Research Article

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Supplementary Text

Antibodies

Human GRO-ß (CXCL-2) ELISA Development Kit (Cat. No: 900-K120, PeproTech), Human IL-1α ELISA Development Kit (Cat. No: 900-K11, PeproTech), Human hBD-1 ELISA Development Kit (Cat. No: 900-K202, PeproTech), Human IL-1RA ELISA Development Kit (Cat. No: 900-K474, PeproTech).

Composition of the TAP

The transdermal analysis patch (TAP) is produced and commercialized by FibroTx. Transdermal Analyses Patch (TAP) consists of a micro-array which is supported by a dermal adhesive plaster (3M White Nonwoven Medical Tape, 9907HTW) for easy fixture to skin. In between the antibody micro-array and the plaster, a layer is positioned that serves as a fluid reservoir for the buffer needed for protein capturing from skin. In addition, this expandable layer serves as a pressure pad to ensure close contact of the micro-array to the skin. Each TAP micro-array contains two spots of positive controls (IgG; Goat anti-human IgG Lab AS, Estonia) to determine the specificity of biomarker measurements, and a panel of capturing antibodies, of which each capturing antibody variant is printed in triplicate (N = 3), as discrete spots on membrane (GE HealthCare, 10600002) by non-contact dispensing. Captured proteins are analyzed, both qualitatively and quantitatively, on the antibody micro-array using spot-ELISA.

Visualization of Captured Proteins Using Spot-ELISA

To create standard curves, capture antibody microarrays were incubated for 20 minutes at 33°C with a mixture of recombinant proteins diluted in PBS + 0.05% (v/v) Tween-20. Unbound proteins were washed from membrane with wash buffer containing washing buffer. For determine the concentrations of skin surface captured biomarkers antibody capturing micro-arrays incubated on skin were removed from plaster and placed into blocked 48-well plates and washed using wash buffer. Antibody capturing micro-arrays subjected for standard curves and skin surface biomarker analysis were blocked for 20 min at room temperature in 5% BSA (w/v) in PBS (pH = 7.4). Biotinylated secondary antibody was added to each capturing antibody micro-array and incubated for 45 min at room temperature. For signal amplification Catalyzed Signal Amplification (CSA) System (Dako, K-1500) was used. For signal visualization Substrate-Chromogen solution diluted to 20% in Substrate Buffer Concentrate was used. Reaction was stopped with milli-Q water. Signals of captured biomarkers were quantified by comparing the signals of these proteins captured from skin of subjects using FibroTx TAP capture antibody micro-array incubated with fixed amounts of recombinant proteins.