Assessment of skin perturbation by means of non-invasive *in vivo* measurement of inflammatory biomarkers and confocal reflectance microscopy

D. Falcone[1], K. Salk[2], M. Peppelman[1], N. Uzunbajakava[3], P. Spee[2], P. van de Kerkhof [1], P. van Erp[1]

[1]Department of Dermatology, Radboud university medical center, Nijmegen, The Netherlands; [2]FibroTX LLC, Tallinn, Estonia;
[3]Philips Research, Eindhoven, The Netherlands

Background

Skin perturbation results in the immediate release of lamellar bodies and cytokines, followed by a cascade of events leading to the restoration of the permeability barrier. *In vivo* data on how cytokines levels change following skin perturbation are scarce, and show large inter-individual differences.

FibroTX Transdermal Analyses Patch (TAP) is a novel and non-invasive technology for measuring skin surface protein biomarkers l*in vivo.* Using TAP, IL-1α, IL-1RA and hBD-1 could be detected on healthy intact skin(1). However, it has not been addressed yet whether TAP can measure changes of skin surface protein biomarkers following skin perturbation.

Two *in vivo* models of skin perturbation are tape stripping, mimicking acute disruption of the skin barrier, and histamine iontophoresis, mimicking acute and local inflammation at minimal skin barrier insult.

(1)Orro K *et al*. Development of TAP, a non-invasive test for qualitative and quantitative measurements of biomarkers from the skin surface. Biomarker research. 2014;2:20

Research question

To explore whether TAP is able to detect dynamic changes in skin surface biomarkers elicited by tape stripping and histamine iontophoresis *in vivo*.

To relate these changes to morphological assessments based on conventional histology (HE) and on confocal reflectance microscopy (RCM).

Methods

Skin perturbation

• Sequential tape stripping was performed on the volar forearm (area: 2.9 cm^2) until complete removal of the stratum corneum.
•Histamine iontophoresis was performed on the volar forearm at 0.4 mA (area: 7.2 cm^2) for 2.5 minutes.

Detection of skin surface biomarkers by TAP

TAP consists of a multiplex capture-antibody micro-array supported by an adhesive bandage for easy fixture to the skin.

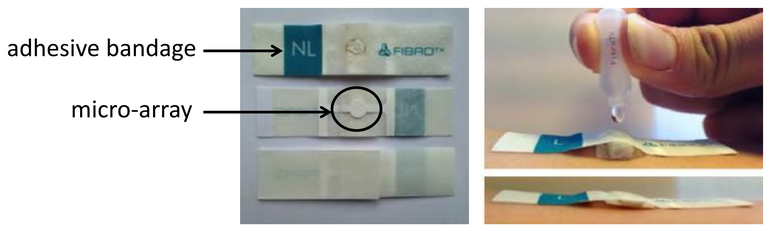

Figure 1. Transdermal Analyses Patch (left) and application to the skin (right). From (1).

Assessment of skin morphology by RCM

RCM images were taken from the skin surface to 150 µm depth (VivaScope 1500 system, Lucid Inc., USA).

Results

Tape stripping → increased levels of IL-1α, IL-1RA and hBD-1, with different dynamics

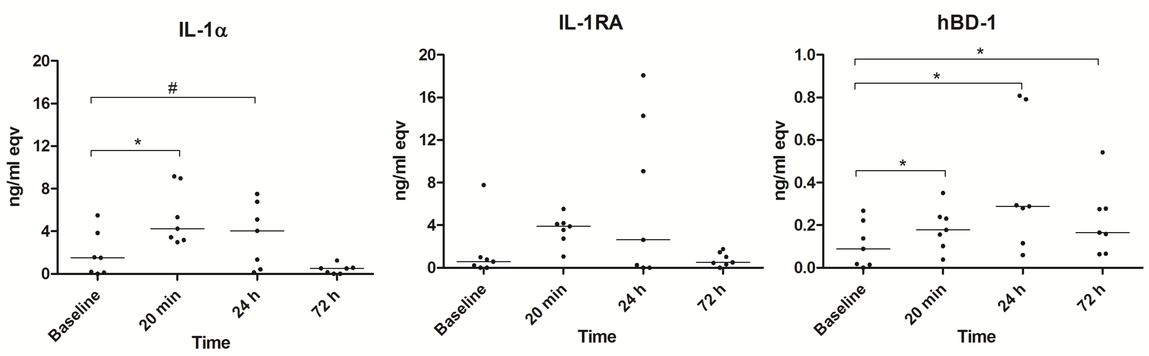

Figure 2. TAP measurements (n=7). #: 0.05<pvalue<0.08; *: pvalue≤0.05. Horizontal bar represents median.

- Increased availability due to barrier disruption might contribute to the increased levels, but the slower upregulation of hBD-1 compared to IL-1α and IL-1RA suggests a true underlying dynamics.

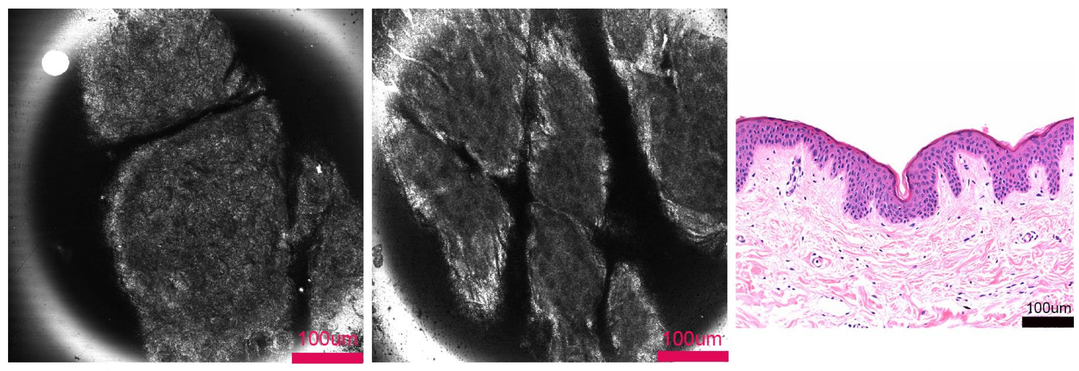

Figure 3. Left: RCM image at baseline, stratum corneum visible. Center: RCM image immediately after tape stripping, nucleated cells of the stratum granulosum visible indicating removal of stratum corneum. Right: HE histology confirms absence of stratum corneum.

Histamine iontophoresis → mostly unchanged or decreased levels of IL-1α, IL-1RA and hBD-1

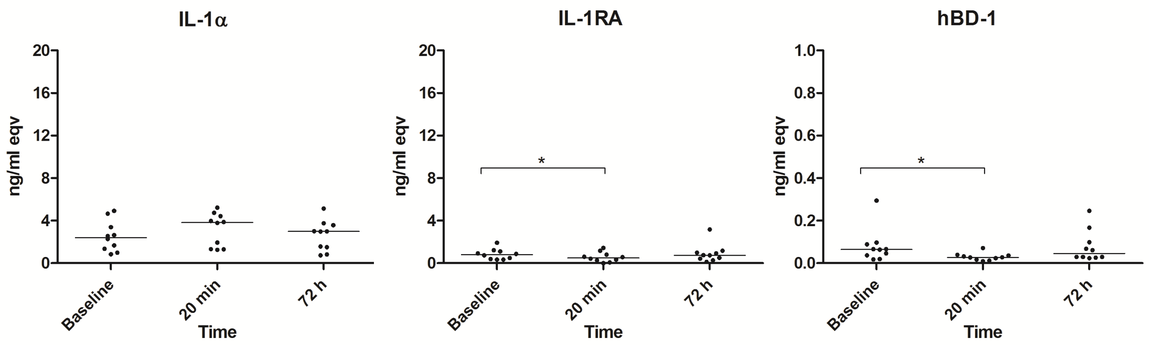

Figure 4. TAP measurements (n=10). *: pvalue≤0.05. Horizontal bar represents median.

- Decreased levels of IL-1RA and hBD-1 at 20 minutes might be due to a swollen stratum corneum, while the unchanged levels of IL-1α suggest a release of this marker after the stimulus.

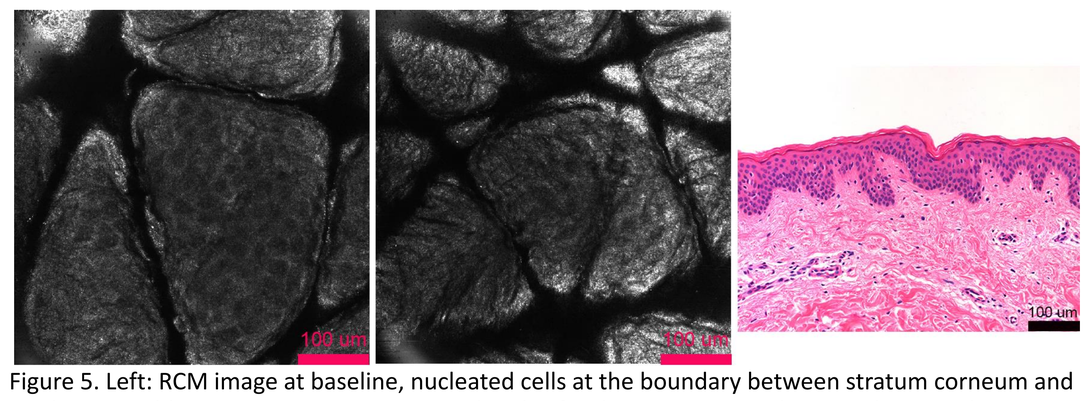

Figure 5. Left: RCM image at baseline, nucleated cells at the boundary between stratum corneum and granulosum visible. Center: RCM image immediately after histamine iontophoresis, decreased contrast in the nucleated cells at the boundary between stratum corneum and granulosum indicates spongiosis. Right: HE histology confirms intact stratum corneum.

Conclusions

• TAP measurement of IL-1α, IL-1RA and hBD-1 from the skin surface was sensitive enough for monitoring dynamic changes *in vivo* after tape stripping and histamine iontophoresis.
• Changes in levels of IL-1α, IL-1RA and hBD-1 could be related to morphological assessments made by RCM and conventional histology, albeit true underlying dynamics measured by TAP following the two models of skin perturbation are probable.
• The functional and morphological measurements with TAP and RCM might represent valuable tools in the non-invasive *in vivo* assessment of skin perturbation.

Presenter: D. Falcone, MSc
6500 HB Nijmegen (The Netherlands)
denise.falcone@radboudumc.nl

Institute for Molecular Life Sciences
Radboudumc