wound appearance and clinical consequences of tinfection.[13,14] All treated and control portions of wound were scored from 0 to 5 for erythema and serous exudate and from 0 to 10 for purulent exudate and deep tissue separation, depending on the percentage of the wound affected by each process (Table 1).

Wounds were scored at each observation time point (T0-T4), and the healing process was classified as follows:

- 0: satisfactory healing;
- 1 to 6: mild healing disorder;
- 7 to 10: lower wound infection;
- 11 to 18: moderate wound infection;
- 19 to 30: severe infection of the wound.

2.2.2 | Histological and immunohistochemical evaluations

Skin biopsies were fixed in 10% formalin, embedded in paraffin, sectioned (3-μm thickness), and stained with hematoxylin and eosin and Masson's trichrome. Two pathologists, unaware of treatement groups, evaluated all sections. A semiquantitative histological evaluation was conducted on each sample with a modified version of a previously described histologic grading system, based on epidermal/dermal changes (Table 2).[15] For each sample, 10 sequential fields (×200) were evaluated, and a histological score based on the sum of the individual morphological parameters (minimum value 0, maximum value 14) was assigned; an average was calculated for both the control and the treated group.

TABLE 2 Scoring system used to evaluate histological appearance of wounds

Feature	Score	Description
Fibroblasts	0	Absent
	1	Mild granulation tissue
	2	Moderate-granulation tissue
	3	Marked-granulation tissue
New vessels	0	Absent
	1	Mild-granulation tissue
	2	Moderate-granulation tissue
	3	Marked-granulation tissue
Polymorphonuclear leucocytes	0	Absent
	1	Mild-surrounding tissue
	2	Mild-granulation tissue
	3	Moderate-granulation tissue
	4	Marked-granulation tissue
Re-epithelialization stage	0	100%
	1	99%–75%
	2	76%–50%
	3	51%–25%
	4	26%–0%

TABLE 1 Scoring system used to assess the macroscopic appearance of wounds at each of the five time points

	PROPORTION OF WOUND AFFECTED					
Wound characteristic	**0%**	**<20%**	**20%–39%**	**40%–59%**	**60%–79%**	**>80%**
Serous exudate, n	0	1	2	3	4	5
Erythema, n	0	1	2	3	4	5
Purulent exudate, n	0	2	4	6	8	10
Deep tissue separation, n	0	2	4	6	8	10

The immunohistochemistry (IHC) sections were mounted on SuperfrostPlus slides (ThermoFisher Scientific, Waltham, Massachusetts), and an avidin-biotin-peroxidase-complex technique, with diaminobenzidine as the chromogen, was performed. A panel of antibodies to the following antigens was used: AE1/AE3 (monoclonal; Dako Denmark A/S, Glostrup, Denmark), factor VIII (FVIII; polyclonal; Antibodies-online.com, Aachen, Germany), epidermal growth factor (EGF; polyclonal; Santa Cruz Biotechnology, Dallas, Texas), decorin (polyclonal; Abcam, Cambridge, United Kingdom), collagen III (polyclonal; Antibodies-online.com), Ki-67 (monoclonal; clone MIB-, Dako Denmark A/S), transforming growth factor-β (TGF-β 1,2,3, monoclonal; ThermoFischer Scientific), tumor necrosis factor-α (TNF-α; monoclonal; Abcam), and fibroblast growth factor-2 (FGF-2; polyclonal, Abcam). For antigen retrieval, a pretreatment was performed with citrate buffer pH 6.0 at 95°C for 15 minutes in a water bath. Appropriate negative and positive controls tissues, including sections of canine skin, were used for the immunohistochemical analysis. The specificity of the different antibodies used for dog tissues was evaluated by using the information available in the data sheet for each antibody.

For scoring of dermal/epidermal cellular expression of EGF, FGF, TGF-β, TNF-α, decorin, Ki67, FVIII, collagen I and III, all cellular types were evaluated under light microscopy (Carl Zeiss, Jena, Germany), with a 40x objective, a 10x eyepiece, and a square eyepiece graticule (10×10 squares, with a total area of 62 500 μm^2). Ten appropriate fields were chosen for each biopsy, and arithmetic means for the number of stained cells were calculated for each skin portion: epidermal, dermal, and hypodermal area. Results were expressed as IHC^+ cells per 62 500 μm^2. For all parameters, cells on the margins of the tissue sections were excluded from the evaluation to avoid inflation of positive cell numbers.

2.3 | Data analysis

Sample size for the clinical trial was determined with a power and sample size analysis performed on preliminary histologic data from treated vs control portions of wounds in the study in G-Power software (version 3.1.9.2; Heinrich-Heini-Universitat Desseldorf, http://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower.html) and a Wilcoxon-Mann–Whitney test. A post hoc power analysis was performed by using the effect size (ES) from the first seven cases per group (ES, d = 1.630279), with α error fixed at .05. An a priori analysis was performed to calculate the suitable sample size to achieve a power of at least 0.90, with α error fixed at .05 and the same ES as previously calculated. After the sample size had been reached, a post hoc power analysis was performed to estimate the power reached by the study with the current ES and with an α error fixed at .05.

The characteristics of the distribution of cardinal data were tested for skewness and kurtosis. Immunohistochemistry scores were compared between treatment and control group with a paired *t* test and Wilcoxon signed-rank test. For the macroscopic assessment, a paired *t* test and Wilcoxon signed-rank test was used for a comparison of C and T areas for each of T0, T1, T2, T3, T4, total, and mean.

3 | RESULTS

Ten dogs ranging from 1 to 10 years in age and of various breeds were prospectively recruited. Orthopedic surgeries performed on these dogs included five tibial plateau leveling osteotomies, three limb alignments, and two femoral head and neck excision. Ten incisional wounds and 20 biopsy samples (10 from the treated portion and 10 from the control portion) were considered. After the intended sample size had been achieved (10 dogs per group), a post hoc power analysis with the current ES (d = 1.821255) yielded a power of 0.96, which was higher than the power planned in the a priori sample size analysis.

No adverse reaction to the treatment was detected throughout the study. At day 13, all wounds seem to be healing well according to macroscopic evaluation, including treated and control areas. In total, 50 clinical (macroscopic) evaluations were performed (5 observations for each dog, 10 dogs; Figure 1, Table 3). Forty-four of

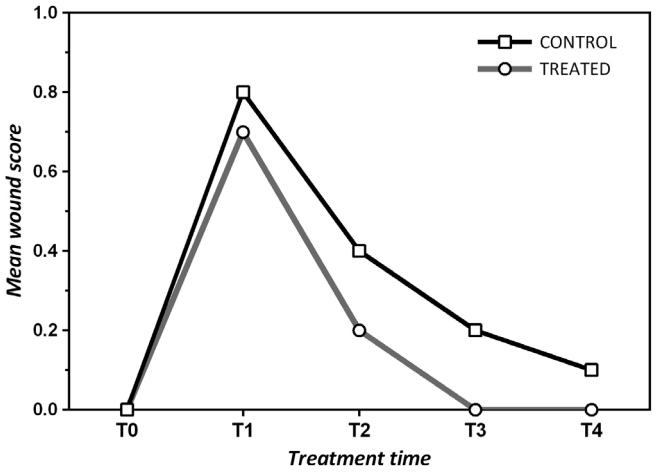

FIGURE 1 Scores assigned to the macroscopic appearance of treated and control wounds

TABLE 3 Macroscopic wound scores obtained at each clinical inspection (5 times)

	T0 (d1)		T1 (d4)		T2 (d7)		T3 (d10)		T4 (d13)		Total		Mean	
Dog	C, n	T, n	C, n	T, n	C, n	T, n	C, n	T, n	C, n	T, n	C, n	T, n	C, n	T, n
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	5	5	2	2	2	0	1	0	10	7	2	1.4
3	0	0	0	0	0	0	0	0	0	0	0	0	1	0.4
4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	0	0	3	2	2	0	0	0	0	0	5	2	1	0.4
9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	0	0	8	7	4	2	2	0	1	0	15	9	3	1.8
Mean	0	0	0.8	0.7	0.4	0.2	0.2	0	0.1	0	1.5	0.9		

Abbreviations: C, control; T, treated.

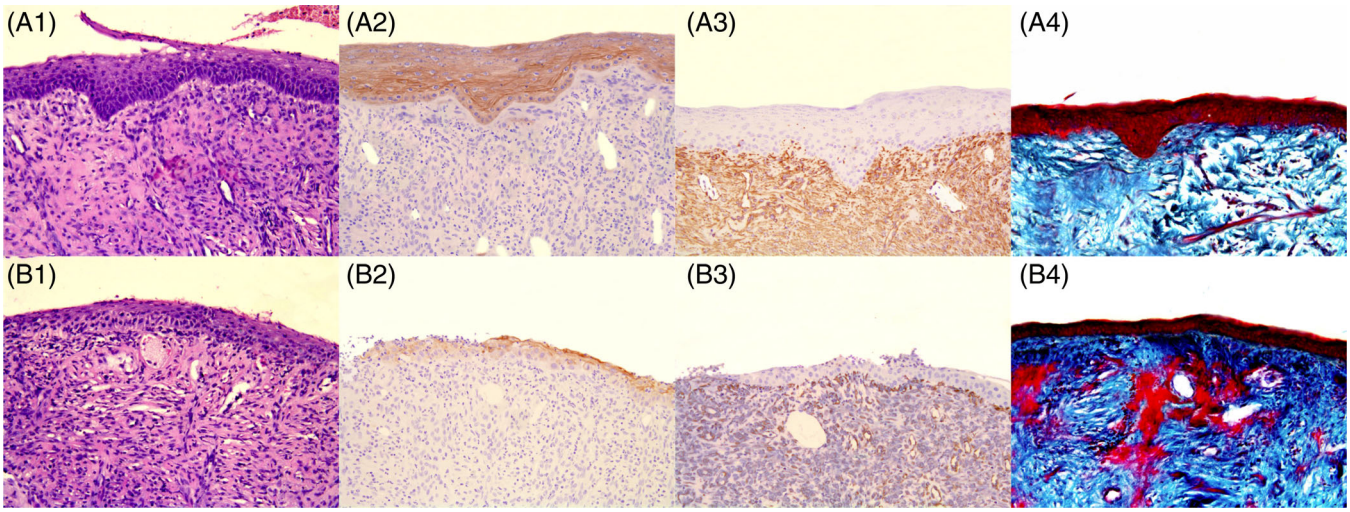

FIGURE 2 Histologic appearance of wounds: (A) portion treated with Phovia, (B) untreated portion. A1,B1, Hematoxylin & eosin coloration. Epidermal integrity and basal activity (greater dermic papillary fever) seemed greater in treated wounds (A1), while no residual phlogosis and less neoangiogenesis were noted compared with the control sample in B1. A2,B2, Immunoblotting for AE1/AE3. Note the strong cytokeratinic expression of the A2 sample compared with the B2 sample, which is consistent with epidermal integrity in A2 compared with a partial re-epithelialization in B2. A3,B3, Collagen immunograde III. The expression of collagen III in A3 is abundant compared with that in the B3 sample. A4,B4, Deposition of collagen is more abundant and regular in A4, whereas greater phlogosis, blood extravasation, and fibrosclerotic processes are present in B4 (blue tendency to black; Masson's trichrome)

50 observations of control portions of the wound were scored 0 (satisfactory healing), and the remaining six did not exceed a score of 5 (mild healing disorder). Forty-seven of 50 of the evaluations of treated portions of the wound were scored 0, and three evaluations achieved a score that did not exceed 5. There was no difference between scores assigned to treated vs control portions of wounds.

Histological examination of Phovia-treated tissue was consistent with complete re-epithelialization, minor inflammation of the dermal layer, and high neoangiogenesis (Figure 2). Treated samples scored lower (5.5 ± 1.34 SE; Table 2) compared with control samples (11.7 ± 0.72, $P = .001$; Figure 3, Table 4). According to immunohistochemistry results, expression of FVIII ($P = .034$), EGF ($P = .008$), decorin ($P = .005$), collagen III ($P = .005$), and

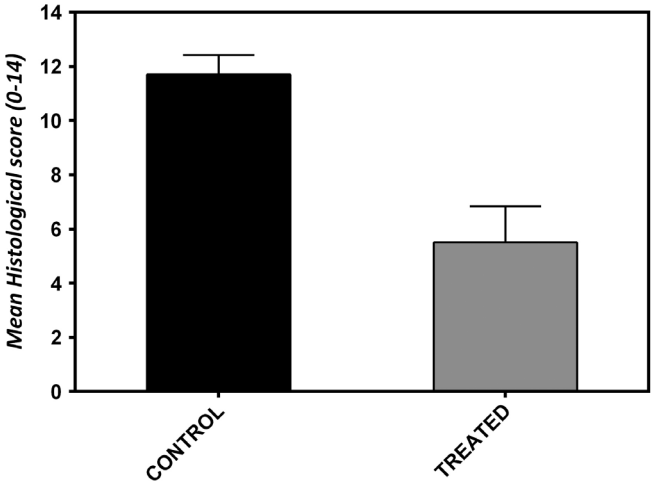

FIGURE 3 Histological scores of treated and control wounds. Black bars indicate standard errors

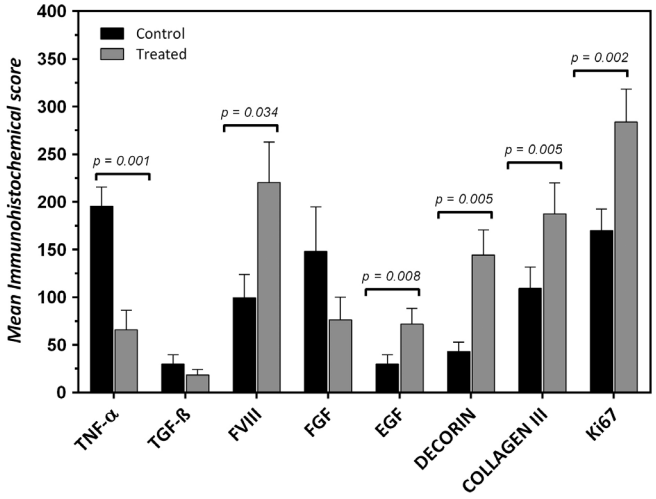

FIGURE 4 Immunohistochemistry scores of treated and control wounds. Black bars indicate standard errors. A panel of antibodies to the following antigens was used: FVIII, EGF, decorin, collagen III, Ki-67, TGF-β, TNF-α, FGF. EGF, epithelial growth factor; FVIII, factor VIII; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α

TABLE 4 Histological scores (0-14) assigned to treated and control wound biopsies

Dog	Control, n	Treated, n
1	14	10
2	13	13
3	8	4
4	13	0
5	9	3
6	11	7
7	14	9
8	13	0
9	9	4
10	13	5

Ki-67 ($P = .002$) was higher and expression of TNF-α ($P = .001$, Figure 4) was lower in treated wounds.

4 | DISCUSSION

The results of our study provide sufficient evidence for us to accept our hypothesis that treatment with Phovia promoted wound healing at day 13 postsurgery by stimulating the cell proliferation (including fibroblasts, keratinocytes) and matrix synthesis. Wound repair is a complex biological process, taking place in several stages and involving many types of cells.[16] The physiological repair process begins with hemostasis and fibrin deposition, leading to an inflammatory cell cascade characterized by the arrival of neutrophils, macrophages, and lymphocytes at the site of the lesion. After this phase, the in situ recruitment of fibroblasts and the deposition of collagen follows (2-10 days after the beginning of the repair process). At 11 to 15 days, tissue remodeling by collagen reticulation and maturation of the scar take place.[17]

Assessing key "signals" required for the influx of connective tissue cells and a new blood supply is essential to evaluate the mechanisms involved in physiologic or pathologic conditions of wound healing, such as fibrosis and chronic nonhealing ulcers.[18] The maturation and remodeling phase of wound healing is characterized by a decrease in the cell population and an increase in collagen organization as well as epidermis regeneration.[19] In our study, we found that, at day 13 postsurgery, collagen III expression was higher in the Phovia-treated wounds than in the control samples. A greater production of growth factors, such as EGF, was simultaneously observed in the treated samples, while others, such as FGF, appeared to be underexpressed compared with the controls. The intense staining of the papillary and reticular dermis for collagen III in the treated samples provided evidence that the wounds were still in the contraction and remodeling phase, as characterized by Schultz et al,[20] despite appearing macroscopically to be healed. This phase is characterized by a reduction in the number of fibroblasts, low levels of FGF, and a balance between production and lysis of collagen. In our study, the presence of abundant collagen III expression at the dermal level is evidence of papillary dermis repair, with an abundance of loose connective tissues that lie just beneath the epithelium and form part of the basal membrane. In contrast, low levels of collagen III in the papillary and reticular dermis are generally associated with early dense, type I collagen-rich, connective tissue deposition,

which provides resistance to the skin but decreases the capacity of epithelium regeneration.[21,22] This process of epithelial growth and collagen deposition/maturation is orchestrated by different and well-characterized growth factors, such as EGF and FGF. Fibroblast growth factor is one of the mediators of the dynamic process of healing produced by keratinocytes, fibroblasts, endothelial cells and smooth muscle cells, among others.[23] Authors describing in vitro and in vivo studies have reported that FGF can help reduce inflammation,[24,25] regulate the synthesis and deposition of extracellular matrix components, and promote the migration of fibroblasts. In our study, EGF expression was strong at day 13 in epidermal cells of samples treated with Phovia, with a continuous and morphologically well-structured epithelium covering the wound. Evidence to support the role of EGF/EGF receptor signaling in the inflammatory phase of wound healing has recently been increasing.[26] Ectogenic EGF can significantly reduce inflammation in vitro,[27,28] accelerate re-epithelialization, and increase tensile strength in wounds while regulating the correct deposition and maturation of collagen.[29] Topical administration of EGF has been found to promote epithelialization and accelerate healing of cutaneous wounds in clinical trials.[30]

Although the macroscopic appearance of treated and control wounds seemed similar in our study, their microscopic appearance differed. Inflammation was reduced in Phovia-treated samples according to histological parameters and as well as lower expression of TNF-α, a cytokine closely related to phlogosis.[31] The increased expression of endogenous decorin in the treated biopsies is another important indicator of improved wound healing process because it correlates with high production of collagen III but not collagen I.[31] The role of decorin in wound healing is supported by in vivo observations in which the administration of exogenous decorin prevented the extracellular matrix accumulation and fibrosis[32] attributed to TGF-β.[33] Conversely, the absence of decorin delays cutaneous wound healing and fibrovascular invasion; furthermore, lower decorin expression was observed in keloid scars compared with healthy skin.[34,35] In this context, strong neoangiogenesis, evidenced by higher levels of FVIII and TNF-α expression, in the Phovia-treated samples compared with controls did not reflect persistent inflammation. Instead, these findings were more likely linked to greater metabolic and proliferative activity of neoformed tissue, which was further supported by the greater mitotic activity of cells present in the treated area.[36] This enhanced proliferative capacity of epithelial and mesenchymal cells in Phovia-treated samples was consistent with the high number of cells that stained for Ki67, a nuclear protein associated with cellular proliferation and ribosomal RNA transcription.[37]

The results of our study provide evidence to support previously published studies in which the effects of PBM therapy on wounds were evaluated.[38,39] For example, PBM has been found to promote keratinocyte activity, turnover of fibroblasts and synthesis and maturation of collagen with better angiogenesis. Güngörmüş and Akyol[40] evaluated the inflammation and re-epithelialization of surgical wounds in diabetic rats undergoing PBM therapy. They proposed that the main mechanism leading to wound healing was the transfer of intracellular energy, leading to activation of adenosine diphosphate (ADP), transformed into adenosine triphosphate (ATP) in the mitochondria.[41] The ADP-ATP cycle provides chemical energy within the cells by inducing the fibroblasts' metabolism and collagen production. The fibroblasts produce the extracellular matrix and collagen. Therefore, during the regulation process of wound repair, greater and more regular collagen deposition improves the elasticity and quality of the wound.[41] Although tensile strength was not measured in the study reported here, previous authors have reported a link between enhanced collagen deposition and tensile strength.[42,43] The anti-inflammatory effects of PBM therapy have also been reported by Mittermayr et al,[44] who found that biological reactions caused by exposure to PBM result in the production of nitric oxide, a vasodilator, powerful painkiller and anti-inflammatory agent. In particular, the anti-inflammatory effects of PBM induce the modulation of the nuclear factor κ-B, which controls both proinflammatory and anti-inflammatory factors.[45]

Although this study provided interesting and significant results, a larger sample of wounds would have increased the statistical strength to the study and could help detect differences in the expression of TGF-β and FGF and macroscopic assessment scores. No conclusions can be formed regarding the effect of this technology on traumatic, chronic, or infected wounds, but the findings provide encouragement for additional research for these types of spontaneous wounds because they are commonly presented to the veterinary clinician and are often difficult to manage clinically.

In conclusion, treating uncomplicated surgical wounds with Phovia did not influence the macroscopic appearance but improved microscopic features and stimulated the release of cytokines promoting would healing. In the maturation phase, the sites treated with Phovia exhibited tissue growth and more complete repair tissues. These histological improvements, orchestrated by several growth factors, should create favorable conditions for the scarring process and may improve the strength of repair tissue. Such effects could reduce the risk of dehiscence, scar formation, keloids, and chronic inflammation. The authors therefore propose that Phovia may be a beneficial to manage incisional wounds, especially those at at risk for dealyed healing. Such wounds may include those

under tension; those close to areas subject to movement, tension or traction; and long surgical wound closures. The encouraging results reported here provide evidence to justify additional research to determine the clinical effects of Phovia on these wounds.

CONFLICT OF INTEREST

The authors report no conflicts of interest in this work.

ORCID

Angela Palumbo Piccionello https://orcid.org/0000-0003-4076-4729

REFERENCES

1. Carmo PenhavelI MV, Tavares Nascimento VH, Ribeiro Durães EF, Pirani Carneiro F, de Sousa JB. Effects of carbon dioxide therapy on the healing of acute skin wounds induced on the back of rats. *Acta Cir Bras.* 2013;28(5):334-339.
2. Feedar A. *Wound Healing Alternatives in Management*. 2nd ed. Philadelphia, PA: Davis; 1995.
3. Burke SF. *The Role of Extracellular Matrix in Development*. New York, NY: Liss; 1984.
4. Shao M, Hussain Z, Thu HE, et al. Emerging trends in therapeutic algorithm of chronic wound healers: recent advances in drug delivery systems, concepts-to-clinical application and future prospects. *Crit Rev Ther Drug Carrier Syst.* 2017;34(5):387-452.
5. Keen MA. Hyaluronic acid in dermatology. *Skinmed.* 2017;15(6):441-448.
6. Tambella AM, Attili AR, Dini F, et al. Autologous platelet gel to treat chronic decubital ulcers: a randomized, blind controlled clinical trial in dogs. *Vet Surg.* 2014;43(6):726-733.
7. Tapiwa CE. Clinical management of nonhealing wounds. *Nurs Stand.* 2018;32(29):48-63.
8. Gammel JE, Biskup JJ, Drum MG, Newkirk K, Lux CN. Effects of low-level laser therapy on the healing of surgically closed incisions and surgically created open wounds in dogs. *Vet Surg.* 2018;47:499-506.
9. Nikolis A, Grimard D, Pesant Y, Scapagnini G, Vezina D. A prospective case series evaluating the safety and efficacy of the Klox BioPhotonic System in venous leg ulcers. *Chron Wound Care Manag Res.* 2016;3:101-111.
10. Romanelli M, Piaggesi A, Scapagnini G, et al. EUREKA study—the evaluation of real-life use of a biophotonic system in chronic wound management: an interim analysis. *Drug Des Devel Ther.* 2017;11:3551-3558.
11. Marchegiani A, Cerquetella M, Laus F, et al. The Klox Biophotonic System, an innovative and integrated approach for the treatment of deep pyoderma in dogs: a preliminary report. In: Proceedings of the 29th ESVD-ECVD Annual Congress; September 7-9, 2017, Lausanne, Switzerland.
12. Tambella AM, Cerquetella M, Attili AR, et al. Klox Biophotonic System, a promising innovative approach to canine chronic otitis externa: preliminary report of a randomized controlled clinical trial. *Vet Surg.* 2017;46(6):E50-E51.
13. Wilson PR, Stirridge MF, Treasure T. A scoring method (ASEPSIS) for postoperative wound infections for use in clinical trials of antibiotic prophylaxis. *Lancet.* 1986;8:331-332.
14. Byrne DJ, Malek MM, Davey PG, Cuschieri A. Postoperative wound scoring. *Biomed Pharmacother.* 1989;43:669-673.
15. Gal P, Kilik R, Mokry M, et al. Simple method of open skin wound healing model in corticosteroid-treated and diabetic rats: standardization of semiquantitative and quantitative histological assessments. *Vet Med (Praha).* 2008;53:652-659.
16. Martin P. Wound healing—aiming for perfect skin regeneration. *Science.* 1997;276:75-81.
17. Diegelmann RF, Evans MC. Wound healing: an overview of acute, fibrotic and delayed healing. *Front Biosci.* 2004;9:283-289.
18. Lawrence WT, Diegelmann RF. Growth factors in wound healing. *Clin Dermatol.* 1994;12:157.
19. Kumar V, Cotran R, Robbins S. *Robbins Basic Pathology*. 7th ed. Philadelphia PA: Saunders; 2003.
20. Schultz GS, Davidson JM, Kirsner RS, Bornstein P, Herman IM. Dynamic reciprocity in the wound microenvironment. *Wound Repair Regen.* 2011;19(2):134-148.
21. Ross RS, Borg TK. Integrins and the myocardium. *Circ Res.* 2001;88(11):1112-1119.
22. Schwartz MA, Ginsberg MH. Networks and crosstalk: integrin signaling spreads. *Nat Cell Biol.* 2002;4:65-68.
23. Niiyama H, Kuroyanagi Y. Development of novel wound dressing composed of hyaluronic acid and collagen sponge containing epidermal growth factor and vitamin C derivative. *J Artif Organs.* 2014;17:81-87.
24. Parker HG, VonHoldt BM, Quignon P, et al. An expressed FGF4 retrogene is associated with breed-defining chondrodysplasia in domestic dogs. *Science.* 2014;325:995-998.
25. Wagner E, Levine M. FGF signaling establishes the anterior border of the *Ciona* neural tube. *Development.* 2012;139:2351-2359.
26. van der Plas MJ, van Dissel JT, Nibbering PH. Maggot secretions skew monocyte-macrophage differentiation away from a pro-inflammatory to a pro-angiogenic type. *PLoS One.* 2009;4:e8071.
27. Yurtçu M, Baba ZF. The effect of epidermal growth factor applied locally for pelviureteral anastomoses. *Int J Clin Exp Med.* 2014;7:4226-4423.
28. Fernández Lorente A, Acosta Brooks S, Neninger Vinageras E, et al. Effect of blockade of the EGF system on wound healing in patients vaccinated with CIMAvax EGF. *World J Surg Oncol.* 2013;11:275.
29. Carrier P, Deschambeault A, Talbot M, et al. Characterization of wound reepithelialization using a new human tissue-engineered corneal wound healing model. *Invest Ophthalmol Vis Sci.* 2008;49:1376-1385.
30. Hardwicke J, Schmaljohann D, Boyce D, Thomas D. Epidermal growth factor therapy and wound healing—past, present and future perspectives. *Surgeon.* 2008;6:172-177.
31. Iozzo RV. Matrix proteoglycans: from molecular design to cellular function. *Annu Rev Biochem.* 1998;67:609-652.
32. Border WA, Noble NA, Yamamoto T, et al. Natural inhibitor of transforming growth factor-beta protects against scarring in experimental kidney disease. *Nature.* 1992;360:361-364.
33. Isaka Y, Brees DK, Ikegaya K, et al. Gene therapy by skeletal muscle expression of decorin prevents fibrotic disease in rat kidney. *Nat Med.* 1996;2:418-423.
34. Järveläinen H, Puolakkainen P, Pakkanen S, et al. A role for decorin in cutaneous wound healing and angiogenesis. *Wound Rep Reg.* 2006;14:443-452.

35. Jumper N, Paus R, Bayat A. Functional histopathology of keloid disease. *Histol Histopathol.* 2015;30(9):1033-1057.
36. David CJ, Huang YH, Chen M, et al. TGF-β tumor suppression through a lethal EMT. *Cell.* 2016;164(5):1015-1030.
37. Bullwinkel J, Baron-Lühr B, Lüdemann A, Wohlenberg C, Gerdes J, Scholzen TJ. Ki-67 protein is associated with ribosomal RNA transcription in quiescent and proliferating cells. *Cell Physiol.* 2006;206(3):624-635.
38. Fernandes KP, Souza NH, Mesquita-Ferrari RA, et al. Photobiomodulation with 660-nm and 780-nm laser on activated j774 macrophage-like cells: effect on m1 inflammatory markers. *J Photochem Photobiol B.* 2015;153:344-351.
39. Sperandio FF, Simoes A, Correa L, et al. Low-level laser irradiation promotes the proliferation and maturation of keratinocytes during epithelial wound repair. *J Biophotonics.* 2015;8(10):795-803.
40. Güngörmüş M, Akyol UK. Effect of biostimulation on wound healing in diabetic rats. *Photomed Laser Surg.* 2009;27(4):607-610.
41. Avci P, Gupta A, Sadasivam M, et al. Low-level laser (light) therapy (LLLT) in skin: stimulating, healing, restoring. *Semin Cutan Med Surg.* 2013;32:41-52.
42. Baneriee P, Suguna L, Shanthi C. Wound healing activity of a collagen-derived cryptic peptide. *Amino Acids.* 2015;47(2):317-328.
43. Chang PJ, Chen MY, Huang YS, et al. Morphine enhance tissue content of collagen and increases wound tensile strength. *J Anesth.* 2010;24(2):240-246.
44. Mittermayr R, Osipov A, Piskernik C, et al. Blue laser light increases perfusion of a skin flap via release of nitric oxide from hemoglobin. *Mol Med.* 2007;13:22-29.
45. Chen AC, Arany PR, Huang YY, et al. Low-level laser therapy activates NF-kB via generation of reactive oxygen species in mouse embryonic fibroblasts. *PLoS One.* 2011;6(7):e22453.

How to cite this article: Salvaggio A, Magi GE, Rossi G, et al. Effect of the topical Klox fluorescence biomodulation system on the healing of canine surgical wounds. *Veterinary Surgery*. 2020;49:719–727. https://doi.org/10.1111/vsu.13415