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Calcium-fluorescence lifetime imaging in *ex vivo* skin II

MJ Behne,[1] N Barry,[2] K Hanson,[2] E Gratton[2] and T Mauro[1] *1 Dermatology, UCSF, San Francisco, CA and 2 Physics - LFD, UIUC, Urbana-Champaign, IL*

In our recent publications, we described Fluorescence Lifetime Imaging Microscopy (FLIM) to assess pH in intact epidermis. Here, we report about our recent progress in measuring and visualizing Ca2+ in ex-vivo biopsies of unfixed epidermis. In our initial Ca-FLIM studies we used Calcium Green-5N which is sensitive in the intermediate range of concentrations expected in epidermis and were able to show overall increasing Ca2+ concentrations from basal (SB) to granular (SG) layers, confirming our prior PIXE and Calcium-precipitation results. We refined and broadened this method to cover the full range of epidermal Ca2+ concentrations, from nanomolar to millimolar values. Using sequential measurements with Calcium Green-1 for low concentrations and Rhod-5N for high concentrations, we find highest Ca-concentrations to be limited to the intracellular domain in an ER-like distribution, specifically in the apical (granulosum and spinosum) layers, while in deeper (basal to dermal) layers this distinction is partially lost. In addition, FLIM demonstrates a shift in the Ca2+ distribution pattern upon barrier disruption: following tape-stripping, Rhod-5N reveals a partial loss of compartmentalization in the high concentrations range and across the depth of epidermis. In the low concentrations range using Calcium Green-1, cells with varying overall Ca-concentration appear, specifically at the SC/SG interface and partially persisting into the SC. Comparison of localized changes in Calcium concentrations and their regulation are especially important to assess functional consequences in barrier homeostasis and repair, differentiation, signaling, and cell adhesion, as well as various pathologic states, e.g., Darier Disease, Hailey-Hailey Disease, and Psoriasis. As extracellular and intracellular absolute Ca2+ concentrations in tissue are unknown, but can be assessed with FLIM, we believe that this method will contribute to elucidating basic physiology as well as pathology in epidermis.