Scopus

Documents

Rossi, F.[a] , Cicchi, R.[b c] , Tatini, F.[c] , Bacci, S.[d] , Alfieri, D.[e] , De Siena, G.[a] , Pavone, F.S.[b c f] , Pini, R.[a]

Healing process study in murine skin superficial wounds treated with the blue LED photocoagulator "EMOLED"
(2014) *Optics InfoBase Conference Papers*, 6 p. Cited 1 time.

DOI: 10.1117/12.2183670

[a] Institute of Applied Physics Nello Carrara, National Research Council, Via Madonna del Piano 10, Sesto Fiorentino, I-50019, Italy
[b] National Institute of Optics, National Research Council, Largo E. Fermi 6, Florence, I-50125, Italy
[c] European Laboratory for Non-linear Spectroscopy (LENS), Via Nello Carrara 1, Sesto Fiorentino, I-50019, Italy
[d] Department of Clinical and Experimental Medicine, Research Unit of Histology and Embriology, Viale Pieraccini 6, Florence, I-50139, Italy
[e] Light4Tech Firenze S.r.L., Via Pisana 316, Scandicci (FI), I-50018, Italy
[f] Department of Physics, University of Florence, Via G. Sansone 1, Sesto Fiorentino, I-50019, Italy

Abstract
A faster healing process was observed in superficial skin wounds after irradiation with the EMOLED photocoagulator. The instrument consists of a compact handheld photocoagulation device, useful for inducing coagulation in superficial abrasions. In this work we present the results of an in vivo study, in a murine model. Two superficial wounds were produced on the back of 12 mice: one area was left untreated, the other one was treated with EMOLED. Healthy skin was used as a control. The animals were sacrificed 3 hours, 12 hours, 1 day, 6 day after treatment. The treatment effects on back skin was monitored by visual observations, histopathological analysis, immuno-histochemical analysis, and nonlinear microscopic imaging performed at each follow up time, finding no adverse reactions and no thermal damage in both treated areas and surrounding tissues. In addition, a faster healing process, a reduced inflammatory response, a higher collagen content, and a better-recovered skin morphology was evidenced in the treated tissue with respect to the untreated tissue. These morphological features were characterized by means of immuno-histochemical analysis, aimed at imaging fibroblasts and myofibroblasts, and by SHG microscopy, aimed at characterizing collagen organization, demonstrating a fully recovered aspect of dermis as well as a faster neocollagenesis in the treated regions. This study demonstrates that the selective photothermal effect we used for inducing immediate coagulation in superficial wounds is associated to a minimal inflammatory response, which provides reduced recovery times and improved healing process. © 2015 SPIE-OSA.

Index Keywords
Cell culture, Coagulation, Collagen, Fibroblasts, Recovery; Collagen organizations, Histochemical analysis, Histopathological analysis, Inflammatory response, Microscopic imaging, Morphological features, Photothermal effects, Visual observations; Tissue

Correspondence Address
Rossi F.; Institute of Applied Physics Nello Carrara, Via Madonna del Piano 10, Italy; email: f.rossi@ifac.cnr.it

Publisher: OSA - The Optical Society

ISBN: 9781628417012
Language of Original Document: English
Abbreviated Source Title: Opt. InfoBase Conf. Pap
2-s2.0-85019517495
Document Type: Conference Paper
Publication Stage: Final
Source: Scopus

Healing process study in murine skin superficial wounds treated with the blue LED photocoagulator "EMOLED"

Francesca Rossi[a*], Riccardo Cicchi[b,c], Francesca Tatini[c], Stefano Bacci[d], Domenico Alfieri,[e] Gaetano De Siena[a], Francesco S. Pavone[b,c,f] and Roberto Pini[a]

[a] Institute of Applied Physics "Nello Carrara", National Research Council, Via Madonna del Piano 10, I-50019, Sesto Fiorentino, Italy
[b] National Institute of Optics, National Research Council, Largo E. Fermi 6, I-50125, Florence, Italy
[c] European Laboratory for Non-linear Spectroscopy (LENS), Via Nello Carrara 1, I-50019, Sesto Fiorentino, Italy
[d] Department of Clinical and Experimental Medicine, Research Unit of Histology and Embriology, Viale Pieraccini 6, I-50139, Florence, Italy
[e] Light4Tech Firenze S.r.L., Via Pisana 316, I-50018 Scandicci (FI) Italy
[f] Department of Physics, University of Florence, Via G. Sansone 1, I-50019, Sesto Fiorentino, Italy

ABSTRACT

A faster healing process was observed in superficial skin wounds after irradiation with the EMOLED photocoagulator. The instrument consists of a compact handheld photocoagulation device, useful for inducing coagulation in superficial abrasions. In this work we present the results of an in vivo study, in a murine model. Two superficial wounds were produced on the back of 12 mice: one area was left untreated, the other one was treated with EMOLED. Healthy skin was used as a control. The animals were sacrificed 3 hours, 12 hours, 1 day, 6 day after treatment. The treatment effects on back skin was monitored by visual observations, histopathological analysis, immuno-histochemical analysis, and non-linear microscopic imaging performed at each follow up time, finding no adverse reactions and no thermal damage in both treated areas and surrounding tissues. In addition, a faster healing process, a reduced inflammatory response, a higher collagen content, and a better-recovered skin morphology was evidenced in the treated tissue with respect to the untreated tissue. These morphological features were characterized by means of immuno-histochemical analysis, aimed at imaging fibroblasts and myofibroblasts, and by SHG microscopy, aimed at characterizing collagen organization, demonstrating a fully recovered aspect of dermis as well as a faster neocollagenesis in the treated regions. This study demonstrates that the selective photothermal effect we used for inducing immediate coagulation in superficial wounds is associated to a minimal inflammatory response, which provides reduced recovery times and improved healing process.

1. INTRODUCTION

EMOLED is a blue LED photocoagulator that was developed to induce immediate coagulation in superficial skin wounds [1]. Blue LED technology offer the potential of a low-cost simple source for targeting particular biological molecules. For example, when considering the absorption coefficient of skin chromophores in the UV-VIS-NIR spectrum (i.e. from 300 nm to 900 nm), hemoglobin exhibits narrow absorption peaks in the blue range (410 nm and 430 nm for oxygenated and non-oxygenated hemoglobin respectively), with an absorption coefficient much higher than other skin chromophores [2- 4]. This particular property of hemoglobin can be used to ensure local temperature increase able to induce hemostasis through a photo-thermo-coagulation process. When irradiating a bleeding wound with the blue LED of proper wavelength, the light is mainly absorbed by hemoglobin naturally present in these areas, causing a local temperature increase within blood. The use of proper irradiation settings in terms of fluence, irradiation time, target spot area and emission pattern allows locally inducing a temperature above the threshold for protein denaturation within blood, resulting in a fast coagulation effect [5]. The hemostasis is thus realized through a light-induced coagulation process, ensured by a spectrally selective and spatially localized photo-thermal effect.

*f.rossi@ifac.cnr.it; +390555225337; bnlab.ifac.cnr.it

Medical Laser Applications and Laser-Tissue Interactions VII, edited by Lothar D. Lilge, Ronald Sroka, Proc. of SPIE-OSA Biomedical Optics, SPIE Vol. 9542, 95420F · © 2015 SPIE-OSA · CCC code: 1605-7422/15/$18 · doi: 10.1117/12.2183670

In this study, we employed a blue-LED photocoagulator device, named "EMOLED", that enables an immediate stop of the bleeding in superficial wounds (e.g. skin abrasions, oral mucosa bleeding, etc.) [1] for treating mechanical abrasions produced on the back of 12 albino mice. Half of the induced wounds were treated with the blue-LED device, while the others were left to naturally recover. The photothermal coagulation effect was monitored during treatment by the use of a thermocamera. Visual observations, non-linear microscopic imaging, histological and histochemical analyses were used to study the healing process 3 hours, 12 hours, 1 day, 6 day after surgery in treated and not-treated wounds.

2. MATERIALS AND METHODS

2.1 The Blue-LED photocoagulator "EMOLED"

The EMOLED is based on the use of two commercially LEDs, emitting at 410 nm and 435 nm, having in total 1 W emission power. The device is a fiber-coupled one (see figure 1). The illuminated area corresponded to a circle with 5 mm radius. The resulting power density was 1.27 W/cm^2. The intensity distribution of the light at the tissue surface was homogeneous, as from a top hat intensity source. During the treatment, the photo-hemostatic optical fiber was kept in motion on the wound, inducing a homogeneous thermal effect over the entire wound. The optimized treatment time was 25 seconds for each wound.

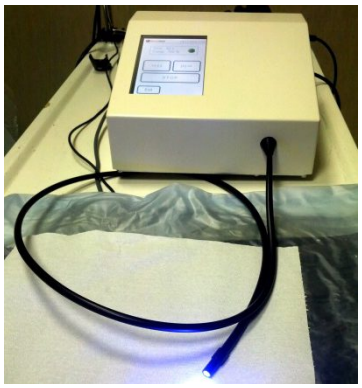

Figure 1: the Blue LED photocoagulator device, "EMOLED".

2.2 Animal model

In this study, 12 adult male mice (Harlan Italy, weighing 15-18 g each) were used. All experiments respected the guidelines of the European Community for the care of the animals (86/609 / EEC). Formal approval to conduct the experiments has been obtained from the animal subjects review board of the University of Florence. The animals were anesthetized before treatment. After anesthesia, hair removal from the back was performed and 2 superficial wounds were produced in each animal. One wound was treated with the photo-hemostatic device; the other one was left to heal without any treatment. During the treatment, the animals were kept at a constant temperature (37.5°C) to reduce stress due to anesthesia. Immediately after treatment, the wound was carried out a topical application of a small amount of Streptosil (Boehringer Ingelheim Italia spa, Milan, Italy), so as to prevent the onset of infections. During follow up, the animals were housed in regulation cages in a controlled lighting (12/12 light and dark) and temperature facility and had free access to water and standard rodent diet ad libitum. After 3 hours, 12 hours, 1 day, 6 day of follow up, the animals were sacrificed by humane methods and from each animal were harvested samples from the two wounds. The biopsies were preserved by the immediate freezing at a temperature of -80 ° C; subsequently were cut by use of the cryostat, the sections were post-fixed in cold acetone and the obtained slides stored at -20 °C.

2.3 IR thermocamera observations

Direct observations of the induced superficial temperature were performed during treatment, using an infrared (IR) thermocamera (ThermoVision A20, FLIR Systems Inc., Wilsonville, OR, USA). The temporal and spatial evolution of the heating in a non-contact configuration was accurately measured.

2.4 Multiphoton Microscopy setup, image acquisition and analysis

In this study, a multiphoton microscopy setup was used for Two Photon Fluorescence (TPF) and Second-Harmonic Generation (SHG) microscopy of thin tissue cross-sections. The experimental setup consisted of a custom-built microscope, made by a compact microscopic head realized in anodized aluminum alloy that hosts the optical component. A detailed description of the experimental setup has been provided in [6].

Both TPF and SHG images were recorded with a pixel dwell time of 20 micros, a field of view of 400 × 400 µm2 and a resolution of 512 × 512 pixels, using an excitation wavelength of 840 nm. Combined TPF-SHG images were presented by coding fluorescence in green and SHG in blue. FFT analysis was performed by taking SHG images of dermal collagen and selecting three different ROI sizes (128, 256, and 512 pixels), respectively corresponding to a ROI size of 100 micrometer, 200 micrometer, and 400 micrometer. The FFT of each selected ROI was calculated using an ImageJ (NIH, Bethesda, US) [7, 8] built-in routine. Collagen anisotropy was then evaluated by means of the aspect ratio of the FFT profile. This parameter, corresponding to the minor to major axis ratio of the elliptic FFT profile was calculated by considering the ratio of the two eigenvalues of the covariance matrix, as described in [9]. The calculation was performed at various ROI size in order to identify the scale level at which a particular organization of collagen fibers and fiber bundles is lost [10]. Statistical t-tests were done on the calculated FFT aspect ratio values at the 0.05 level using Microcal Origin Pro9 software (OriginLab Corporation, Northampton, US). GLCM matrices were calculated from SHG images according to [11] using a Matlab (Mathworks, Natick, US) routine for a neighbor index ranging from 1 to 100. GLCM Correlation was then calculated and displayed versus distance in order to provide useful information on periodic structures or on the sudden change or regularity of a linear structure, such as breakage of a fibrillar structure [12, 13].

2.5 Histology and immunofluorescence analysis

For histological evaluations, specimens were embedded in freezing tissue medium (Killik; BioOptica, Milan, Italy), cryosections were post fixed in cold acetone. Sections from each case (one section per staining) were stained with haematoxilin and eosin or labeled with the following antigens: Heat Shock Protein (Hsp) 47 for fibroblasts [14] (Hsp47+; Abcam, Cambridge, UK), alpha Smooth Muscle Actin (SmA) for myo-fibroblasts [15] (Acta2+; Sigma, Milan, Italy) by using monoclonal antibodies diluted 1:50, 1:100 respectively. Appropriate fluorescein isothiocyanate labeled polyclonal antibodies from rabbit or mouse (1:32; Sigma, Milan, Italy) were used as secondary ones. Primary antibodies were applied overnight at 4°C, secondary ones for 2 h at 37°C. Control samples from healthy back rat skin were also used for the histological and fibroblast-targeted immunofluorescence examination.

3. RESULTS

3.1 Direct observation during treatment

Thermal analysis during treatment evidenced that there is an immediate temperature rise in the irradiated bleeding area (see Figure 2): a maximum temperature of 49.4±2.6°C is reached in the first 5 seconds and it is maintained almost constant for 20 s. No thermal damage to surrounding intact tissues are observed. No collateral effects are evidenced during treatment.

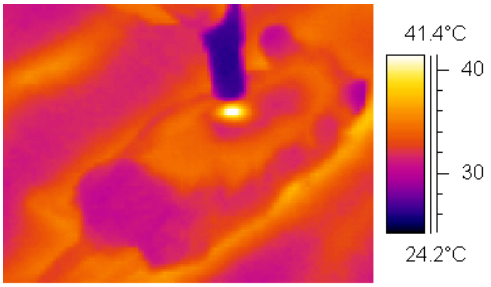

Figure 2: Thermal image of EMOLED treatment, showing the temperature rise in the superficial wound of a mouse back.

3.3 Combined TPF-SHG imaging

TPF and SHG images are presented by coding fluorescence in green and SHG in blue, as shown in the example represented in Figure 3. The difference in terms of dermal morphology is clearly distinguishable from the images. In the NTW the inflammatory response is still ongoing, as demonstrated by the large presence of vessels and cells in the dermis. In both NTW and TW, the epidermal morphology is fully recovered at 6 days follow up. However, in the NTW area there is still the presence of crusts, as resulted by the bright signal in some regions beneath the epidermal surface. Collagen orientation is quite different as well, with TW showing a more isotropic distribution of collagen fiber bundles with several intersections in comparison to NTW where intersections are not clearly identifiable yet. Similar results were found at each follow up times.

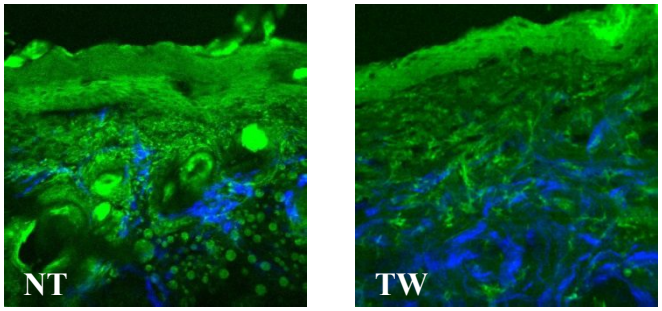

Figure 3. TPF (green) and SHG (blue) images of treated (right) and not treated (left) wounds, 6 days after treatment.

3.4 Histology and Immunofluorescence examination

In order to evaluate the extent of the inflammatory reaction in untreated and treated wounds, the hematoxylin-eosin staining was performed. The light microscopy evaluation of stained section of dermis in NTW showed a rich cellular infiltrate. In comparison with NTW, sections of TW tissue showed, by examination of grading scale, a significant decrease of inflammatory cellular infiltration. The level of inflammatory cellular infiltration in TW was found closer to that one found in a control sample at 6 follow up days, than that of NTW.

To determine whether the population of the cellular types involved in wound healing were different in NTW and TW, light microscopy and immunofluorescence analysis were performed. The presence of fibroblasts and myofibroblasts was assessed.

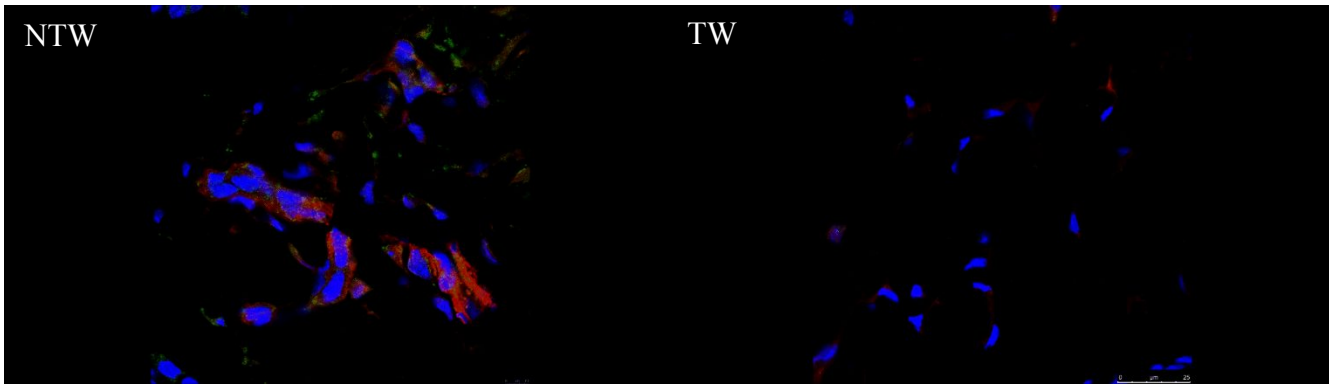

Figure 4 Confocal microscopies of not treated (left) and treated (right) wounds, 6 days p.o.

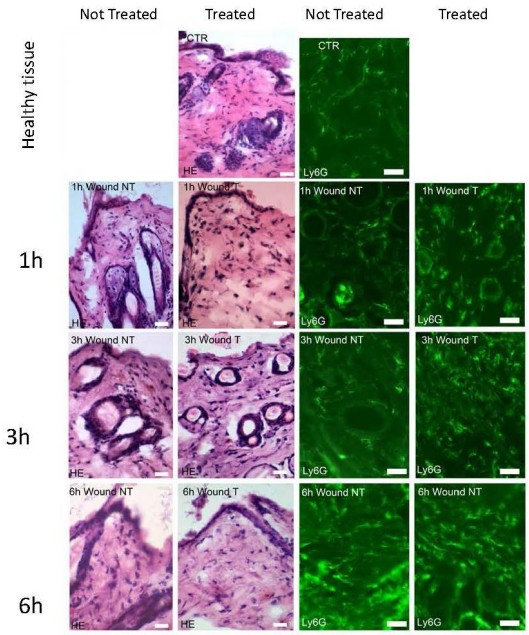

Figure 5 Histologies (left) and Immunofluorescence images (right) of not treated and treated wound, soon after treatment. Comparison is performed with healthy tissue (top).

4. CONCLUSIONS

In conclusion, a faster wound healing process was found in treated abrasions with respect to the control wounds 3 hours, 12 hours, 1 day, 6 day after treatment. Visual observations, non-linear microscopic imaging, and histological/histochemical analyses demonstrated that the treated wounds showed a higher collagen content, a better recovered morphology and organization of dermal collagen, minimal inflammatory response, no adverse reaction neither thermal damage in both wound areas and surrounding tissue. All the results obtained from these analyses constitute a strong support to the hypothesis that the selective photo-thermal effect used for inducing hemostasis in superficial skin wounds is associated to a minimal inflammatory response and to a faster healing process.

5. ACKNOWLEDGMENTS

The research leading to these results has received funding from Tuscany Region and EU FP7 BiophotonicsPlus projects “LighTPatcH” (Led Technology in Photo Haemostasis) and “LITE” (Laser Imaging of The Eye), from the European Union Seventh Framework Programme (FP7/2007–2013) under grant agreement number 284464, from the Italian Ministry for Education, University and Research in the framework of the Flagship Project NANOMAX, from Fondazione Pisa, and from Ente Cassa di Risparmio di Firenze.

REFERENCES

1. F. Rossi, R. Pini, G. De Siena, D. Massi, F. S. Pavone, D. Alfieri, and G. Cannarozzo, "A blue-LED-based device for selective photocoagulation of superficial abrasions: Theoretical modeling and in vivo validation," in *Progress in Biomedical Optics and Imaging - Proceedings of SPIE*, 2010),
2. M. J. C. Van Gemert, S. L. Jacques, H. J. C. M. Sterenborg, and W. M. Star, "Skin Optics," IEEE Trans. Biomed. Eng. **36**, 1146-1154 (1989).
3. J. Mobely and T. Vo-Dinh, *Optical properties of Tissue* (CRC Press, Boca Raton, 2003), Vol. Biomedical Photonics Handbook.
4. A. N. Bashkatov, E. A. Genina, V. I. Kochubev, and V. V. Tuchin, "Optical properties of human skin, subcutaneos and mucous tissues in the wavelength range from 400 to 2000 nm," J. Phys. D: Appl. Phys. **38**, 2543-2555 (2005).
5. M. H. Niemz, *Laser-Tissue Interactions* (Springer Verlag, Berlin, 1996).
6. R. Cicchi, D. Kapsokalyvas, and F. S. Pavone, "Clinical Nonlinear Laser Imaging of Human Skin: A Review," BioMed research international **2014**, 903589 (2014).
7. W. S. Rasband, ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA **1997-2012**(http://imagej.nih.gov/ij/).

8. C. A. Schneider, W. S. Rasband, and K. W. Eliceiri, "NIH Image to ImageJ: 25 years of image analysis," Nat. Methods **9**, 671-675 (2012).
9. R. Cicchi, D. Kapsokalyvas, V. De Giorgi, V. Maio, A. Van Wiechen, D. Massi, T. Lotti, and F. S. Pavone, "Scoring of collagen organization in healthy and diseased human dermis by multiphoton microscopy," Journal of biophotonics **3**, 34-43 (2010).
10. R. Cicchi, N. Vogler, D. Kapsokalyvas, B. Dietzek, J. Popp, and F. S. Pavone, "From molecular structure to tissue architecture: collagen organization probed by SHG microscopy," Journal of biophotonics **6**, 129-142 (2013).
11. M. Hall-Beyer, "The GLCM Tutorial Homepage," (2007) (http://www.fp.ucalgary.ca/mhallbey/tutorial.htm).
12. R. Cicchi, D. Kapsokalyvas, M. Troiano, P. Campolmi, C. Morini, D. Massi, G. Cannarozzo, T. Lotti, and F. S. Pavone, "In vivo non-invasive monitoring of collagen remodelling by two-photon microscopy after micro-ablative fractional laser resurfacing," Journal of biophotonics (2013).
13. R. Cicchi, C. Matthaus, T. Meyer, A. Lattermann, B. Dietzek, B. R. Brehm, J. Popp, and F. S. Pavone, "Characterization of collagen and cholesterol deposition in atherosclerotic arterial tissue using non-linear microscopy," Journal of biophotonics **7**, 135-143 (2014).
14. T. Goodpaster, A. Legesse-Miller, M. R. Hameed, S. C. Aisner, J. Randolph-Habecker, and H. A. Coller, "An immunohistochemical method for identifying fibroblasts in formalin-fixed, paraffin-embedded tissue," Journal of Histochemistry and Cytochemistry **56**, 347-358 (2008).
15. G. Gabbiani, "The myofibroblast in wound healing and fibrocontractive diseases," Journal of Pathology **200**, 500-503 (2003).
16. S. Bacci, B. De Fraia, L. Cinci, L. Calosi, D. Guasti, L. Pieri, V. Lotti, A. Bonelli, and P. Romagnoli, "Immunohistochemical analysis of dendritic cells in skin lesions: correlations with survival time," Forensic Sci Int **244**, 179-185 (2014).