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Calcium-fluorescence lifetime imaging in ex-vivo skin

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Abstract

Calcium controls several key events in keratinocytes and epidermis: epidermal barrier homeostasis and repair, neonatal barrier establishment, differentiation, signaling, cell adhesion, and various pathologic states. Yet, tissue- and cellular Ca2+ concentrations in physiologic and diseased states are only partially known. The very barrier properties of epidermis pose a major obstacle in determinations of the Ca2+ concentrations in intact skin. Methodology to measure and localize Ca2+ in intact and unfixed tissue has been lacking, while so far Ca2+-precipitation electron microscopy, or proton-induced x-ray emission were used. Both techniques are limited in that they can determine Ca2+ in only very small sample volumes, at or below light microscopic resolution levels, require fixed tissue and a chemical precipitation, or determine only total Calcium, irrespective of ionization or binding. So far, neither cellular and/or subcellular localization can be determined through these approaches. In cells, fluorescent dyes have been used extensively for ratiometric measurements of static and dynamic Ca2+ concentrations, and in recent publications, we described Fluorescence Lifetime Imaging Microscopy (FLIM) to assess pH in intact epidermis. Here we report a method to measure and visualize Ca2+ in ex-vivo biopsies of unfixed epidermis, via FLIM. Using Calcium Green as the calcium sensor and the phasor-plot approach to separate raw lifetime components, we could establish a protocol to assess dynamic changes of Calcium throughout epidermis. We believe that this method will contribute to elucidating basic physiology as well as various pathologic situations in dermatology.