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Mapping Inter-Cellular Water in Skin

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The main functions of skin's outer skin, stratum corneum (SC), are to prevent the water loss from underlying tissue, limit penetration of extraneous chemicals and save the viable cells of skin from environmental insults. Normal skin cells embark upon a well-orchestrated differentiation pathway that eventually results in cell desquamation. Proper desquamation depends upon the degradation of intercellular plugs, desmosomes, which in turn is believed to depend upon skin hydration [1]. Water, therefore, plays a critical role in maintaining skin's function and is also important for skin's physical and even optical properties. Earlier *in vitro* measurements of water distribution in skin [2] have relied on indirect measurement of water requiring dehydration of the sample in the microscope column. Here we report the application of electron energy-loss spectroscopy (EELS) and spectrum imaging (SI) [3] to measure cellular water in skin maintained in its native state. The main advantage of the EELS based measurements is that the specimen is maintained in frozen state during analysis, and therefore dehydration-induced artefacts are avoided and it also provides a direct measure of water in hydrated skin.

Thin slices (~200 μm) of freshly excised porcine skin (a by-product) were high pressure frozen using Leica Impact. Vitrified tissue blocks were trimmed and cut into 150 nm thick sections using Leica Ultracut EM FCS (at -150 C). Frozen samples were cryo-transferred at –170C to a Philips CM20 FEG STEM, operating at beam energy of 200 kV and equipped with a Gatan spectrometer Model CCD *ENFINA*. Spectrum-images were acquired using the Emispec data acquisition system. Data was collected at 5-15 kX using a probe size of about 40 nm, dwell time 20 ms, at 0.1 eV /channel and using a collection angle 10 mrad. The electron dose was about 2000 electrons/nm^2. The images were transferred to a Macintosh G4 500MHZ dual processor for further processing. Pixel wise spectral contribution in spectrum images was computed after spectral pre-processing and by multiple least squares fitting of the reference spectra to SI data set [3].

The frozen-hydrated skin, as expected, appears predominantly featureless (Fig. 1a) but the computed water map distinctly shows water-rich SC cells (Fig. 1b). White in this map corresponds to high water content. Water was found to reach a plateau of about 60% concentration at about 24 micron from the skin surface. The presence of a water gradient is seen in the outer layers of skin (Fig. 1c). Outermost region of the water map displays alternating, ~ 0.5 micron wide, regions of high water content, corresponding to intracellular regions of SC, separated by dark regions corresponding to intercellular lipid rich regions. Desmosomes that are clearly visualized in conventionally fixed tissue (Fig. 2a) appeared as water-rich (60-65%) dense pockets punctuating the lipid-rich intercellular regions of skin (Fig. 2b, ellipses). Such regions were predominantly present in the compactum, immediately above the viable cells.

1. A. Watkinson et al., Arch. Dermatol. Res. 293 (2001) 470
2. R. Warner et al., J. Invest. Derm. 100 (1993) 528.
3. S. Sun et al., J. Micros., 177 (1995) 18.

Fig. 1 Frozen hydrated section (a) water map (b) and computed water gradient (c) from SC to outer viable skin cells.

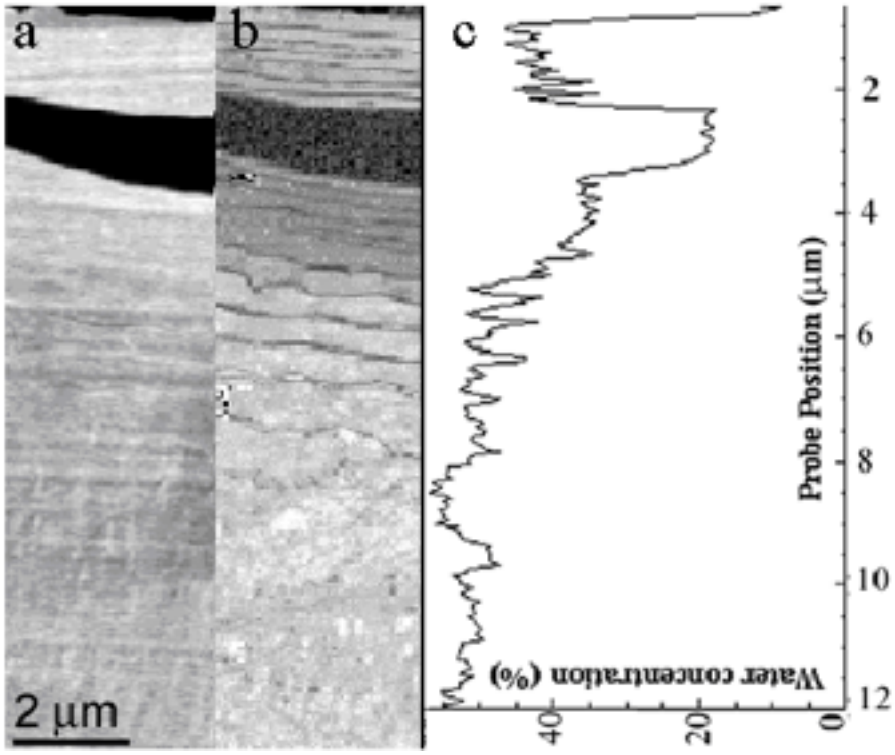
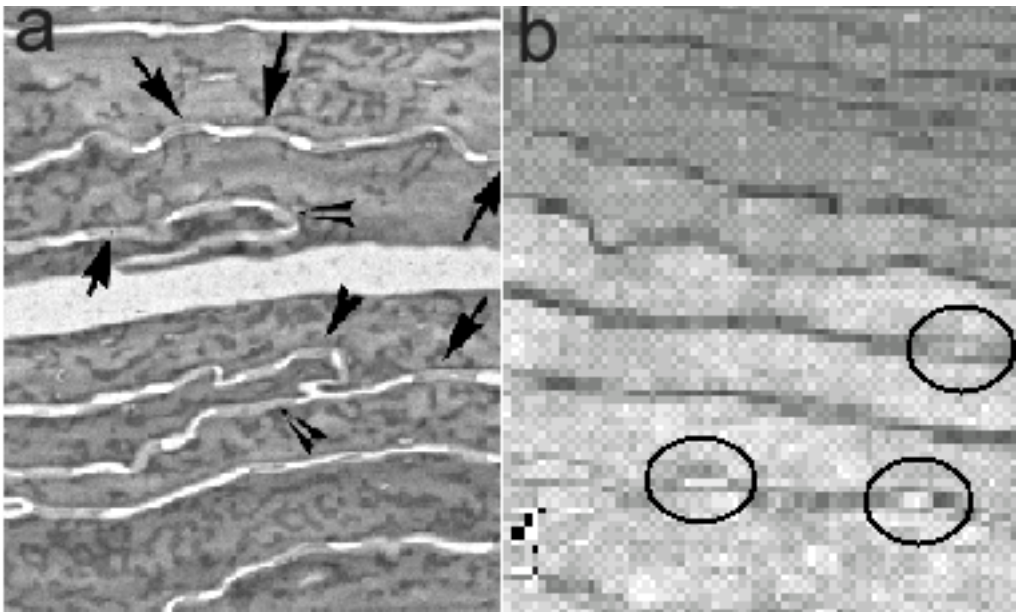


Fig. 2 Conventionally prepared micrograph of skin (a) showing inter- (arrows) and intra-layer (arrowheads) desmosomes and water map of frozen-hydrated skin showing inter-layer (circles) desmosomes (b).



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