Lipid analysis of the Phenion® FT Skin Model and the OS-REp Model – the molecular basis for a functional skin barrier

Cuevas, A.J[1]., Vierkotten, L[2]., Fischer, A[2]., Merkel, M[2]., Blasius, N[2]., Görtz, G[2]., Beyer, T[2]., Petersohn, D[2]., Mewes K.R[2].

Henkel AG & Co. KGaA: [1]Henkel Corporation Beauty Care North America, Stamford CT, USA; [2]Biophysics & Biological Research, Düsseldorf, Germany

Introduction

Any reactive agent, e.g. cosmetic or pharmaceutic ingredients or environmental noxious factors, must penetrate the skin barrier to elicit reactions in epidermis and dermis, respectively. The skin barrier is mainly defined by the stratum corneum, comprising of crosslinked corneocytes embedded in highly structured layers of lipids. Synthesis and deposition of the various lipids are orchestrated by specific intra- and extracellular enzymes synthesized by the keratinocytes of the upper epidermal layers. Structural proteins like involucrin stabilize the corneocytes by generating the so-called cornified envelope.

Three-dimensional tissue models of the skin are a state-of-the-art alternative to animal testing for toxicological safety assessment, product development and basic research. Using 3D *in vitro* skin models methods, like skin irritation or dermal absorption assays, requires an in-depth understanding about their barrier properties and the underlying molecular processes, which is the subject of the following study.

Two (2) different 3D skin equivalents: the **Phenion Full-Thickness (FT) Skin Model**, and the **Open Source Reconstructed Epidermis (OS-REp)** were used to investigate the following:

- epithelial **lipid** composition
- expression of 2 key enzymes of dermal lipid synthesis, **serine palmitoyl transferase (SPT)** and **β-glucocerebrosidase (GBA)**
- expression of **involucrin (IVL)**, a component of the cornified envelope

3D Tissue models

The Phenion Full-Thickness Skin Model comprises both a dermis and an epidermis, both generated from primary human skin cells (Mewes et al., 2007; **Fig. 1a**). The dermal fibroblasts, embedded in a mechanically stable collagen sponge, synthesize major extracellular matrix proteins like collagens, elastin and fibrillin-1. The epidermis consists of all 4 layers typical for native human skin, including a multi-layered stratum corneum. All tissues are metabolically active, a prerequisite for the development of the 3D Skin Comet assay for the *in vitro* genotoxicity assessment (Reisinger et al., 2018).

Figure 1a

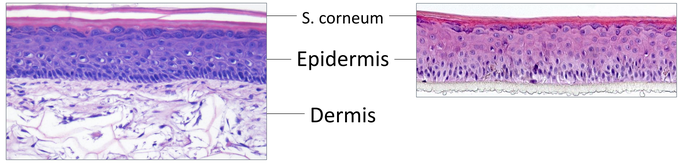
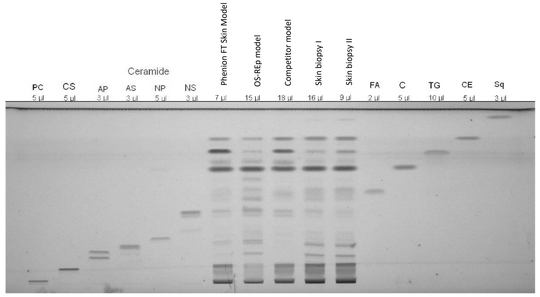


Figure 1b

The Open Source Reconstructed Epidermis model (OS-REp) is generated from primary human keratinocytes cultured in co-culture inserts under air-liquid interface conditions (Mewes et al., 2016; **Fig. 1b**). Its tissue architecture is similar to native human skin and to the FT skin model epidermis. The OS-REp model was validated for *in vitro* skin irritation testing according to OECD TG 439 (Groeber et al., 2016).

Lipid profiles of 3D skin equivalents



Abbreviations:

CE	cholesterol ester
C	cholesterol
TG	triglycerides
FA	fatty acid
NS	non-hydroxy-FA- sphingosin
NP	non-hydroxy-FA- phyto sphingosin
AS	hydroxy-FA-sphingosin
AP	hydroxy-FA-phyto sphingosin
CS	cholesterol sulfated
PC	phosphatidylcholin
Sq	squalen

Figure 2: All major lipid classes were present in the epidermal compartments isolated from skin models and human skin. The lipid profile seen in the Phenion FT Skin Model and the OS-Rep Model matched the lipid pattern found in native human skin (biopsies I and II), with some ceramides being more prominent in the OS-REp model. In contrast, most ceramides were lacking in the epidermis of a competitor skin model.

Methods: Lipids from the isolated epidermal tissues of the skin models were extracted according to Bligh & Dyer (1959). The lipids were then separated by automated multiple development thin-layer chromatography (AMD- TLC). For identification of the dermal lipids, pure lipids, representing the major lipid classes found in native human skin, were applied to the TLC, too. All samples contained equal amounts of tissue homogenate.

SPT, GBA and IVL in monolayer keratinocytes

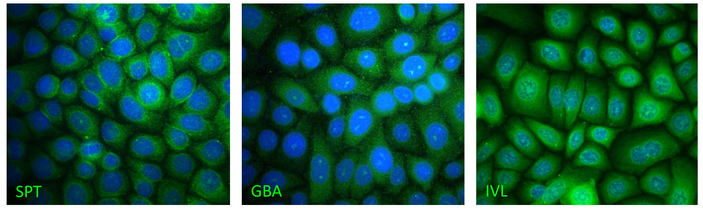


Figure 3: Serine palmitoyl transferase (SPT) and glucocerebrosidase (GBA), key enzymes of the lipid metabolism in the terminally differentiating epidermis, were detected intracellularly in keratinocytes under 2D culture conditions with specific antibodies directed against the enzymes. Both enzymes reveal a granular pattern within the cytoplasm (green signals), with a more intense expression adjacent to the nuclei seen for SPT. Involucrin (IVL) is homogeneously distributed in the cytoplasm. The nuclei remain free of signals.

Methods: Primary human keratinocytes were grown in monolayer cultures until sub-confluency before being used to construct the 3D skin models. Immunostaining with anti-serine palmitoyl transferase, anti- glucocerebrosidase and anti-involucrin antibodies, the nuclei were counterstained with DAPI (blue).

SPT, GBA and IVL in 3D skin equivalents

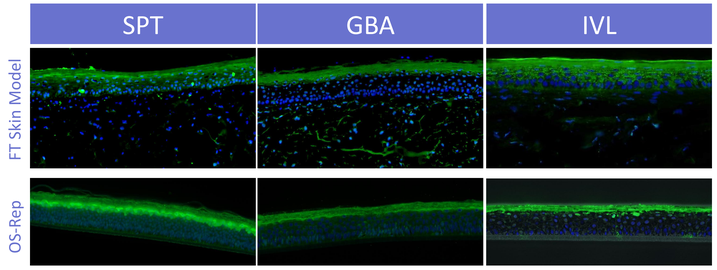


Figure 4: In both, the Phenion FT Skin Model and the OS-REp model, glucocerebrosidase, serine palmitoyl transferase and involucrin were detected in the epidermal compartment. GBA was confined to the S. corneum, SPT- positive signals were visible in the S. granulosum and the S. corneum. Involucrin was prominent in the suprabasal keratinocytes in the FT skin model and in S. granulosum and corneum in the OS-REp-model.

Methods: The full-thickness and epidermal models were cultured under air-liquid interface conditions until the epidermis was fully differentiated. Immunostaining on cryosections with anti-serine palmitoyl transferase, anti- glucocerebrosidase and anti-involucrin antibodies, the nuclei were counterstained with DAPI (blue).

Summary and conclusions

- The epithelial lipid patterns of the Phenion FT Skin Model and the OS-REp Model resemble the pattern found in healthy human skin. All major lipid classes are present in the respective skin equivalents.
- The enzymes SPT and GBA as well as the structural protein involucrin are expressed in the upper strata of both 3D tissue models.
- All 3 proteins can already be detected in human keratinocytes cultured under 2D conditions. The granular structure indicates towards enzyme localization in the endoplasmic reticulum (for SPT) and in the lamellar bodies (for GBA).

Taken together, this study reveals the presence of pivotal physiological features essential for the generation of a distinct barrier function in the 3D equivalents of the human skin. Thus, both skin equivalents are well suited for conducting *in vitro* tests like dermal absorption studies and skin irritation and sensitization assays, respectively, which depend on a functional dermal barrier.

Cited literature:

Mewes KR et al., Skin Pharmacol Physiol. 2007; 20:85-95; Mewes KR et al., Toxicol In Vitro. 2016; 36:238-53; Groeber F et al., Toxicol In Vitro. 2016; 36:254-61; Reisinger K et al., Mutat Res. 2018; 827:27-41; Bligh EG and Dyer WJ, Can J Biochem and Physiol, 1959, 37(8): 911-917