Expression of metabolic systems in a newly developed full-thickness skin equivalent

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Introduction

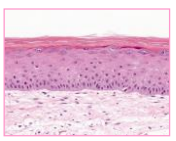
Reconstructed human skin equivalents gain importance as animal-free alternative to assess toxicological effects and the efficacy of raw materials, cosmetics and pharmaceuticals. We developed a multilayered human full-thickness skin equivalent consisting of a fully stratified epidermis and a dermis rich in extracellular matrix (ECM). In order to show resemblance to native skin we analyzed the expression of two separate enzyme systems: the ECM-degrading matrix metalloproteinases (MMPs) and the phase I and II enzymes of the xenobiotic metabolism.

MMPs are synthesized as zymogens (pro-MMPs) and become activated by defined proteolytic cleavage after secretion into the extracellular space. There they play a major role in the metabolism of the ECM and hence in skin ageing [1-3].

The skin metabolism does have a great influence on several toxicological endpoints particularly those which integrate the skin as first site of contact like sensitization or genotoxicity. Several phase I and phase II enzymes are involved in metabolism of a wide range of exogenous compounds [4].

Methods

Production of the skin model:
The skin equivalents consisted of a lyophilized collagen matrix and fibroblasts and keratinocytes isolated from human foreskin tissue. After 3 weeks of cultivation under submersed conditions the skin models were cultivated at the air-liquid interface (ALI) for another 2 weeks [5]. Epidermal models were generated according to Poumay et al. (2004) [6].



Immunofluorescence:
Cryosections (8 µm) were blocked with 10% normal goat serum (NGS) and incubated with the 1st antibody. The sections were washed and incubated with the 2nd antibody, coupled to Alexa Fluor 488®. Both antibody solutions contained 10% NGS for effective background reduction. Nuclei were counterstained with DAPI (2 µg/ml).

Western Blotting
Cell culture media were subjected to SDS-PAGE on gradient gels (4-12.5%; Invitrogen). For molecular weight determination the MagicMark™ XP Standard (Invitrogen) was used. The separated proteins were transferred onto nitrocellulose membranes by electroblotting at 170 mA for 2h. After blocking with 5% milk and incubation with the 1st antibody the blots were further processed for chemiluminescence detection using the ECL-Plus-Kit (Amersham).

Compartment separation:
To separate the dermis from the epidermal part Phenion® Full Thickness Skin Models or human foreskin were incubated with dispase for 3h at 4°C.

Quantitative Real-Time PCR:
2µg of total extracted RNA were transcribed to cDNA with Omniscript®-RT Kit (Qiagen). Finally 1µl cDNA was amplified using the TaqMan gene expression assays (Applied Biosystems). All data were normalized to beta-actin.

Conclusions

The presence of MMPs and several phase I and II enzymes corroborate the high grade of similari-ty between the bioartificial skin equivalents and the native human skin. Therefore our model represents a promising tool to study questions of toxicology and efficacy related to skin *in vitro*.

MMP expression after UVA irradiation

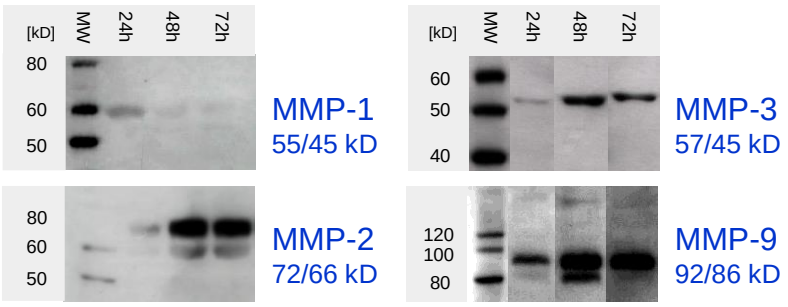


Fig. 1: MMPs were detected by immunoblotting in the supernatants of cultured and UVA-irradiated skin equivalents (30 J/cm² UVA). MMP-1, -2, -3 and -9 could be detected at a basal level either as the proenzyme or both, proenzyme and active enzyme, under standard cultivation conditions (not shown here). After UVA irradiation the amount of secreted inactive pro-MMP-2, -3 and -9 increased during cultivation with an optimum around 48h. Also the fractions of converted activated enzymes increased markedly for MMP-2 and -9. Pro-MMP-1 revealed its highest expression 24 h after irradiation, then the concentration in the medium decreased. For MMP-3 only the inactive zymogen could be detected.
These results indicate that under environmental stress situations MMPs are inducible in our *in vitro*-system in a pattern which is discussed in native human skin as well.

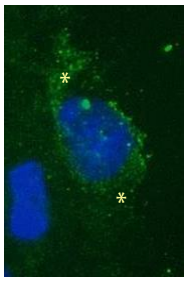


Fig. 2: MMP-2 (gelatinase A) could be detected in the dermal fibroblasts of the full-thickness skin model after UVA irradiation (30 J/cm²). The MMP-2-positive material appears in the shape of tiny granules scattered throughout the cytoplasm of the fibroblasts.
Immunostaining on cryosections with MMP-2 antibody (green, asterisks); nuclei counterstained with DAPI (blue).

Xenobiotic Metabolism

Basal gene expression

	Phenion® Full Thickness Skin Model		Phenion	Native Skin	
	Dermis	Epidermis	Epidermal Model	Dermis	Epidermis
CYP1A1	-/-/-	+/-/-	++/+	+/+	++/++
CYP1B1	+++/++/+++	+/+/++	++/+++	++/+++	++/++
CYP2B6	-/-/-	-/-/-	-/-	-/-	-/-
CYP2D6	-/-/-	-/-/-	-/-	-/-	-/-
CYP2E1	+/+/+	++/++/++	+/+	++/+	++/++
CYP2S1	+/+/+	+/+/++	++/++	++/++	++/++
CYP3A4	+/+/+	+/+/+	-/+	+/+	+/+
CYP26A1	-/-/-	-/-/-	-/-	-/-/+	-/-/+
FMO1	++/+/++	+/+/++	-/+	++/++	+/++
FMO3	+/+/+	-/-/-	-/-	++/++	-/-
FMO5	+/+/+	++/++/++	++/++	++/++	++/+++
GSTp1	++/++/+++	++/++/+++	+++/+++	++/++	++/++

Fig. 3: Comparison of basal gene expression levels between tissues, - (not detected), + (ΔC_T to beta-actin control > 10), ++ (ΔC_T = 5-10), +++ (ΔC_T < 5). Full Thickness data are from 3 donors; Epidermal Model and native skin data are from 2 donors. The dermis and the epidermis show distinct metabolic characteristics. Particularly the FMO3 is not expressed in the epidermal compartments. Altogether these results show that both skin models display metabolic expression patterns similar to native human skin.

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Literature

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