Supplementary material

Physiological and molecular effects of *in vivo* and *ex vivo* mild skin barrier disruption

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IN VIVO EXPERIMENTS

In vivo skin physiology measurements

Non-invasive assessment of skin physiology was performed by measuring transepidermal water loss (TEWL, Tewameter TM® 300), skin surface pH (Skin-pH-Meter® PH 905), stratum corneum hydration (SCH, Corneometer® CM 825) and skin elasticity (Cutometer® dual MPA 580) before and at different time points (up to 48h) after the procedure. Measurements were performed using a Multi Probe Adapter MPA (Courage-Khazaka, Cologne, Germany). For an overview of the measurements please refer to suppl. Figure 1.

Skin surface protein harvesting and protein extraction

Stratum corneum material was collected at different time points to quantify cytokines using Sebutape (S100 Sebutape patches, CuDerm Corporation). Sebutapes were attached to the defined test area on the upper arm and remained attached for 5 minutes. After that, they were removed using a forceps and carefully placed in pre-cooled 15mL tubes (Falcon Tubes, BD Biosciences Europe). The tubes were placed in dry ice and immediately after stored at -80^{o}C.

For protein extraction, tubes were kept on ice, and 2mL of phosphate buffer (PBS, 1x, Biochrom, Merck Millipore) was carefully added to each tube, in order to cover the whole strip area. Tubes were incubated for 3h on iced water and subsequently sonicated for 10 min (35kHz, Sonorex Super PK102H, Bandelin electronic GmbH & Co KG, Germany). After sonicating, they were vortexed for 30s (1800rpm) and centrifuged for 1 min, 1500rpm. Supernatant containing the protein extract was carefully aliquoted and frozen at -80^{o}C for further analysis.

EX VIVO EXPERIMENTS

Skin physiology and analysis of superficial cytokine production

Ex vivo skin samples

Fresh skin samples were obtained from 7 healthy volunteers undergoing plastic surgery of the breast (n=1) or the abdomen (n=6). All skin samples were taken after informed consent approval by the Institutional Ethics Committee of the Charité Universitätsmedizin Berlin, according to the ethical rules stated in the Declaration of Helsinki Principle. All donors (n=7) were female between 32 and 63 (average age 45.8) years of age and had phototypes between I and III according to the Fitzpatrick classification.

Ex vivo skin preparation

Directly after surgical excision, skin samples were conserved in a dry and sterile container for 2 to 4 hours. Skin samples were cleaned with a wet paper towel using unsterile PBS (PBS Dulbecco, Biochrom GmbH, Germany). Extensive subcutaneous fat was removed using scissors so that the skin lay plainly. The skin was fixated on a block of Styrofoam using needles and a disinfection of the surface was performed using Softa-Man skin disinfection solution (B.Braun Melsungen AG, Germany). Test areas were sized 1cm x 1cm and were 1 cm apart from each other.

Ex vivo skin physiology measurements

Skin physiology was measured using following devices: Transepidermal water loss – TEWL (Tewameter TM® 300, Courage & Khazaka Electronics GmbH, Germany), stratum corneum hydration (capacitance, Corneometer® CM 825, Courage & Khazaka Electronics GmbH, Germany), sebumetry (Sebumeter® SM 815, Courage & Khazaka Electronics GmbH, Germany), pH (Skin-pH-Meter® PH 905, Courage & Khazaka Electronics GmbH, Germany), skin temperature (Skin-Thermometer ST 500, Courage & Khazaka Electronics GmbH, Germany) and epidermal thickness (VivoSight, Michelson Diagnostics, United Kingdom).

Protein extraction from diagnostic Sebutape and CSSS

To quantify different cytokines and chemokines protein material was extracted from the diagnostic stripes (Sebutape and mini-zone CSSS). Tapes were incubated for 3 hours with 2mL of PBS with 0.005% Tween-20 (Sigma-Aldrich Corp., catalogue number P1379-25ML, USA) on ice. Afterwards the tubes were sonicated in ice water for ten minutes (35kHz, Sonorex Super PK102H, BANDELIN electronic GmbH&Co. KG, Germany), vortexed for 30 seconds and subsequently centrifuged for 1 minute at 1500rpm. The extracted solution was aliquoted and frozen at -80°C.

Total protein quantification and ELISA from ex vivo samples

Total protein quantification: To quantify the protein amount in the solution extracted from Sebutapes, a bicinchoninic acid-assay was used (Micro BCA Protein Assay Kit, catalogue number 23235, Thermo Scientific Pierce Protein Biology Products, USA). For CSSS extracts a Bradford assay was used (Coomassie Plus Assay Kit, catalogue number 23236, Thermo Scientific Pierce Protein Biology Products, USA). Protein extracted from whole tissue samples were quantified using a Coomassie-based Bradford assay (660nm Protein Assay Reagent, catalogue number 22660, Thermo Scientific Pierce Protein Biology Products, USA). BSA was used as the standard in all assays.

ELISA: ELISA assays were performed on Sebutape extracts for IL-1α, IL-1β, and receptor antagonist (R&D Systems Inc, catalogue numbers DY200, DY201, DY280 respectively, USA) according to the kit protocol.

qRT-PCR skin samples and skin preparation

For qRT-PCR experiments, fresh skin samples were obtained from 6 healthy volunteers undergoing plastic surgery of breast (n=4) or abdomen (n=2) (Service de chirurgie plastique, reconstructrice et esthétique-Centre de brûlés, Saint-Louis hospital, Paris, France). All skin samples were taken after informed consent approval by the Institutional Ethics Committee of the Saint Louis Hospital (IRB 00003835), according to the ethical rules stated in the Declaration of Helsinki Principle. All donors (n=6) were female between 23 and 50 years old and had phototypes between II and V after the Fitzpatrick classification. Directly after surgical excision, skin tissues were conserved in NaCl for 2 to 4 hours. Skin samples were washed sequentially in povidone-iodine (Betadine® dermique 10 %, MEDA pharma, France) and PBS 1X. Subcutaneous fat and hypodermal tissue were removed using scissors; the skin was shaved carefully if visible hairs were present.

Skin treatment and incubation

Six fields of 2cm^2 were marked on the skin. On three of them Cyanoacrylate Stripping (CSSS) was performed as previously described and 62.4µL PBS applied. The skin fields were cut out and conserved in RPMI 1640 (Invitrogen, Thermo Scientific, USA) medium supplemented with 10 % Fetal Calf serum (FCS), glutamine and a mix solution of antibiotic-fungicide (Invitrogen, Thermo Scientific, USA) for incubation. For the time points of 4 hours, 16 hours (over night) and 24 hours respectively, one field of untreated skin and one field of skin after CSSS with PBS was incubated.

Human skin cell suspension

After incubation skin samples were cut in small pieces (about 0.3 mm^2) and incubated in dispase II (2.4 U/mL) in RPMI 1640 medium at 4°C over night to separate epidermal sheets from dermis, using tweezers. Epidermal cell suspensions were obtained by Trypsin (Trypsin-EDTA 0.2%, Sigma) treatment for 10 min. FCS was added to inactivate the enzymes. The digested tissue was crushed and passed through a 70 µm filter. Skin cell extracts were resuspended in PBS-1% FCS and then centrifuged. Epidermal cell pellets were frozen at -80°C.

Supplementary figure captions

Suppl. Figure 1. Schematic view of study procedures. In A, the single tape stripping is depicted, and in B, the CSSS procedure. Note that the CSSS procedure removes around 30% of the stratum corneum and opens hair follicles. In C, timeline of study procedures performed are depicted. In C, left, the location of CSSS and tape stripping performed in the clinical trial is shown. The upper part of the 4cm x 4cm test area was used to collect Sebutape. In the bottom part of the test area skin physiology measurements were performed before, directly after, after 2h, after 4h, after 24h and after 48h disruption.

Suppl. Figure 2. Pain perception of subjects for CSSS and Tape procedure, compared to intra muscular injection. Subjects were asked to mark on a scale (0=no pain at all; 10=maximum pain) their pain perception after CSSS and tape procedure. As well they were

asked to mark their perception for a standard vaccination intra muscular injection, n=12, error bars show standard deviation.

Suppl. Figure 3. Schematic overview of experimental setup for qRT-PCR. Skin samples were left untreated or CSSS was performed, with the application of phosphate buffer. After 4 hours, 16 hours (overnight stay) and 24h, qRT-PCR was performed.

Suppl. Figure 4. PCR data where no significant changes were observed. A selection of molecules analysed by qRT-PCR in epidermal cell suspensions not treated or after a single CSSS are shown. A normalization sample was used on every plate to account for interpolate variability. For the statistical analysis, one-tailed paired Wilcoxon tests were performed.