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Major translocation of calcium upon barrier insult: calcium dynamics visualized by -fluorescence lifetime imaging

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Calcium controls an array of key events in keratinocytes and epidermis: localized changes in Ca2+ concentrations and their regulation are therefore especially important to assess in epidermal barrier homeostasis and repair, neonatal barrier establishment, in differentiation, signaling, cell adhesion, and in various pathologic states. Yet, tissue- and cellular Ca2+ concentrations in physiologic and diseased states are only partially known, and difficult to measure. Here we report a method using Calcium Green as the calcium sensor and the phasor-plot approach to separate raw lifetime components. This enables us to quantitatively assess and visualize dynamic changes of Ca2+ in ex-vivo biopsies of unfixed epidermis, exploiting fluorescence lifetime imaging. Our first results comparing undisturbed epidermis with epidermis following a barrier insult revealed major shifts from intra- to extracellular, and, more importantly, a mobilization of high amounts of Ca2+ shortly following barrier disruption, presumably from intracellular stores. These results partially contradict the conventional view, where barrier insults abrogate a Ca2+ -gradient towards the SG. Methodologically, the latter is based on Ca2+-precipitation followed by electron microscopy, or proton-induced x-ray emission. Both techniques require fixed tissue, for electron microscopy also a chemical precipitation and are limited in that they can determine Ca2+ in only very small sample volumes, at or below light microscopic resolution levels, or, in the case of PIXE, determine only total calcium, irrespective of ionization or binding. So far, neither cellular and/or subcellular localization can be determined through these approaches. We believe that our approach will overcome these limitations in the observation of epidermal Ca2+ dynamics, and contribute to elucidating basic physiology as well as various pathologic situations in epidermis.