

Characterization of burned skin ablated with ultrashort pulses laser using nonlinear microscopy technique

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Nonlinear microscopy imaging technique enable to take both images of collagen fibers in dermis through second harmonic generation (SHG) signal and elastic fibers by two-photon emission fluorescence microscopy (TPEFM). These techniques are the most commonly used to characterize turbid and thick tissues and also to image biological samples which presents highly ordered structural proteins with no need of any exogenous label. The objective of this study is to characterize dermis of third-degree burned skin by TPEFM and SHG technique. A mode-locked laser (Spectra Physics) source was used with pulse width of approximately 100 fs at 80 MHz. It was coupled into a multiphoton microscope using a laser scanning unit (Olympus Fluoview 300), mounted on an inverted confocal system microscope (Olympus IX81), with focusing objective (40X, NA = 1.30). The samples were obtained from Wistar rats, male, adults. Dorsum areas were submitted to burn caused by water vapour exposure. At 5 day post-exposure, one lesion of each rat was irradiated with Ti:Sapphire laser pulses centered at 800 nm, with pulse width of 30 fs and energy densities in range of 4.54 to 34.1 J/cm^2 . Punch biopsy of lesion was excised at 0, 1, 2, 7 and 10 days post-irradiation. The biopsies obtained were cryosectioned in slices of 20 μm width. Selected area of interface between the irradiated and healthy subdermal burned skin were imaged by TPEFM and SHG technique. Two different autofluorescence signals are observed as a function of excitation wavelength. The autofluorescence observed at 760 nm and 690 nm refers to components of extracellular matrix at different depths. In SHG images, collagen fibers were visible and can be quantified during the healing process. According to the images obtained, these methodologies can be used to characterize dermis of burned tissue as its healing process with reduced out-of-plane photobleaching and phototoxicity. Acknowledgement: FAPESP CEPID (05/51689-2), Instituto Nacional de Fotônica/CNPq (573.916/2008-0), FAPEAM - Programa RH-POSGRAD.

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