Imaging of nonlinear microscopy of burned skin treated by ultra-high intensity laser pulses

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The techniques of two-photon excitation of fluorescence microscopy (TPEFM) and second harmonic generation (SHG) shown as powerful tools to investigate the components of cells and extracellular matrix[1]. Collagen is a major component of the skin, and is known to undergo denaturation at the elevated temperatures associated with burns. Due to its structure that possess regions that lack a centre of symmetry, the collagen is considered an effective signal generating second harmonic, allowing the use of technique to investigate the degree of organization and degradation of collagen in tissue. In two-photon excitation, the signal of collagen arises from proteins that make up the triple helix structure of molecule[2]. After the approval of the Animal Research Ethics Committee, the samples were obtained from Wistar rats, male, adult. The rats were anaesthetized with ketamine and xylazine combination. Two dorsum areas were submitted to burns caused by water vapour exposure during 20 s. The burned areas after third, fifth, seventh and fourteenth days post treatment with ultra-high intensity laser pulses were biopsied. The burn skin collected were cryosectioned in slices of 20 μm width and mounted on glass slides and covered with coverslip for viewing. The samples were excited with a wavelength centered at 950 nm and the emitted signal at 485 nm was collected for the SHG signal and in the range of 520 nm to 560 nm for the TPEFM signal. The images obtained by TPEFM and SHG are lacked markers of exogenous cells (Figure 1). On the day 3 shows low intensity of SHG signal from collagen and the presence of hypodermis outside the region. On the last day, the collagen fibers have great contribution to the signal, however no specific structures in the layer was seen. The TPEFM and SHG images allowed evaluating the morphological regeneration of tissue, by collagen orientation, and establishing hypotheses based on the emission band collected by the microscope, on which chromophores contribute to the fluorescence.

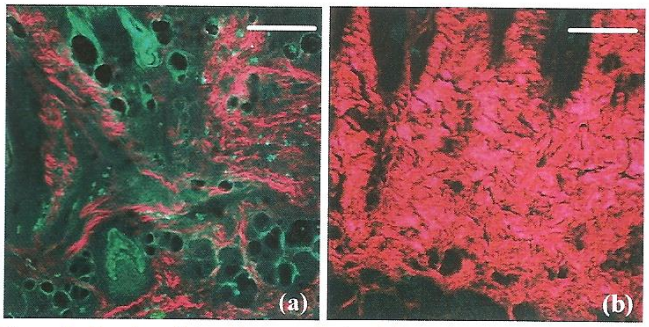

Figure 1: Overlay of two images obtained from the same sample site each, using TPEFM and SHG technique. Images of treated dermis at (a) day 3 and (b) day 14. SHG signal are shown in red and TPEFM are shown in green. Scale bar = 100μm.

References

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[2] Aikaterini Zoumi, Alvin Yeh and Bruce J. Tromberg. *Imaging cells and extracellular matrix in vivo by using second-harmonic generation and two-photon excited fluorescence*. PNAS 2002, **99** (17) pp. 11014-11019.