# Thermographic analysis of photodynamic therapy with intense pulsed light and needle-free injection photosensitizer delivery: an animal study

Michelle B. Requena, Mirian D. Stringasci, Sebastião Pratavieira, José Dirceu Vollet-Filho, Andrigo B. de Nardi, et al.

Michelle B. Requena, Mirian D. Stringasci, Sebastião Pratavieira, José Dirceu Vollet-Filho, Andrigo B. de Nardi, Andre Escobar, Rozana W. da Rocha, Vanderlei S. Bagnato, Priscila F. C. de Menezes, "Thermographic analysis of photodynamic therapy with intense pulsed light and needle-free injection photosensitizer delivery: an animal study ," Proc. SPIE 10489, Optical Biopsy XVI: Toward Real-Time Spectroscopic Imaging and Diagnosis, 104891F (21 February 2018); doi: 10.1117/12.2300407

SPIE.

Event: SPIE BiOS, 2018, San Francisco, California, United States

# Thermographic analysis of photodynamic therapy with intense pulsed light and needle-free injection photosensitizer delivery: an animal study

Michelle B. Requena*[a], Mirian D. Stringasci[a], Sebastião Pratavieira[a], José Dirceu Vollet-Filho[a], Andrigo B. de Nardi[b], Andre Escobar[b], Rozana W. da Rocha[b], Vanderlei S. Bagnato[a], Priscila F. C. de Menezes[a]

[a] São Carlos Institute of Physics, University of São Paulo, São Carlos, SP, Brazil;
[b] College of Agricultural and Veterinary Sciences, São Paulo State University, Jaboticabal, São Paulo, Brazil

## ABSTRACT

The photodynamic therapy (PDT) is a therapeutic modality that depends mostly on photosensitizer (PS), light and molecular oxygen species. However, there are still technical limitations in clinical PDT that are under constant development, particularly concerning PS and light delivery. Intense Pulsed Light (IPL) sources are systems able to generate pulses of high energy with polychromatic light. IPL is a technique mainly used in the cosmetic area to perform various skin treatments for therapeutic and aesthetic applications. The goals of this study were to determine temperature variance during the application of IPL in porcine skin model, and the PDT effects using this light source with PS delivery by a commercial high pressure, needle-free injection system. The PSs tested were Indocyanine Green (ICG) and Photodithazine (PDZ), and the results showed an increase bellow 10 °C in the skin surface using a thermographic camera to measure. In conclusion, our preliminary study demonstrated that IPL associated with needle-free injection PS delivery could be a promising alternative to PDT.

**Keywords:** Thermographic Analysis, Needle-Free Injection, Photodynamic Therapy, Intense Pulsed Light, Photodithazine, Indocyanine Green.

*requenamichelle@gmail.com; http://cepof.ifsc.usp.br/

## INTRODUCTION

Photodynamic therapy (PDT) comprises a series of photochemical processes involving mainly light, phototosentizer (PS) and molecular oxygen. The PS is a molecule that absorbs the light and transfers part of the energy to oxygen, promoting the cell death. PDT can be used as a treatment option for a number of modalities of cancer[1] and when applied to kill bacteria or fungi is called Photodynamic Inactivation (PDI) or antimicrobial PDT (aPDT).[2]

For clinical PDT applications, the PS is usually either applied topically or intravenously injected, and it takes time to accumulate in the tissue. The primary clinical use nowadays is for the non-melanoma skin lesions treatment with topical application of PS precursors.[3,4] One of the main problems related to the PS in the lesion is its limited penetration that makes the treatment of thick tumors difficult. To deal with this, an intradermal application with a needle-free injection system[5] was already explored for PS precursors delivery.[6,7] In this study, we use needle-free injection for delivery of Photodithazine and Indocyanine green (ICG) into the skin to be used as phototosentizers.

Regarding the light source, PDT can be performed with laser or LED, depending on the application.[8] To get more light penetration in the lesion, the red-infrared excitation is usually a better option.[9]

IPL is a light source mainly used in the cosmetic area to perform skin treatments for therapeutic and aesthetic applications, with relevant results as a light source in PDT for actinic keratosis. [10,11] Although Photodithazine (PDZ) and Indocyanine green (ICG) are PSs already used in several PDT procedures, these PS are mainly used with monochromatic light sources.[12] To promote a multichromatic excitation including the red-infrared region, the intense pulsed light (IPL) can be an interesting alternative. Using IPL to activate PDZ can be a good option because we can activate the several visible absorptions bands, and makes the process more efficient. ICG has absorption in the near-infrared region, and IPL can also be a useful light source for its activation as well.[13]

A thermographic camera can detect the infrared radiation emitted from the body surface (wavelengths between 7 and 14 μm) and converts to temperature unit.[14] In the thermographic images, each pixel corresponds to a temperature represented in color scale. The thermography is a tool that can be used to measure the temperature at the body surface

Optical Biopsy XVI: Toward Real-Time Spectroscopic Imaging and Diagnosis, edited by Robert R. Alfano, Stavros G. Demos
Proc. of SPIE Vol. 10489, 104891F · © 2018 SPIE · CCC code: 0277-786X/18/$18 · doi: 10.1117/12.2300407

and has the advantage to be a noninvasive technique and entirely safe for the patient since it only detects the infrared radiation naturally emitted by the body.[14,15]

The light irradiation in the biological tissue can increase the temperature since the light can be absorbed by its molecules and the energy absorbed can be converted into heat.[16] Also, the tissue response to PDT can change the local temperature, due to factors such as healing and inflammation processes able to modify the vascular system functioning within the region.[17] Tissue heating is a significant concern because a cellular damage can be caused if the cells were exposed to temperatures above 44 °C.[18]

Aiming for a new protocol for PDT, we evaluated both thermal and PDT effects using a multichromatic light source of IPL, with PDZ and ICG in porcine skin, since this model has optical properties similar to those of human skin.[19,20]

## MATERIAL AND METHODS

### Animal model

The experiments had the Animal Ethics Committee approval (nº 019546/13) and were conducted at College of Agricultural and Veterinary Sciences (FCAV) at São Paulo State University in Jaboticabal, São Paulo, Brazil. We used three two-month-old pigs with 20 kg each, maintained with food and water *ad libitum*. During the experiments, the animals were kept under general anesthesia with inhalational anesthetic (isofluorane with oxygen), and after the procedures, the animal dorsum was protected from light, and dipyrone and methadone were applied intramuscularly three times per day for 48 hours. Two of the pigs had the euthanasia 48 hours after PDT and one after 24 hours.

### Photosensitizers

Photodithazine (PDZ) from VETA-GRAND (Moscow, Russia) at 1 mg/mL concentration and Indocyanine Green (ICG) at 0.15 mg/mL from OPHTHALMOS (Sao Paulo, Brazil) were used as photosensitizers. Groups were administered each PS alone or as a mixture of both solutions (PDZ+ICG), with the purpose to investigate the simultaneous PS excitation both in the red (approximately 665 nm) and in the near-infrared (approximately 800 nm) absorption bands aiming for the increase in PDT efficacy in greater depths, was also used. The injection volume for PDZ+ICG sample was prepared with 50% PDZ solution 50% ICG solution, in volume. The spectra of normalized solutions absorbance and IPL light source emission are presented in Figure 1.

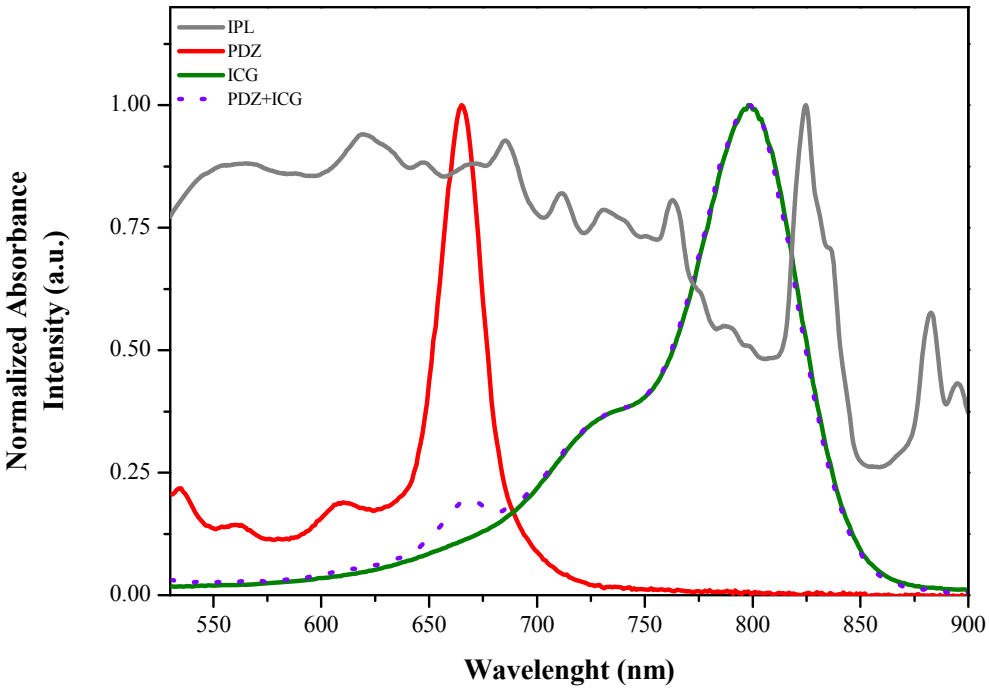



Figure 1 – Normalized Photosensitizers absorbance spectra (PDZ, ICG and, PDZ+ICG) and normalized IPL emission spectrum (IPL).

**Intradermal application**

For the drug delivery, we used a needle-free injection system (INJEX PHARMA AG. SAFE-INJECT, Germany) that allow an application by an orifice with 0.17 mm in diameter, in contrast to conventional injections in which the needle diameter is around 0.39 mm. The solution volume injected was 0.1 mL, taking care to always place the injection in the tissue with the same angulation (syringe perpendicular to the skin) and to perform the same pressure on the skin.

**Light Source**

We used a commercial system of Intense Pulsed Light (HKS801 Intense Pulse Light, Ningbo Ruipu Medical Equipment Co., Ltd., China) with a high pass filter (560-1200 nm) to promote the excitation in longer wavelengths, aiming increased light penetration into the skin, and the excitation of the higher absorptions bands in both PSs (at 660 nm and 780 nm for PDZ and ICG, respectively). Each "flash" was set to have 15 light pulses with 30 milliseconds width, 1 milliseconds of delay, 4 seconds of release and 50 J of energy. An automatic optical power and energy meter specific for IPL devices was used for calibration (Fit IPL®, LaserPoint, Italy).

**Photodynamic Therapy**

As PDT protocol, we applied using 15 flashes, with 10 seconds interval among them adjusted to provide 5.7 J/cm² fluence per flash (85.5 J/cm² of total fluence), with the device positioned at 10 mm from the skin with a handmade celeron coupler and aluminum masks with 8 mm of diameter to delimit the irradiation area. The drug-light interval (DLI), the time between the PS application and the irradiation, was 90 minutes.[21]

**Thermographic analysis**

The thermographic camera used in the experiments was FLK-Ti400 model (Fluke®, Everett, USA), with a sensitivity lower than 0.05 °C at 30° C, ± 2 °C accuracy and 320 x 240 pixels of resolution. The thermal imager allows registering the visible light and infrared images simultaneously. This device also has manual and automatic focus options, and an image acquisition is demonstrated in Figure 2.

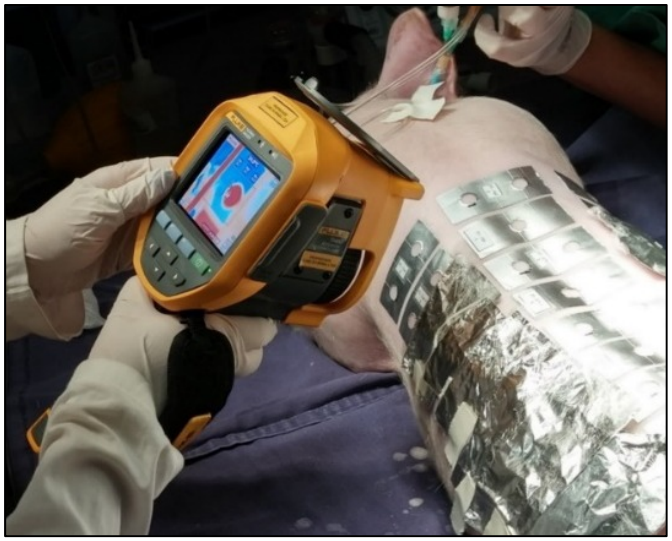


Figure 2 – Thermographic image acquisition with FLK-Ti400 model (Fluke®, Everett, USA) demonstration.

The thermal monitoring of treated areas was performed to verify if irradiation could produce enough heating to cause cell damage. The heating monitoring was performed in two situations: after each flash at the non-sensitized skin, also before and after the PDT at the skin with PS, checking the temperature differences. The room temperature was maintained at 24 °C. A black paper mask was positioned on top of the aluminum mask during the measurements to avoid the infrared radiation reflected in the aluminum to interfere in the images collection. SmartView® software (Fluke, USA) allowed saving infrared, white light images and the matrix reporting the temperature value for each pixel of the image. These data were analyzed by an algorithm written in MATLAB® (The MathWorks, USA), in which the mean temperature and standard deviation were calculated after a manual delimitation of the region of interest.

## RESULTS AND DISCUSSION

Figure 3 shows examples the thermographic images recorded after each IPL flash (15 flashes total per region) in a non-sensitized region. Through these images, it is possible to evaluate the skin heating caused only by the light. The color scale (from 32 to 40 ºC) positioned on the left side has the red color to represent the higher temperature values, and the blue represents lower temperatures. The IPL source of light has emission in the infrared region up to 1200 nm, which can promote the molecules agitation and consequent increase in temperature. In fact, it is possible to observe this temperature increase in the region after the irradiation with a sequence of flashes.

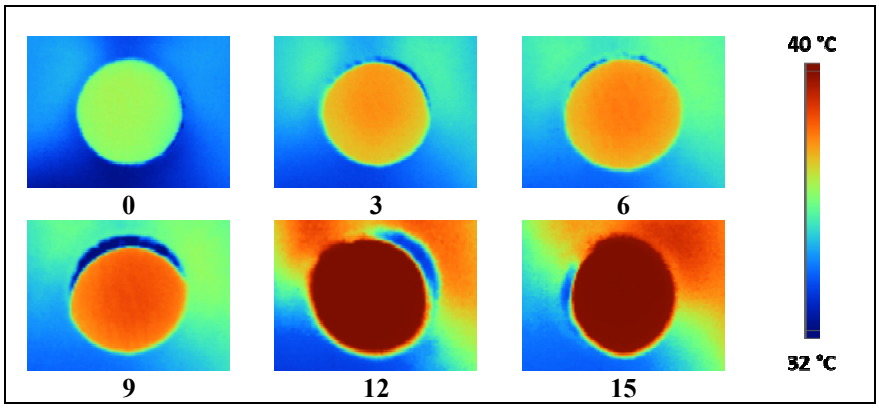
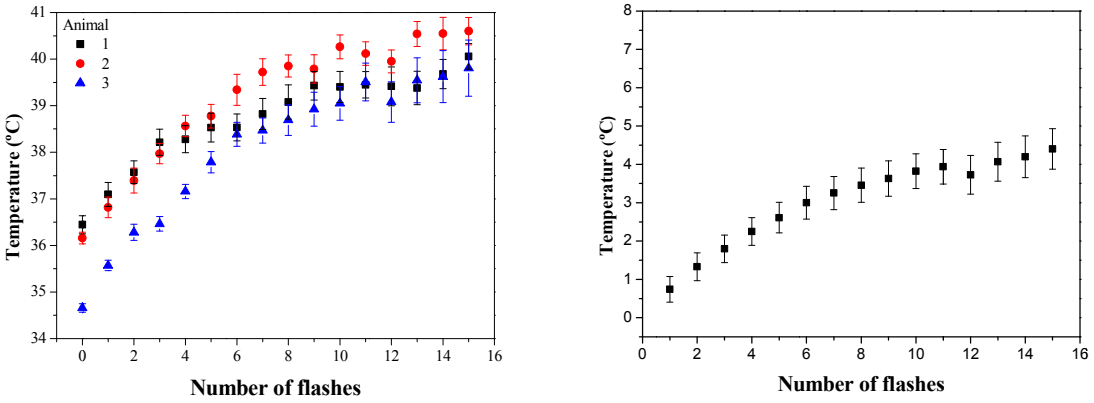



Figure 3 - Thermographic images with 8 mm of diameter recorded before irradiation, after 3, 6, 9, 12 and 15 IPL flashes. The color scale is positioned on the left side and the red color to represent the higher temperature values, and the blue represents lower temperatures.

Figure 2A shows the temperature of each animal throughout the IPL flashes delivery. The error bars were obtained from the propagation of errors related to the standard deviation of the temperature values in the images before and after the flashes irradiations. The irradiated regions replicated in the three animals showed the same behavior, with very similar growth curves. Figure 2B shows the average temperature variation between the three animals and their respective deviation.




(A) (B)

Figure 4 – (A) Temperature of each animal over the IPL flashes on the non-photosensitized skin; (B) average temperature variation of non-photosensitized skin during irradiation.

In three individual temperature curves of the animals and the average temperature variation among them, we can observe the most substantial increase occurs during the initial eight flashes, as the temperature tends to stabilize after them. The temperature increase caused by the IPL flashes was not enough to cause thermal damage in any of the regions since in

any case the temperature never exceeds 44 °C.[18,22] The maximum value observed was 40.6 ºC for the animal number 2 after the fifteenth flash.
The thermal monitoring was also performed in the PDT groups, in those groups the PS used were PDZ, ICG and the mixture of them. The mean temperature variation before and after PDT procedure is shown in Figure 4, the three sensitized groups (PDZ, ICG, PDZ+ICG) and the control group (IPL – irradiation on the non-photosensitized skin).

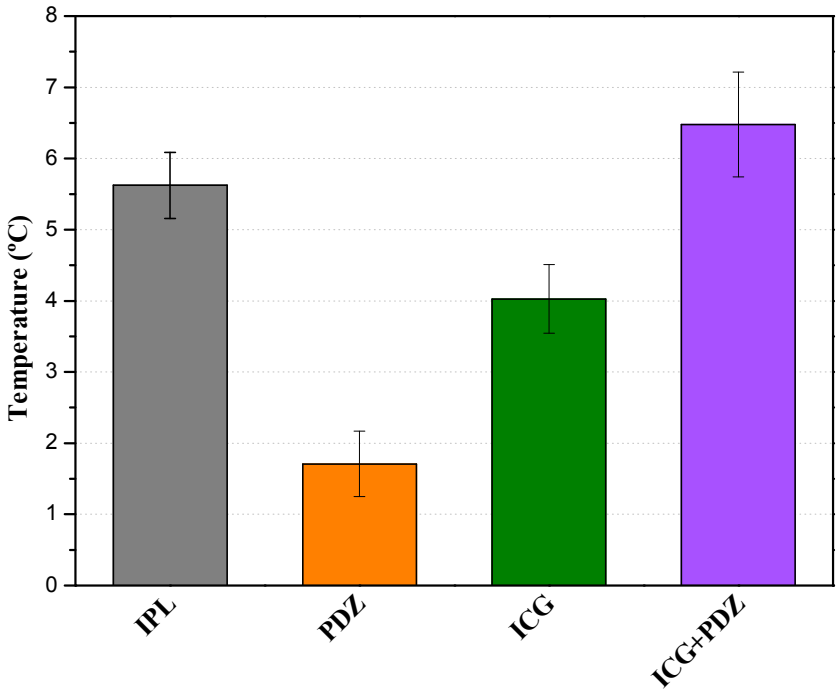



Figure 5 - The mean temperature variation during the before and after PDT, the three sensitized groups (PDZ, ICG, PDZ+ICG) and the control group (IPL – irradiation on the non-photosensitized skin).

This monitoring shows that no group had enough average temperature to promote thermal damage (greater than 44 °C). The increase in temperature in the groups sensitized with PDZ and ICG is lower when compared to the increase in temperature of the non-sensitized tissue. This behavior is due to the fact the tissue containing PS consumes part of the energy in photodynamic effect while on the tissue without PS more energy is converted into heat because there is no photodynamic action. No apparent cause for the substantial increase of the temperature for the PSs association (PDZ+ICG) was identified. We suggest that such an increase may be related to the simultaneous promotion of different photodynamic effects caused by each PS, or that the interference between PSs may facilitate deactivation of excited states by vibrational levels, resulting in non-radiative decay and thus heating. Further investigation must be performed to elucidate that.
The damage caused to the tissues was according to the temperature variation of each group: the highest damage was observed in the group that presented the highest temperature variation and the lowest damage in the group with the lowest. In this case, the highest temperature in this group may be related to the tissue response to the procedure as the development of an inflammatory process.
In pilot studies, the PDT evaluation in normal skin of pigs was carried out for 7 days, and it was noticed that after 48 hours the tissue was already in the advanced macroscopic healing process. These data suggest the time of 48 hours to evaluate the damage caused by PDT, allowing observing the tissue changes caused by the effects of the treatment. However, when the damage promoted by PDT is not expressive (in the sense that it is small proportionally to the animal size, and does not compromise relevant portions of tissue), it was not possible to observe macroscopically the damage caused after 48 hours. To evaluate the temporal evolution of the damage up to 48 hours, we performed the euthanasia of one animal with 24 hours, in the expectation of observing any little expressive damage caused by the therapy before the

complete macroscopic recovery was established. Figure 6 has the representative images of the superficial lesions 24 and 48 hours after PDT.

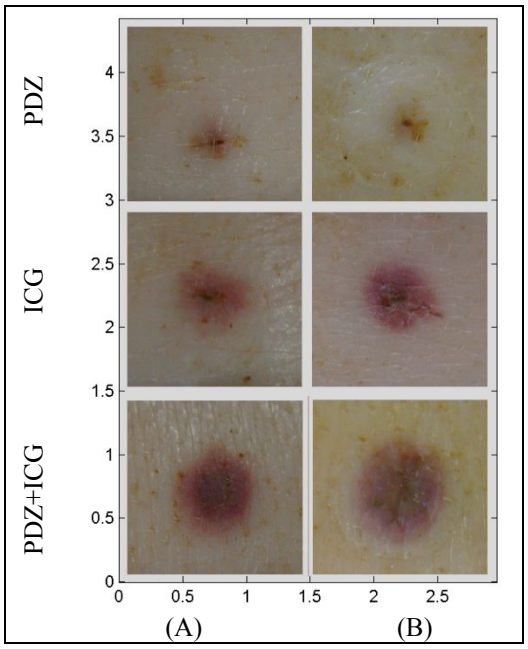



Figure 6 - Widefield images (with scale in centimeters) of the superficial lesions with the application of PDZ, ICG, and PDZ+ICG; (A) after 24 and (B) 48 hours of PDT with IPL

Macroscopically, the superficial damage was lower with the PDZ, ICG, and PDZ+ICG, respectively, agreeing with the thermal images. There is no significant difference visually between the lesions at times of 24 and 48 hours, which can be an indication of a slow macroscopic healing process. Figure 7 shows histological images of the tissues treated with the PSs 24 and 48 hours after the PDT to analyze microscopically the tissue characteristics.

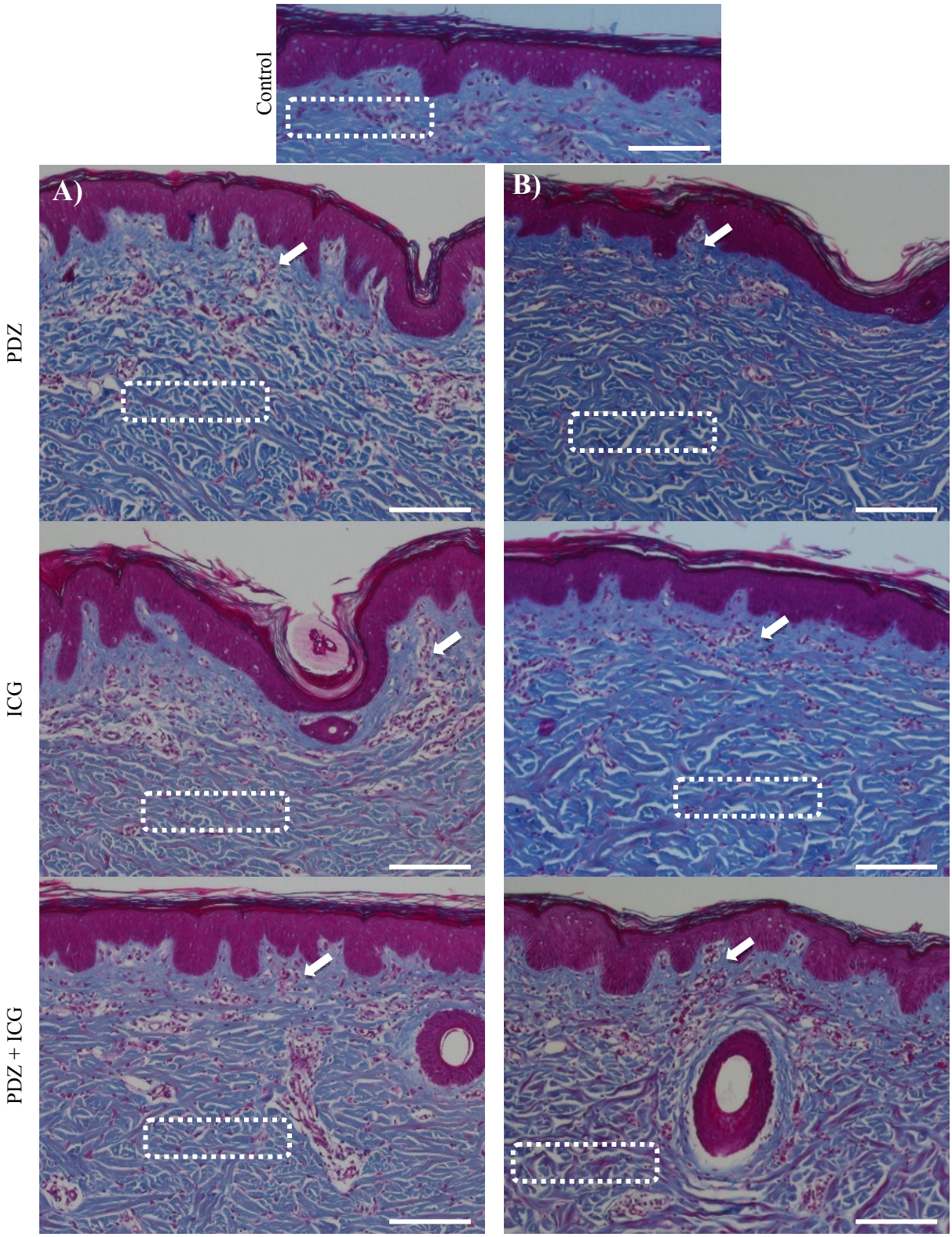



Figure 7 - Histological images of slides with 10x magnification and staining with Masson's Trichrome for PDZ, ICG, PDZ+ICG after (A) 24 and (B) 48 hours of PDT. A white line at the bottom right of the images (100 μm) represents the scale. The white arrows indicate inflammation characteristics and the white dotted rectangles indicate regions of conformation and cohesion of the collagen fibers.

Regarding histological aspects, all the conditions in 24 hours are quite similar to inflammatory and proliferation process. At 48 hours, for the use of the PSs isolated, the remodeling is in progress (collagen fibers are organized and more densely packed, with fewer inflammatory infiltrates than in 24 hours). However, the PDZ+ICG after 48 hours still shows inflammation (infiltrates infamous as present in 24 hours) and proliferation signs (degradation of collagen fibers more expressive than in 24 hours).

## CONCLUSION

A relevant aspect concerning thermal effects of IPL and IPL-PDT was discussed in this study. As a rule-of-thumb, IPL does not provoke relevant changes in temperature, particularly for PDT using a single PS. On the other hand, larger thermal effects were observed for the association of PSs in IPL-PDT, which may be caused by interference between PSs or by different competing PDT mechanisms still unknown. Single-PS use showed interesting outcome concerning tissue heating and stage of regeneration, which is evidence that such a light source may contribute with faster and more efficient PDT outcome. Thus, the results encourage further investigation on aspects related to PDT using IPL irradiation. Interestingly, the results obtained for PSs combination should also be further investigated, to understand how the association of PSs interferes with heating.

## ACKNOWLEDGEMENTS

The authors acknowledge the support provided by Brazilian Funding Agencies: Capes; CNPq and São Paulo Research Foundation (FAPESP) grants: 2013/07276-1 (CEPOF); 2009/54035-4 (EMU); 2014/50857-8 (INCT).

## REFERENCES

[1] Allison, R. R. and Sibata, C. H., "Oncologic photodynamic therapy photosensitizers: A clinical review," Photodiagnosis and Photodynamic Therapy **7**(2), 61–75 (2010).

[2] Romano, R. A., Pratavieira, S., Silva, A. P. da, Kurachi, C. and Guimarães, F. E. G. G., "Light-driven photosensitizer uptake increases Candida albicans photodynamic inactivation," Journal of Biophotonics **10**(11), 1538–1546 (2017).

[3] Griffin, L. and Lear, J., "Photodynamic Therapy and Non-Melanoma Skin Cancer," Cancers **8**(10), 98 (2016).

[4] Ramirez, D. P., Kurachi, C., Inada, N. M., Moriyama, L. T., Salvio, A. G., Vollet Filho, J. D., Pires, L., Buzzá, H. H., de Andrade, C. T., Greco, C. and Bagnato, V. S., "Experience and BCC subtypes as determinants of MAL-PDT response: Preliminary results of a national Brazilian project," Photodiagnosis and Photodynamic Therapy **11**(1), 22–26 (2014).

[5] Inoue, N., Todo, H., Iidaka, D., Tokudome, Y., Hashimoto, F., Kishino, T. and Sugibayashi, K., "Possibility and effectiveness of drug delivery to skin by needle-free injector," International Journal of Pharmaceutics **391**(1–2), 65–72 (2010).

[6] Rodrigues, P. G. S., de Menezes, P. F. C., Requena, M. B., Kurachi, C., Escobar, A., da Rocha, R. W., de Nardi, A. B. and Bagnato, V. S., "New alternatives to improve the transdermal application of ALA, M-ALA in photodynamic therapy using needle-free injection," Photodiagnosis and Photodynamic Therapy **12**(3), 360 (2015).

[7] Barolet, D. and Boucher, A., "No-needle jet intradermal aminolevulinic Acid photodynamic therapy for recurrent nodular Basal cell carcinoma of the nose: a case report.," Journal of skin cancer **2011**, 790509 (2011).

[8] Grecco, C., Buzzá, H. H., Stringasci, M. D., Andrade, C. T., Vollet-Filho, J. D., Pratavieira, S., Zanchin, A. L., Tuboy, A. M. and Bagnato, V. S., "Single LED-based device to perform widefield fluorescence imaging and photodynamic therapy," SPIE Proceedings, 953121:1–10 (2015).

[9] Sordillo, L. A., Pu, Y., Pratavieira, S., Budansky, Y. and Alfano, R. R., "Deep optical imaging of tissue using the second and third near-infrared spectral windows," Journal of Biomedical Optics **19**(5), 56004 (2014).

[10] Kim, H. S., Yoo, J. Y., Cho, K. H., Kwon, O. S. and Moon, S. E., "Topical photodynamic therapy using intense pulsed light for treatment of actinic keratosis: clinical and histopathologic evaluation.," Dermatologic surgery : official publication for American Society for Dermatologic Surgery [et al.] **31**, 33-36-37 (2005).

[11] Gold, M. H., Bradshaw, V. L., Boring, M. M., Bridges, T. M. and Biron, J. A., "Split-face comparison of photodynamic therapy with 5-aminolevulinic acid and intense pulsed light versus intense pulsed light alone for photodamage," Dermatologic Surgery **32**(6), 795–801 (2006).

[12] Stranadko, E. P., Ponomarev, G. V, Mechkov, V. M., Ryabov, M. V, Ivanov, A. V, Reshetnickov, A. V and Koraboyev, U. M., "First experience of photodithazine clinical application for photodynamic therapy of malignant tumors," Optical Methods for Tumor Treatment and Detection: Mechanisms and Techniques in

Photodynamic Therapy IX, 138 (March 29, 2000) **3909**, 138–144 (2000).
[13] Geralde, M. C., Pratavieira, S. and Bagnato, V. S., "Stability of indocyanine green for clinical use," Progress in Biomedical Optics and Imaging - Proceedings of SPIE **10417** (2017).
[14] Kennedy, D. a, Lee, T. and Seely, D., "A comparative review of thermography as a breast cancer screening technique.," Integrative cancer therapies **8**(1), 9–16 (2009).
[15] Kerr, J. and Zealand, N., [Review of the effectiveness of infrared thermal imaging (thermography) for population screening and diagnostic testing of breast cancer] (2004).
[16] Ishimaru, A., "Diffusion of light in turbid material" (1989).
[17] Orenstein, A., Kostenich, G., Tsur, H., Kogan, L. and Malik, Z., "Temperature monitoring during photodynamic therapy of skin tumors with topical Saminolevulinic acid application," 227–232 (1995).
[18] Yarmolenko, P. S., Moon, E. J., Landon, C., Manzoor, A., Hochman, D. W., Viglianti, B. L., Dewhirst, M. W. and 1Department., "Thresholds for thermal damage to normal tissues: An update," Int J Hyperthermia **27**(4), 320–343 (2013).
[19] Zamora-Rojas, E., Aernouts, B., Garrido-Varo, A., Saeys, W., Pérez-Marín, D. and Guerrero-Ginel, J. E., "Optical properties of pig skin epidermis and dermis estimated with double integrating spheres measurements," Innovative Food Science and Emerging Technologies **20**, 343–349 (2013).
[20] Schmook, F. P., Meingassner, J. G. and Billich, A., "Comparison of human skin or epidermis models with human and animal skin in in-vitro percutaneous absorption," International Journal of Pharmaceutics **215**(1–2), 51–56 (2001).
[21] Li, X., Wang, X., Gu, J., Ma, Y., Liu, Z. and Shi, Y., "Needle-free injection of 5-aminolevulinic acid in photodynamic therapy for the treatment of condylomata acuminata," Experimental and Therapeutic Medicine **6**(1), 236–240 (2013).
[22] Moritz, A. R. and Henriques, F. C., "Studies of thermal injury: the relative importance of time and surface temperature in the causation of cutaneous burns," American Journal of Pathology **23**, 695–720 (1947).