The molecular basis for a functional dermal barrier in two biotechnologically produced human skin equivalents: the Phenion® FT Skin model and the OS-REp model

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Introduction

Biotechnologically produced 3D skin equivalents are the state-of-the-art tools to study human skin physiology and pathology under standardized conditions *in vitro* and to replace animal experiments for the toxicological assessment of chemicals. In healthy human skin a functional and selective barrier, mainly located in the stratum corneum, discriminates between chemicals which penetrate the skin and subsequently reach the deeper tissue layers, or which remain on the tissue surface without any effect on the skin. Thus, lipid composition and structure of the dermal barrier are crucial for the access of chemicals into the skin and subsequently influence all downstream reactions, both in vivo and in 3D tissue models. Gaining a deeper understanding about the molecular basis of the barrier in 3D skin models is the subject of this study.

Two (2) different 3D skin equivalents, the Phenion Full-Thickness (FT) Skin Model, and the Open Source Reconstructed Epidermis (OS-REp), were analyzed in respect of their

- epithelial lipid composition
- expression of 2 key enzymes of dermal lipid synthesis, serine palmitoyl transferase (SPT) and β-glucocerebrosidase (GBA)
- expression of involucrin (IVL) and loricrin (LOR), components 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 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).

Figure 1a

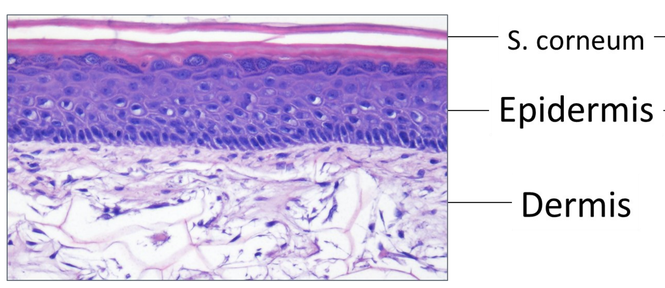
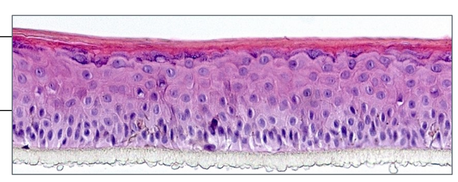


Figure 1b



SPT, GBA and IVL in monolayer keratinocytes and 3D skin equivalents

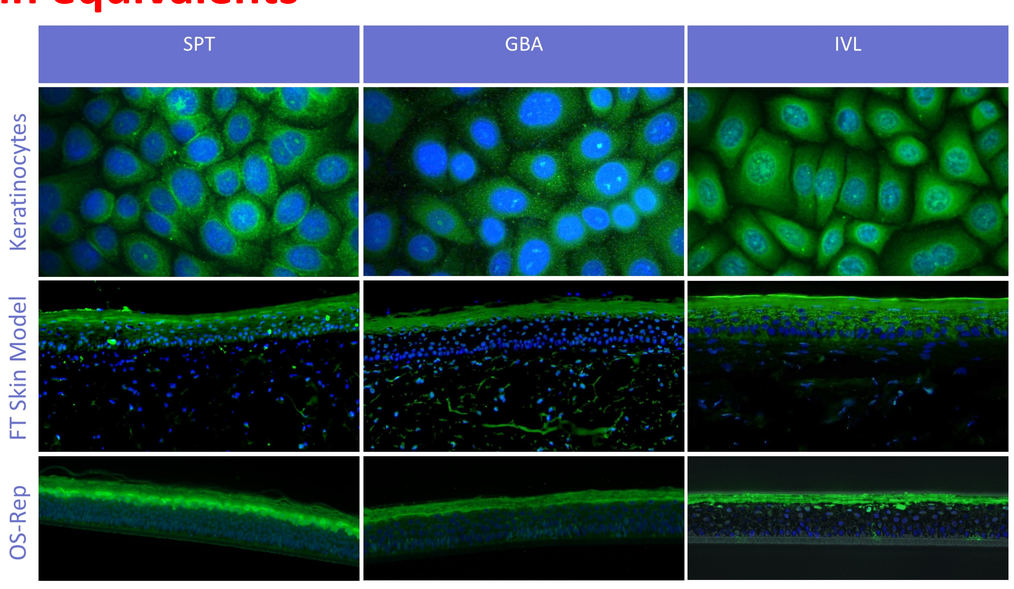


Figure 2: Serine palmitoyl transferase (SPT) and glucocerebrosidase (GBA), key enzymes of the lipid metabolism in the terminally differentiating epidermis, as well as involucrin (IVL) were detected intracellularly in keratinocytes under 2D culture conditions with specific antibodies directed against the enzymes (green fluorescence). The nuclei remain free of signals.
In both 3D skin models, 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: Primary human keratinocytes were grown in monolayer cultures until sub-confluency before being used to construct the 3D skin models. The full-thickness and epidermal models were cultured under air-liquid interface conditions until the epidermis was fully differentiated. Immunostaining with anti-serine palmitoyl transferase, anti- glucocerebrosidase and anti-involucrin antibodies (green), the nuclei were counterstained with DAPI (blue).

Expression of SPT, GBA, and LOR genes in OS-REp models and native human epidermis

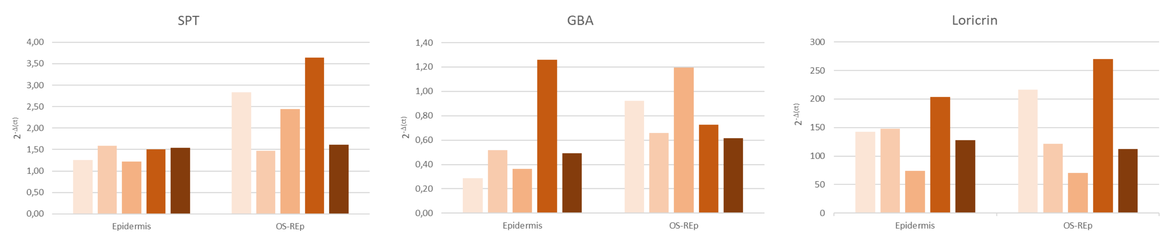
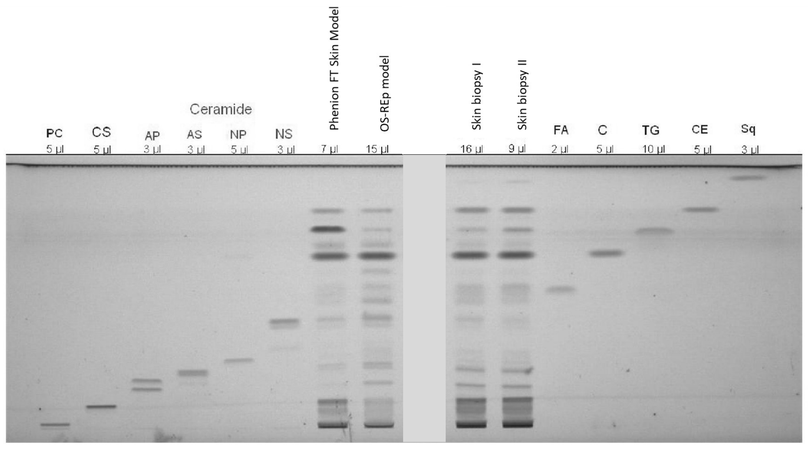


Figure 3: The expression of the SPT, GBA, and LOR genes was determined for OS-REp models generated from keratinocytes of 5 different donors and compared with the gene expression in the respective native epidermal tissues before keratinocyte isolation. All three genes are expressed in the epidermis as well as in the OS-REp models, with LOR being expressed at a much higher level than SPT and GBA. For all three genes, the expression levels in the native epidermis and the OS-REp are comparable. However, they reveal significant donor variabilities, which can have an impact on the OS-REp performance in *in vitro* assays.

Methods: mRNA was extracted from the samples, reverse transcribed, and subjected to real- time PCR. All expression data were normalized against G6PDH.

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 4: 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.

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. Applied volumes of homogenates were adjusted referring to tissue weight.

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.
- SPT, GBA and LOR genes are expressed in the differentiated OS-REp models at comparable levels as in the native human epidermis. The gene expression profiles show donor variabilities.

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.

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