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How the skin is affected by environment or actives: A molecular perspective using solid-state NMR

In the development of topical and transdermal therapeutics, as well as for cosmetics and personal care, in-depth knowledge regarding cutaneous barrier function is paramount. Solid-state NMR is a highly sensitive method that can reveal atomic level information regarding this barrier and its interaction with topical formulations. In particular, the technique reveals the balance between the solid and fluid components that make up the stratum corneum. The fluid components are crucial for partitioning of any molecules into the skin.

Skin anatomy and barrier function

The barrier function of the skin is attributed to the outer horny layer of the skin epidermis – the stratum corneum (SC) (Fig 1). The SC is approx. 15 to 20 µm thick and is a protein-lipid composite where the corneocytes are embedded in a continuous lipid matrix much like a "brick and mortar" structure (Fig 1). The SC lipids mainly consists of ceramides, cholesterol, and free fatty acids and together they can form multilamellar stacks. Unlike phospholipids (commonly found in the cell membrane), which are typically fluid at ambient condition, the skin lipids are crystalline and thus provides a robust barrier.

Importance of SC fluid lipid

A tiny fraction of fluid lipid coexists with the solid lipids in the SC. The presence of fluid lipid is crucial for partitioning of drugs and other small molecules applied onto the skin. The balance between fluid and solid components in the SC can be tuned by changing its ambient condition, for example, by changing the hydration level and can be leveraged in topical product development.

Role of NMFs in the skin barrier

The skin must be intact and moist in order to maintain a healthy barrier. Natural moisturizing factors (NMFs), a group of tiny polar molecules found in the skin such as urea, glycerol, and amino acids, help to keep the skin wet by hydrating and lubricating it. Lack of NMF in the skin may result in dry, brittle skin with compromised barrier, necessitating supplementation to restore a healthy barrier. The development of NMF formulation is facilitated by the use of solid-state NMR (ssNMR).

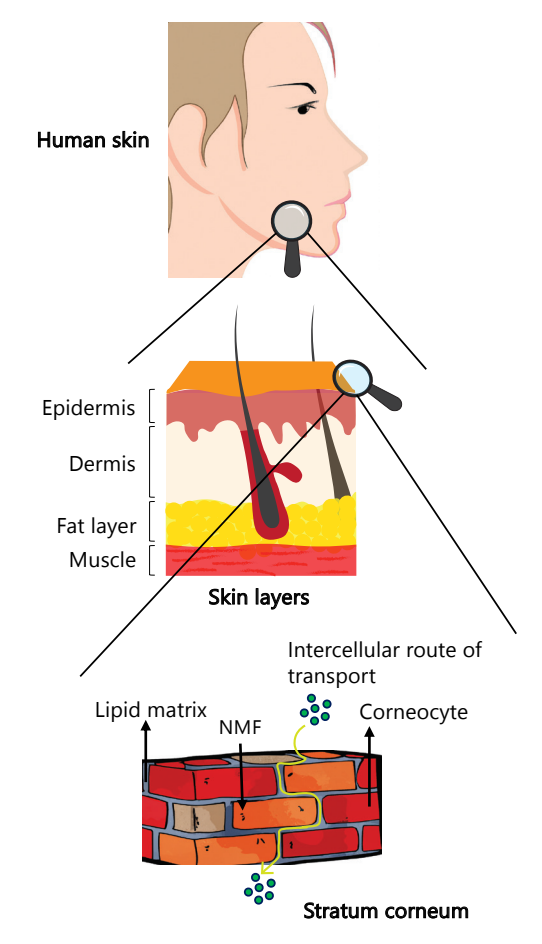

Figure1: *Illustration of human skin and its constituent layers. The outer layer of the skin epidermis - stratum corneum (SC) provides the primary barrier. The barrier needs to be intact and moist in order to be healthy and efficient.*

key aspects of the skin that can be examined with ssNMR –

- Effect of humidity, pH, and temperature.
- Effect of UV rays and the use of sunscreen.
- Consequence of burning, irritation, e.g., usage of shaving razors.
- Effect of enhancers, additives, co-solvents, API's, excipients etc.
- Molecular dynamics of applied ingredients alone & in the skin.

Complementary techniques –

- Trans-epidermal water loss (TEWL).
- FTIR and Raman spectroscopy.
- Small and wide-angle X-ray diffraction (SAXD/WAXD).
- In vitro Franz/flow-through cell diffusion studies.
- Imaging techniques - confocal microscopy/tomography.

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Application of ssNMR in the skin: effect of skin hydration and enhancers

Herein, an application is provided how ssNMR can be exploited to examine the effect of penetration enhancers in the skin, but similar experiments can be performed to investigate the hydration effect of urea or glycerol and can be aapplied to hair and nails, as well.

ssNMR spectra of dry SC

A representation of ^{13}C solid-state NMR spectra of native SC in dry condition is shown in Fig 2A. The three individual experiments of solid-state NMR are DP, CP and INEPT. In the spectra, the major signal arising from the lipids dominates in the lower chemical shift region (approx. 10-35 ppm), whereas the main contribution from the protein prevails at slightly higher chemical shift (approx. 40-80 ppm). In Fig 2A, the prevailing CP signal with the absence of INEPT demonstrate the solid nature of the SC lipid and protein components in the dry SC.

ssNMR spectra of hydrated SC

When the dry SC is exposed to humid condition, the INEPT signal start to appear (Fig 2B), which is an indication of hydration-induced mobility in the SC.

ssNMR spectra of SC with enhancers

Adding enhancers to the hydrated SC further increases mobility (Fig 2C). Furthermore, the dynamics of the enhancers when partitioning in the SC could also be visualized.

ssNMR allows mobility identification

It is possible to identify where in the SC mobility is observed - either in the lipids or in the protein or both compartments. This is highly dependent on the physio-chemical nature of the molecules and their interaction with the SC components. The increment of fluid components in the SC at different treatment conditions is presented in Fig 2D.

Overall, a detailed molecular picture regarding SC lipid and protein components and their interaction with formulation ingredients can be probed, leading to deeper mechanistic understanding of concepts such as drug uptake, hydration, and water-loss. for more details, see the article *Pham et al., JCR, 2016.*

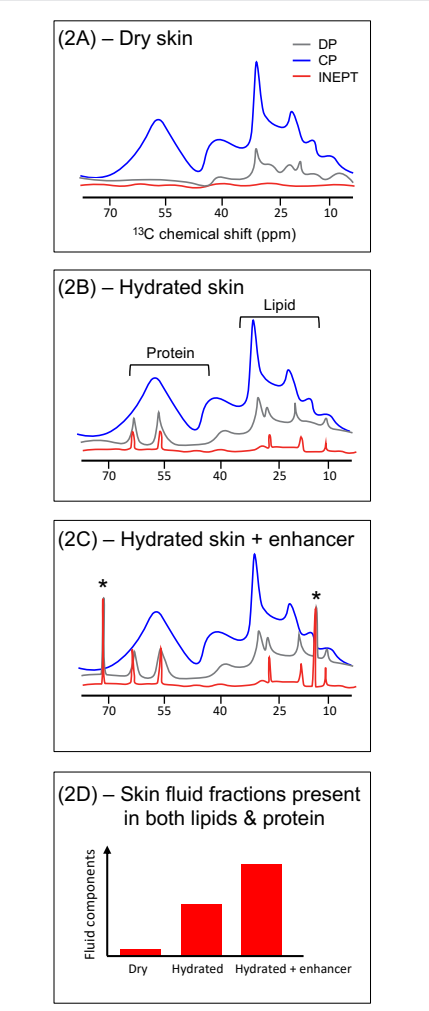

Figure 2: *Schematic of ^{13}C solid-state NMR spectra. Individual DP (grey), CP (blue) and INEPT (red) signals are labeled and overlaid for comparison purpose. The chemical shift region where the signals from lipids and proteins in the SC prevail are also indicated. The '*' in Figure C denotes signals arising from the enhancer.*

Solid-state NMR

ssNMR is a spectroscopic technique that can be used to probe molecular structure and dynamics in solid and semi-solid systems, e.g., powders, amorphous materials etc. The ^{13}C solid-state NMR method in focus is a combination of three individual experiments performed sequentially on the same sample. The three experiments are; direct polarization (DP), cross polarization (CP), and insensitive nuclei enhanced by polarization transfer (INEPT).

DP is a normal pulse experiment, where the signals from all the carbons in the sample is recorded and used as a reference. CP is selectively boosting signals for solid components whereas INEPT is doing so for liquid fractions in the system. When the signals from DP, CP and INEPT are combined, detailed molecular level information regarding SC lipid and protein molecular mobility and rigidity in the skin can be obtained.

The unique ability of this method to distinguish small fluid fractions from the majority of rigid materials is crucial for understanding skin barrier and is not possible to examine with other traditional methods, such as X-ray diffraction.

From Lab to Boardroom and Beyond

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+46 702 599 755 or anna@crcom.se