A full thickness skin equivalent for efficacy, toxicity and penetration studies

Mewes, K. R.[1], Bock, A.[1], Raus, M.[1], Goerg, K.[1], Prießner, A.[1], De Wever, B.[1], Zoeller, N. N.[1,2], Bernd, A.[2]

1 Phenion GmbH & Co.KG, Max-von-Laue-Str. 9, 60438 Frankfurt/Main, Germany,
2 Dept. of Dermatology and Venerology, University Hospital, Frankfurt/Main, Germany
contact: k.mewes@phenion.uni-frankfurt.de, phone: +49-69-798-29812

Introduction

In the framework of the EU cosmetics directive 76/768/EEC the 7th amendment sets a deadline concerning the marketing of cosmetic products which have been tested on animals. Therefore new test systems must be developed which, in the light of the 3Rs, will be used as alternatives to animal testing. They must fulfill the legal requirements for product safety and be able to assess and predict the efficacy of new products. Promising tools in this context are bioartificial skin models consisting of both an epidermis and a dermis. To contribute to the need to establish advanced non-animal test methods, we have developed a three-dimensional skin equivalent based on a new robust matrix material with properties comparable to native human skin.

Methods

Production of the skin model:
The skin equivalents consisted of a lyophilized and stabilized collagen matrix, which became successively inoculated with fibroblasts and keratinocytes isolated from human foreskin tissue. After a 3 weeks cultivation period under submersed conditions the skin models were cultivated at the air-liquid interface (ALI) for another 2 weeks.

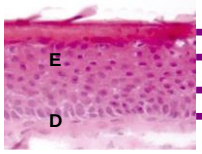
Immunohistochemistry:
Cryosections (8 µm) were fixed in ice-cold acetone, washed with TBS and incubated with the 1st antibody. The sections were washed again and incubated with the 2nd antibody, coupled to ALEXA Fluor 488®. The 2nd antibody solution also contained 0.1% Evans Blue for background reduction and DAPI (2 µg/ml) for nuclear staining.

Irritation of the skin models:
From each SDS solution (0.2 –1.0%) 100 µl were applied topically onto the skin models. After a 4 h incubation period at 37°C the models were washed gently with PBS and cultured 48 h at the air-liquid interface. Then the culture medium was harvested and stored for further analysis.

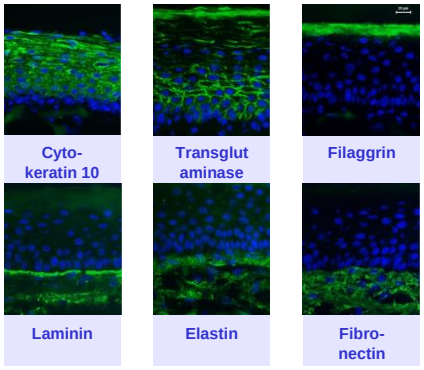
Penetration study:
Penetration studies were performed with Cy3-labelled oligonucleotides of different chain length (CpG-1, 20-mer, CpG-9, 6-mer; Biospring, Frankfurt, Germany) on a fluorescence microscope.

Penetration properties were also assessed by topically applying tritiated retinyl palmitate in two different formulations on skin models mounted in a "PermeGear In-Line Cell". Skin integrity was assessed with ^3H_2O. Tissue absorption and penetration were determined in a scintillation counter.
The Phenion Skin Model was compared with the "Damour-Model", a full-thickness skin equivalent based on a collagen-chitosan-glycosaminoglycane matrix (manufactured at Henkel KGaA).

Results



Skin model after 12 days of culture at the air-liquid interface.
An **epidermis** (E) consisting of the characteristic cell layers including a cornified layer has formed. The **dermal compartment** (D) contains fibroblasts in a newly synthesized network of extracellular matrix proteins.

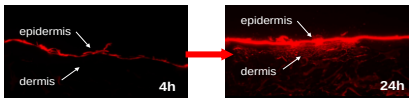


Immunofluorescence analysis of differentiation markers in the skin model (day 12 of ALI).

Epidermis: CK10 and transglutaminase are expressed in suprabasal keratinocytes, filaggrin in the late S. granulosum and S. corneum.

Basement membrane: Laminin 5, a major BM protein, is expressed at the dermo-epidermal junction.

Dermis: Elastin-containing fibres and fibronectin fibres are deposited in the dermal compartment of the skin model.



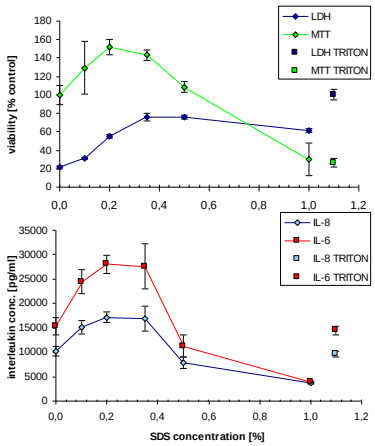
Uptake of CpG-1 (long chain) after topical application by the Phenion® Skin Model.

Long-chain CpGs (20-mers) penetrate the skin model slowly. After 4 h, most molecules are retained in the S. corneum, only after 24h the molecules can be detected in the viable cell layers.

Short-chain CpGs (6-mers) penetrate the epidermis in 4h (not shown).

	Epidermis [%]	Dermis [%]	Receptor fluid [%]	Recovery [%]
Retinyl palmitate [^{3}H] in Cetiol B	0.13	0.07	1.03	102.5
Retinyl palmitate [^{3}H] in Diadermine	0.21	0.43	1.49	93.9
^3H_2O in PBS	0.01	0.64	90.0	91.5

Penetration rates and percutaneous absorption for **retinyl palmitate** are comparable to those of a skin model based on the "Damour Model". Absorption from Diadermine is better than from Cetiol B. **Water** penetrates at half the rate of the "Damour Model", but at a much bigger rate compared to pig skin (not shown).



Influence of topically applied irritant solutions (SDS) on the viability and interleukin secretion of the skin model.
Viability was assessed with an MTT assay (mitochondrial enzyme activity) and with the Cytotoxicity Detection Assay (leakage of LDH; *Roche Applied Science*).
Secretion of **IL-6 and IL-8** simultaneously increased with increasing SDS concentrations, but then dropped abruptly at concentrations more than 0.35% SDS. Both interleukins were detected with ELISA kits (*Bender MedSystems*).
positive control: 1% TRITON X-100
negative control: culture medium (0.0%)
data point: mean ± SD (n = 3)

Conclusions

The Phenion® Full Thickness Skin Model shows properties comparable to the human skin.

- The epidermis consists of all four layers typical for healthy human skin including a well-developed S. corneum.
- All differentiation markers are properly expressed in the appropriate compartment of the skin equivalent.
- An elastic network is established in the dermal compartment.
- Viability and IL secretion are valuable parameters to assess the irritating and cytotoxic potential of topically applied substances. The physiological response of the skin model towards substances of different irritating potential varies in a concentration-dependent manner.
- Topically applied substances can penetrate the skin model dependent on their physico-chemical properties.

Therefore the Phenion® skin equivalent represents a promising tool to study toxicological and efficacy aspects related to skin.

Topical application of formulations on the skin equivalent