Title
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10

MULTI-PHOTON EXCITED FLUORESCENCE MICROSCOPY AND SECOND-HARMONIC GENERATION SIGNAL EVALUATION AFTER APPLICATION OF OPTICAL CLEARING AGENTS

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Objective: To study the effects of topically applied optical clearing agents (OCA), a chemical combination of the lipophylic poly-propylene glycol (PPG) and the hydrophilic poly-ethylene glycol (PEG), on human skin using multi-photon excitation fluorescences microscopy (MPM) and second harmonic generation (SHG).

Material and Methods: Freshly excised skin samples were trimmed to 10mm square and divided into 4 groups. The skin samples were immersed for 2 hours in 1 of the following: 1) saline; 2) OCA (increased lipophylic concentration, PPG > PEG); 3) OCA (equal concentrations of PPG:PEG); 4) glycerol. The skin samples were imaged using native fluorescence (providing information on the cellular content and elastin presence) and SHG (providing selective information on collagen fibrils).

Results: Samples immersed in saline provided a strong SHG signal and clear visualization of the collagen fibrils to a depth of 150 μm. Immersion in the OCAs and glycerol did not change noticeably the intensity of intrinsic fluorescence signals. However, SHG signal intensity was diminished progressively in the OCA 1, OCA 2 and glycerol samples.

Conclusion: Tissue immersion in our OCAs and glycerol resulted in diminution of SHG signal intensity indicating loss of collagen fibrillar structure. The ratio of overall native fluorescence intensity and SHG signal may be used as a measure of "optical clearing" effectiveness.