

Detecting Reactive Oxygen Species in Skin Using Two-Photon Fluorescence Imaging Microscopy.

Kerry M. Hanson¹, Patrick J. Hayden², Joseph Kubilus,² and Robert M. Clegg¹.

¹Laboratory for Fluorescence Dynamics, Department of Physics, University of Illinois at Urbana-Champaign. ²MatTek Corp. 200 Homer Avenue, Ashland, MA 01721.

Reactive oxygen species (ROS) contribute to skin photodamage including photoaging, immunomodulation, actinic keratosis and skin cancers. Because these highly-reactive derivatives of molecular oxygen are extremely short-lived and essentially non-emissive, they are difficult to detect directly. In addition, until recently with the realization of two-photon excited fluorescence (TPEF) imaging, the opaque and heterogeneous environment of the skin has inhibited detection and quantification of UV-induced ROS within the skin. We have developed a TPEF imaging method to detect the presence of ROS with 0.5 μm spatial resolution and $>100 \mu\text{m}$ depth penetration. Dihydrorhodamine (DHR, 100 μL , 50 μM) is applied to the skin surface (Epiderm-200 (epidermis) and Epiderm-200FT (epidermis/dermis) (MatTek Corp.)) and incubated for 1 hr (37 $^{\circ}\text{C}$, 5% CO_2). DHR is non-fluorescent until it reacts with ROS and forms fluorescent rhodamine-123 (R123). Samples are imaged before and after UV irradiation (200–1600 J m^2 , 280–400 nm, solar simulator, Solar Light Co.). A detailed description of the instrument and experimental parameters will be presented. ROS are generated predominately in the lipid rich extracellular matrix of the corneocytes. With increasing depth, R123 fluorescence is detected primarily in the cytoplasm of the keratinocytes within each epidermal layer. In the dermis, fibrous regions of R123 fluorescence are detected. The data show that at commonly obtained UV doses, detectable ROS are generated within all layers of the epidermis and in the dermis. Both *ex vivo* and skin equivalent tissues yield similar results, with the advantage of the latter being reduced variability (scattering coefficients, pigmentation differences) between samples that *ex vivo* tissue affords. By coupling the TPEF microscopy method with skin equivalent tissue, the effects of topical applications like antioxidants and sunscreens upon UV-induced ROS levels can be studied.

For presentation at the 2004 Society of Investigative Dermatology Meeting, Providence, RI, May 2004.