SUCTION BLISTER METHOD

THE *IN VIVO* > *EX VIVO* > *IN VITRO* APPROACH FOR YOUR INNOVATIVE CLAIM SUPPORT

In addition to standard *in vitro* methods including cell culture techniques and organotypic skin models, we offer an innovative approach that allows us to combine *in vivo* treatment via *ex vivo* sampling with a large range of available *in vitro* analyses. The obtained samples (tissue and tissue fluids) can be subjected to biochemical tests such as ELISA tests, immunohistological, histological and molecular tests:

IN VIVO **TOPICAL TREATMENT**

▼

EX VIVO **SAMPLES**
(Complete viable epidermis with intact basal cell layer plus interstitial fluid within the blister)

▼

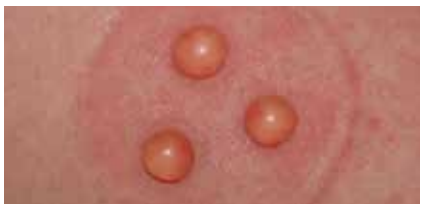
IN VITRO **ANALYSES**
(Biochemistry, cell biology, immunology, molecular biology, histology)

Sampling via punch biopsies provides epidermal and dermal material but requires a local anaesthetisation and leaves scars. The suction blister method, initially developed by Kiistala *et al.* in 1968, is a valuable alternative that is considered to be non-invasive.

Approved by the Ethics Committee in a general vote, we have established this method as a standard trial that allows us to obtain epidermal material for subsequent *in vitro* analyses.

As the name implies, blisters are artificially induced. Custom-made plexiglass chambers with round openings are attached to the respective test areas and connected to a vacuum pump:

Within approximately 2 hours, the epidermis is slowly detached from the underlying dermis. The induced blister fills with fluids from the surrounding tissue (interstitial fluid):



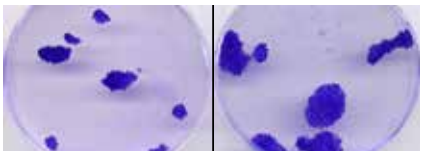
The procedure is painless – causing itching at worst – and the wounds heal without forming scars within 7 to 10 days. However, some subjects develop hyperpigmentation on the former wound that can last for some months or longer. After removal of the chambers, the blister fluid (interstitial fluid) can be extracted with a sterile syringe and the blister roof carefully removed using sterile instruments.

For the *in vitro* analyses, a large variety of methods such as immunohistological stainings and a continuously extended list of commercially available analysis kits are available.

Parameters that can be estimated using *in vitro* enzyme-linked immunosorbent assays (ELISA), immunohistological or other methods:

- Anti-pollution (smoke/UV model):
  Analysis of markers such as MMPs and/or interleukins (ELISA) or analysis of the lipid barrier (REM, TEM, HPTLC, in cooperation with Microscopy Services Dähnhardt GmbH)
- Structural proteins:
  Pro-collagen I as a measure for the *de novo* synthesis of collagen I, fibrillin and elastin (ELISA)
- Extracellular matrix:
  Hyaluronic acid, MMPs such as Matrix Metallo-proteinase-1 (ELISA)
- Oxidative stress:
  8-isoprostane as a marker for lipid peroxidation, carbonyl proteins as a marker for protein oxidation (ELISA)
- Cytokines:
  e.g. IL-6, IL-8 and TNF-alpha (ELISA)
- UV-induced DNA damage:
  Immunohistological detection of DNA damage induced by UV light (thymine dimers)
- Differential gene expression via Real Time Quantitative PCR
- Stem cell activation:
  Number of p63-positive epidermal stem cells by immunohistological analysis, colony forming efficiency by cultivation of epidermal stem cells (colony-forming assays)

Estimation of colony-forming efficiency by cultivation of epidermal stem cells (colony-forming assay)



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Please contact us for further information, consultation or a detailed proposal.

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