Skin absorption developments

Relevance of current test systems to man

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„Skin Models“

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Monolayer cultures (2D)
keratinocytes
fibroblasts (phototoxicity)
immune cells (sensitization)

Organotypic constructs (3D)
- reconstructed normal
  - human epidermis (RHE)
  - full-thickness skin (RHS)
- disease models

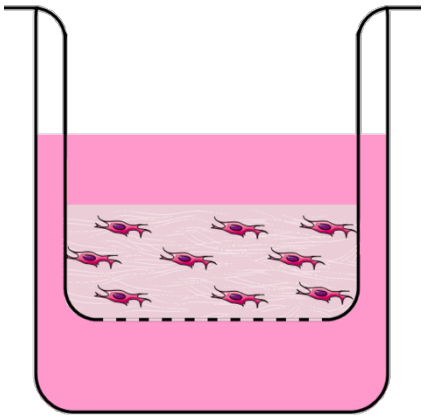
Human skin *ex vivo*

Pig skin

(Per-)cutaneous absorption rat skin

	Hydro-cortisone	Testo-sterone
log P	**1.61**	**3.3**
% Permeation, hairless rat skin, *ex vivo*		
normal	4.8	39.0
scarred	0.8	2.8
% Penetration hairless rat skin, *ex vivo*		
normal	3.3	12.0
scarred	2.0	5.1

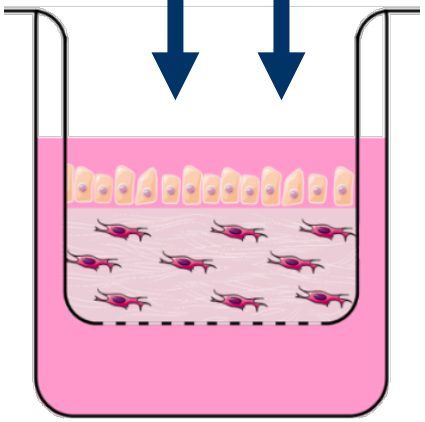
Reconstruction (RHS, RHE)



1: Embedding fibroblasts in collagen matrix

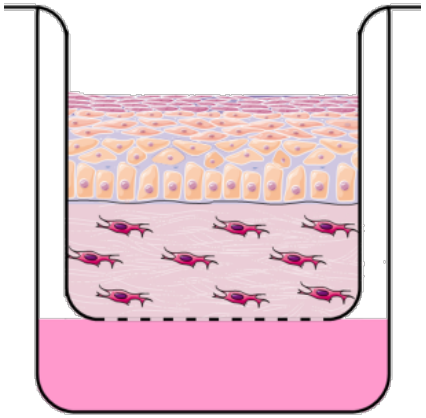
2: Seed keratinocytes

Co-culture
Tumor cells / microorganisms



3: Airlift

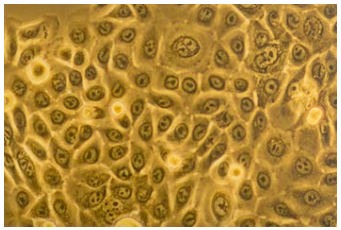
exposure to cytokines



Disease models:

(Lesional skin)

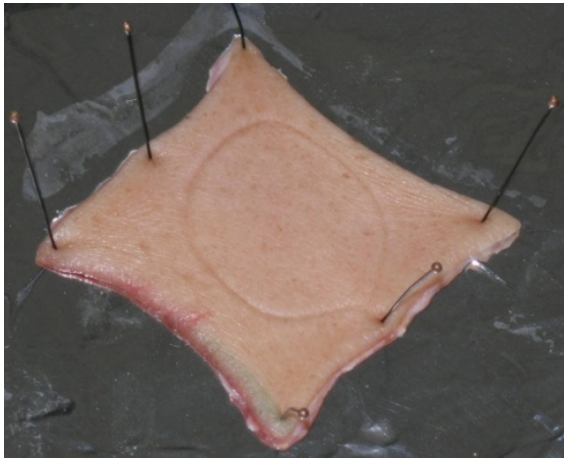
Physical/chemical damage
Co-culture
Transfection



Transfection/siRNA

Skin Penetration (OECD 428)
Applicability to RHE

Compound	Mol. Mass	log D
Mannitol	182.2	-4.67
Benzoic acid	122.1	-1.25
Caffeine	194.2	-0.08
Nicotine	162.2	0.02
Digoxine	780.9	1.14
Flufenaminic acid	281.2	2.03
Testosterone	288.4	3.47
Clotrimazole	344.8	5.74
Ivermectin	875.1	6.82



BMBF funded study

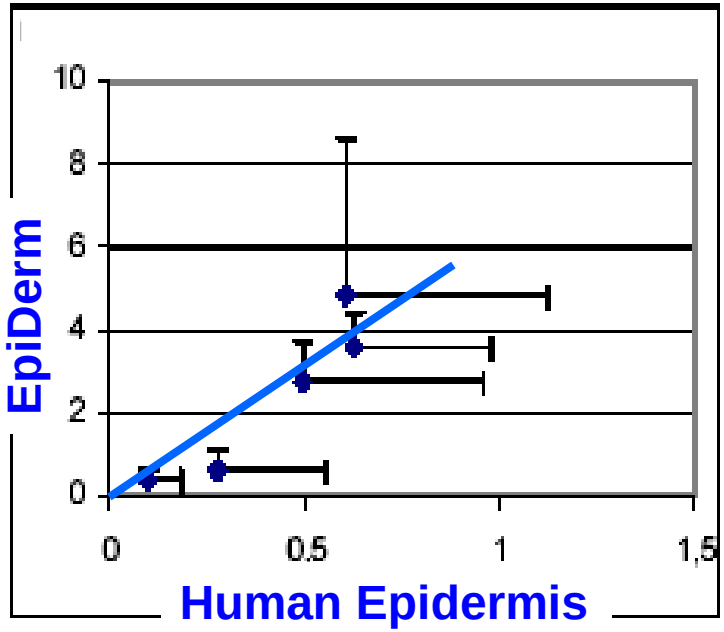
Schäfer-Korting et al, ATLA 2008

Results Infinite-Dose-Study:

P_{app} values

Pearson Correlation		r^2
Pig skin	EPISKIN®	0.920
Pig skin	SkinEthic®	0.918
Pig skin	EpiDerm™	0.842
Pig skin	HES	0.861
HES	EPISKIN	0.707
HES	SkinEthic	0.803
HES	**EpiDerm**	**0.932**
EpiDerm	EPISKIN	0.637
EpiDerm	SkinEthic	0.853
SkinEthic	EPISKIN	0.906

Scatter Plot



Schäfer-Korting et al., ATLA 2008

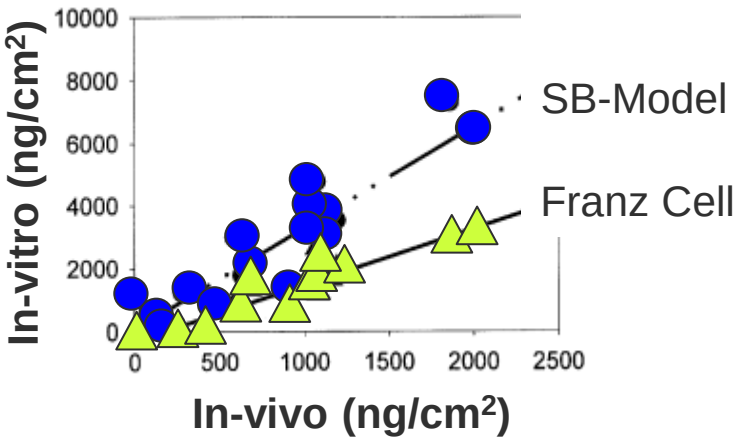
Antimicrobial peptides + cleavage products penetrate human skin

Do et al, J Exp Dermatol 2014

Predictability for Human

Human skin *ex vivo* is predictable:
Bronaugh et al, J Pharm Sci 1986
Hotchkiss et al, Food Chem Toxicol 1992
Van de Sandt et al, Toxicol Sci 2002
Wagner et al, JID 2002
Herkenne et al, JID 2007

Flufenaminic acid penetration of deeper abdominal skin layers; pre- and post surgery

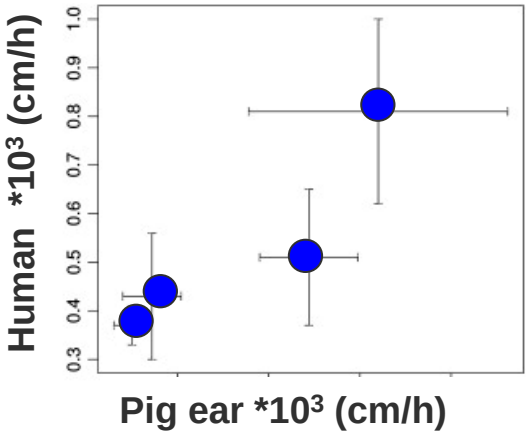


Wagner et al, JID 2004

Rat skin *ex vivo* is not:
Human skin is less permeable, differences not determined by mol. mass, logP, aq. solubility

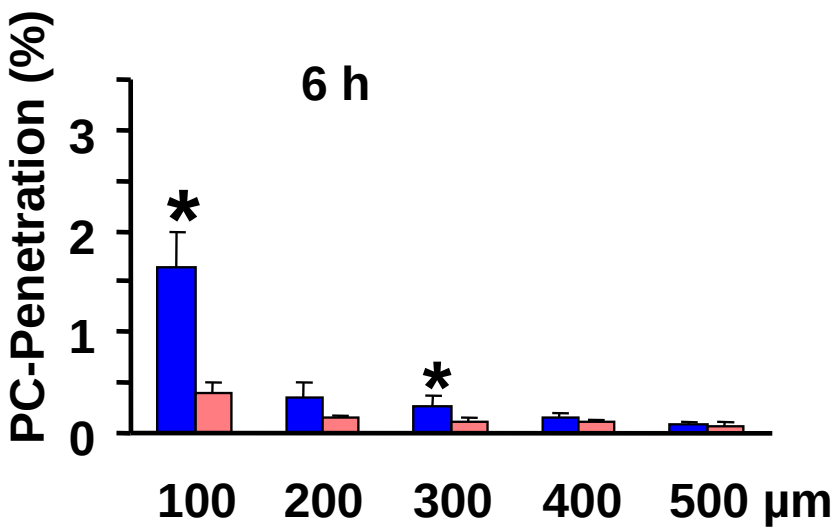
Van Ravenzwaay & Leibold, TiV 2004

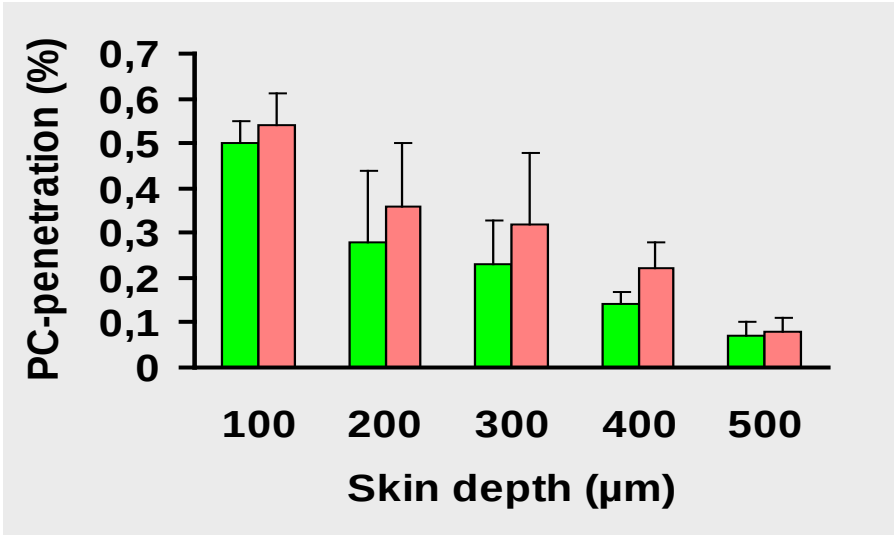
In vivo/in vitro ibuprofen permeability coeff. (4 formulations)



Herkenne et al, JID 2007

Skin Targeting





Clinical Studies:

Clobetasol propionate
Kalariya et al., 2005

TCA Liposomes
Fesq et al, 2003

Santos Maia et al.,
J Drug Target 2002

Biotransformation capacity

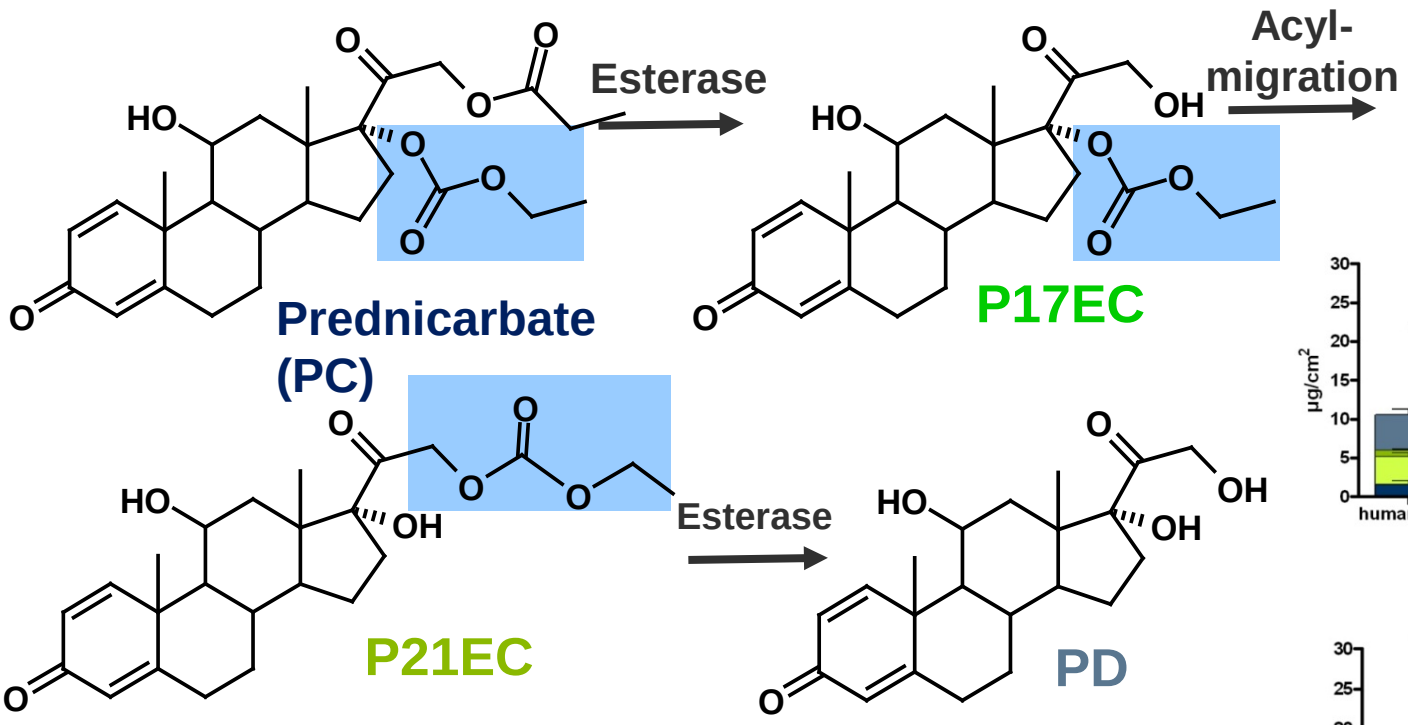
Testing in the Franz cell is inadequate –
mechanical stress induced results in major decline of enzyme activity

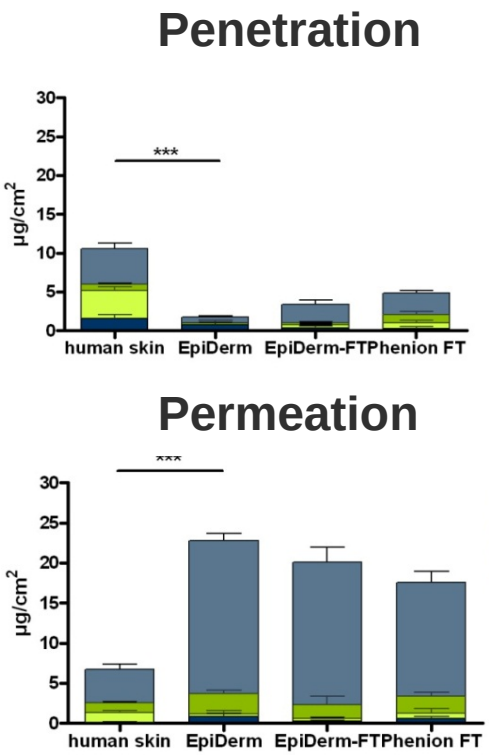
human keratinocytes, RHE, RHS and human skin ex vivo:
Benzo{a}pyren biotransformation results in DNA-adducts and genotoxicity (Micronucleus Test)

Henkler et al, 2012; Brinkmann et al (2013)

BMBF funded study on agrochemicals, drugs, consumer products

Prednicarbate Biotransformation

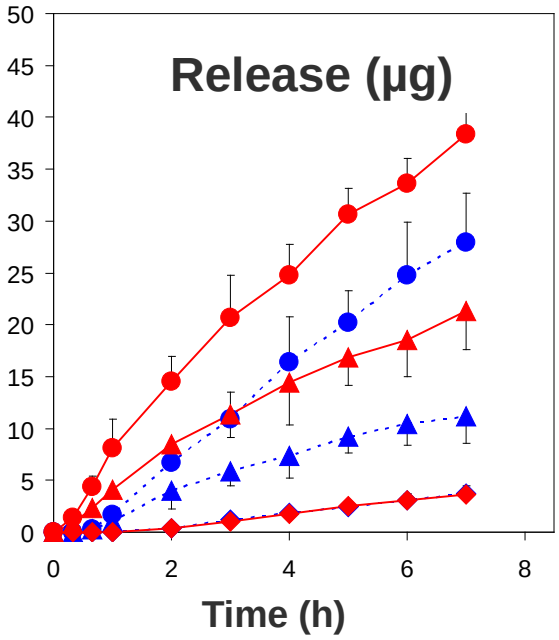


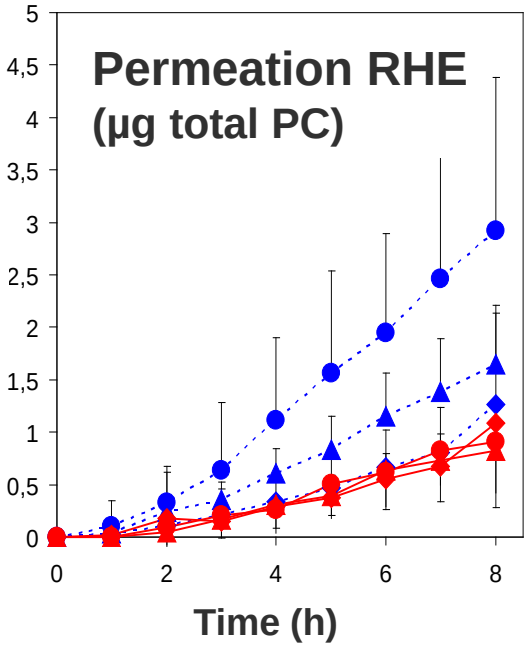


Keratinocytes > > Fibroblasts
EpiDerm ≈ EpiDerm FT ≈ Phenion-FT ≥ Human Skin

Gysler et al, Pharm Res (1999); Bätz et al EJPB (2013)

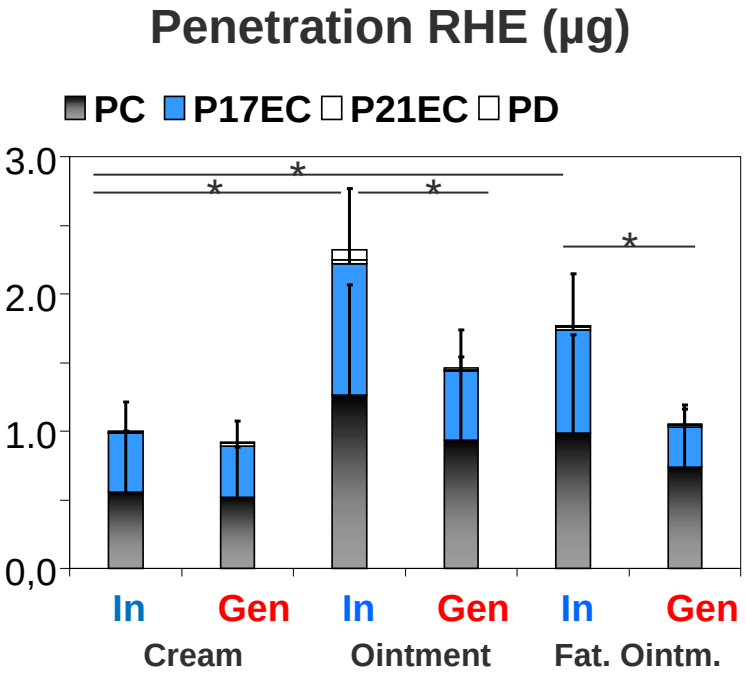
Influences on Dermal Absorption:
Formulation & Prednicarbate Biotransformation





Cream ◆◆, Ointment ●●, Fatty Ointment ▲▲

Prednicarbate 1.25 mg



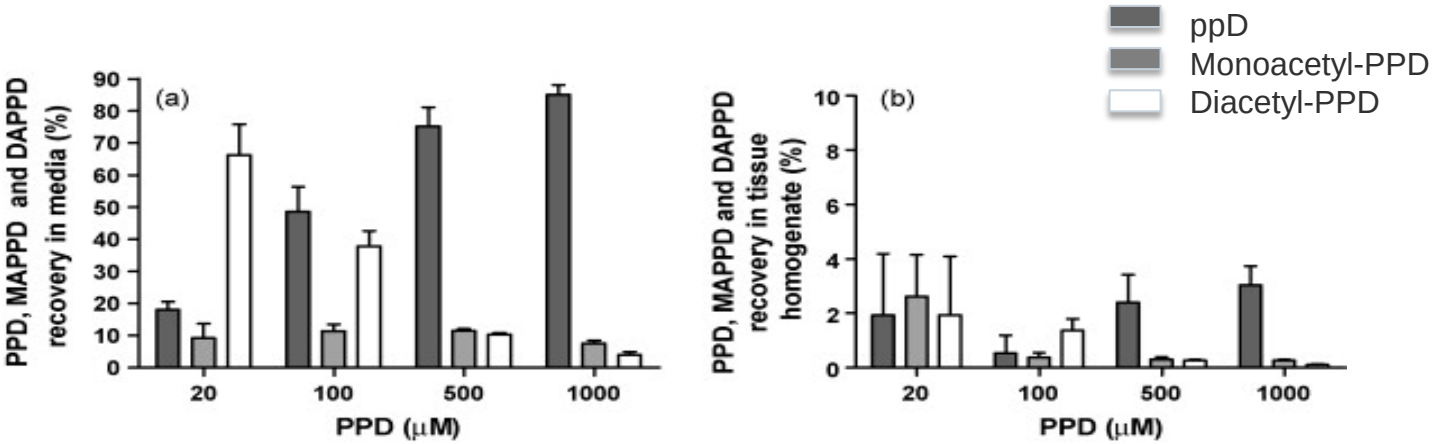
Lombardi Borgia et al, EJPB (2008)

p-Phenylendiamine
RHE penetration and acetylation

EpiDerm (in vitro)

PPD penetration is 10-20% after 0.5 h topical application of the hair dye ingredient

PPD and its acetylated metabolites in media and tissue following 24 h exposure via cell culture media

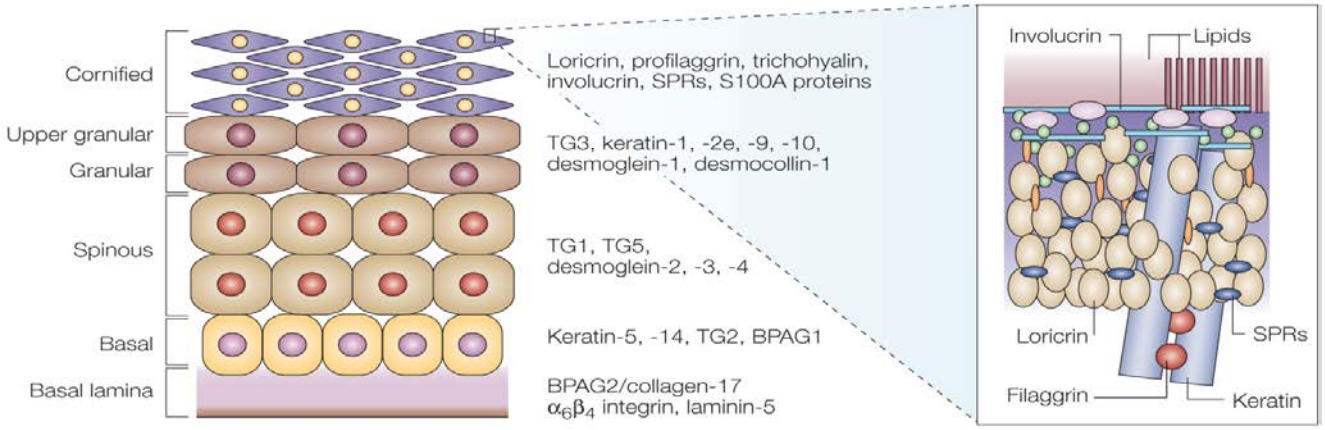


Penetration of human skin in vivo after 0.5 h: < 0.25 %

T. Hu et al, Toxicol Lett 2009

Diversity: Skin Disease

Epidermial Differentiation and Apoptosis



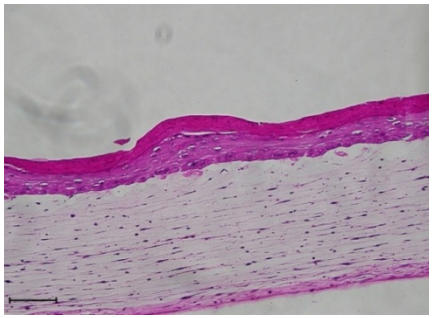
Candi et al, Nat Rev Mol Cell Biol 2005

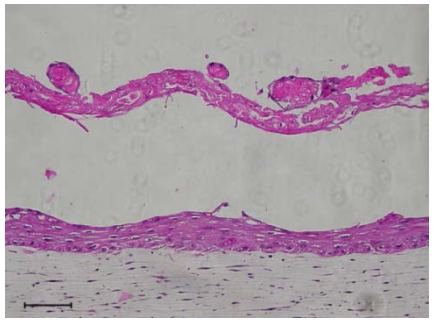
Atopic Dermatitis

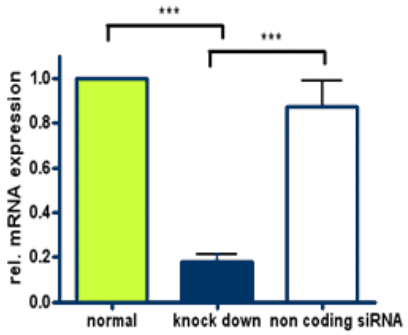
Filaggrin Gene	**Non-functional**
Skin lipids	**Less ordered**
Cytokines	**IL-4, IL-13,** IL-25, IL-31, **TNF**
Environmental factors	Dry air **Irritants** (water; tape stripping) stress, age, bacteria, fungi

Thyssen & Kezic, J Allergy Clin Immunol 2014

RHS: Barrier deficient Model
FLG kd (Atopic dermatitis “like”)

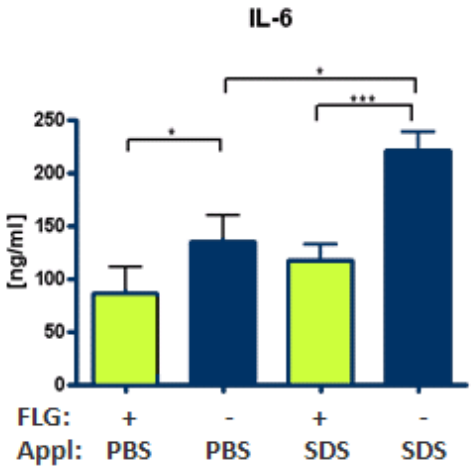






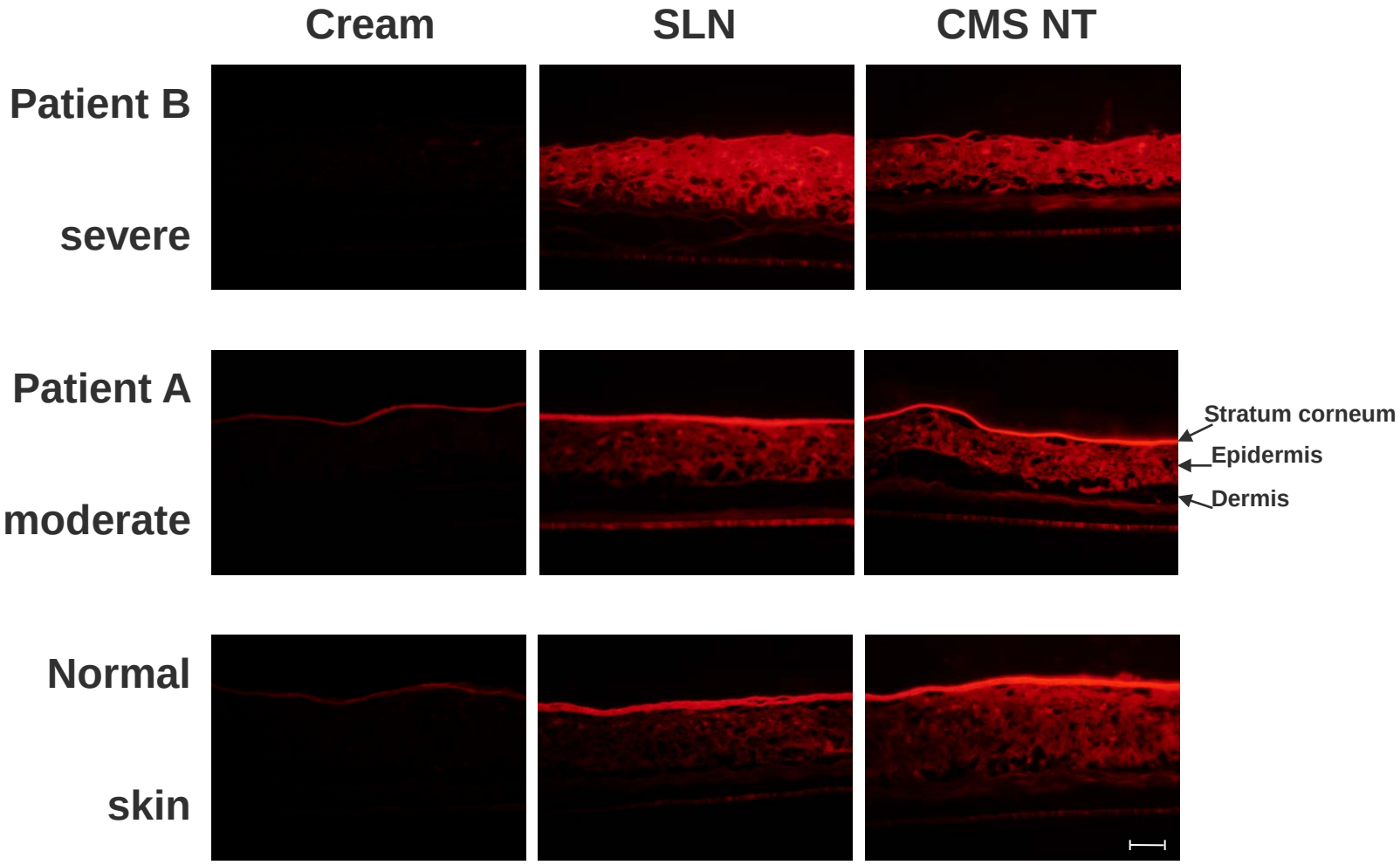
Stratum corneum:
- free fatty acids enhanced
- lipids less ordered

Skin surface pH 5.5



Küchler et al., ATLA (2011)
Vávrová et al, JID (2014)

Skin disease:
Congenital Ichthyosis Model



Do et al, Exp Dermatol 2014

Key-Take-Aways

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(Per-)cutaneous absorption

- In vitro studies on human skin ex vivo, RHE, RHS and pig (ear) skin predict human in vivo
- Skin integrity is essential, no surface contamination
- Viability is not essential, if dermal biotransformation is of no relevance
- Nanoparticles can enhance skin penetration and induce targeting

Biotransformation capacity of human skin

- Lower than the liver, yet relevant and often still neglected
  can detoxify and toxify xenobiotics and activate prodrugs
- Enzymes deteriorate under prolonged storage and stressful test conditions (no Franz Cell, acceptor: culture media)
- Predictable by RHS, product specific differences may exist

Neglected topic is diversity

Sex, age, disease ...

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