PKDerm
Innovative solutions

Use of alternative skin models for animal-free safety and efficacy testing in dermatology research

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SaferWorldbyDesign Webinar

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PKDERM company

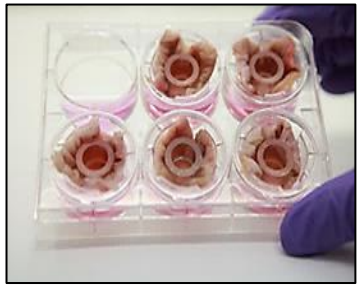
PKDERM is a French SME committed to help developing the most efficacious and safest product to the patient and consumer

Based on honesty, transparency and agility, PKDERM provides smart innovative *in vitro* solutions to evaluate the efficacy and safety of products likely to come into contact with the skin

Our partners: pharmaceutical, cosmetic, chemical and agrochemical industries

www.pkderm.com

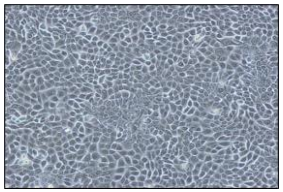
https://www.linkedin.com/company/40768033/admin/



EXPERTISE & SERVICES IN DERMAL EFFICACY AND SAFETY TESTING

In-vitro Efficacy
Dermal absorption
Anti-inflammation
Skin ageing, Skin Pigmentation
Wound Healing

In vitro Safety
Irritation (skin and eye)
Sensitization (partnership with SenzaGen)
Phototoxicity & Cytotoxicity

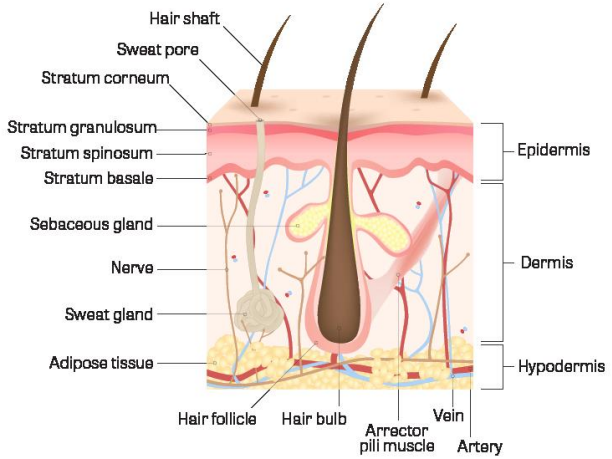


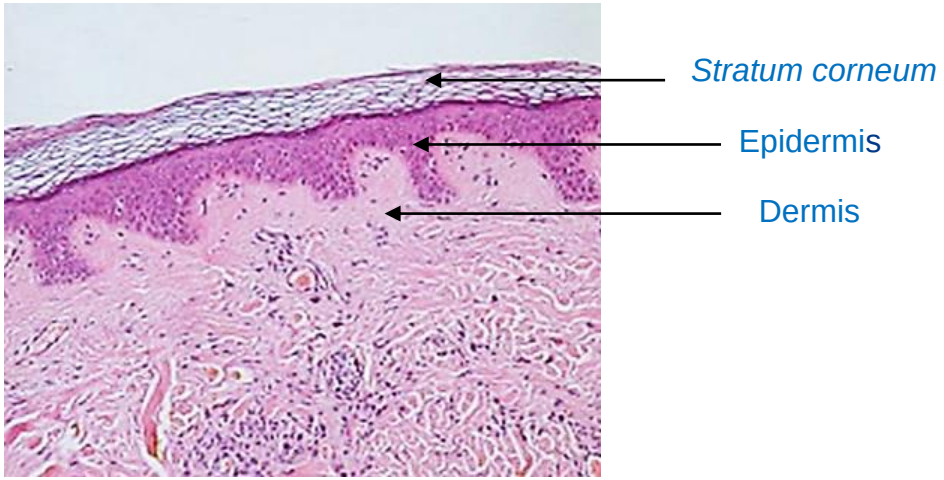
AVAILABLE SKIN MODELS

Excised human skin
3D human skin equivalent
3D human skin microtissues
2D human skin cell culture (Keratinocytes, fibroblasts, melanocytes)

Human skin

- Skin is the largest organ of the body
  - 2 m² surface area
  - 0.5 – 4 mm thickness
- Skin is the boundary between the environment and the organism, plays a crucial role in body protection





https://www.uihere.com/free-cliparts/human-skin-anatomy-hair-follicle-human-body-hair-6543385/download

Strategies for skin protection and defense

- Today a lot of active ingredients are commonly incorporated into skin care products to combat the effects of pollution and protect human skin against environmental pollution
- Skin care products represent the largest segment of the global beauty industry and can have different claims:
  - Anti-inflammation
  - Anti-pollution
  - Anti-ageing
  - Skin lightening
  - Sun protection
  - …

Cosmetic products European regulations

- The 7th Amendment to the Cosmetics Directive 2003/15/EC
  - Prohibition to test finished cosmetic products and cosmetic ingredients on animals (testing ban)

- Regulation EC 1223/2009
  - Prohibition to supply a cosmetic product that may cause damage to human health
  - Cosmetic products are required to be effective when used by Consumers

- Regulation EC 655/2013
  - Claims for cosmetic products shall be supported by adequate and verifiable evidence regardless of the types of evidential support used to substantiate them, including where appropriate expert assessments.

Topical product criteria

- When developing a topical product, we should ensure:

What kind of *in vitro* safety testing?

- Dermal absorption
- Skin & eye irritation
- Skin sensitization
- Genetic toxicology
- Photo-irritation
- Photo-sensitization
- Skin metabolism & drug transporters

SENZA GEN

GENEVOLUTION

What kind of *in vitro* efficacy testing?

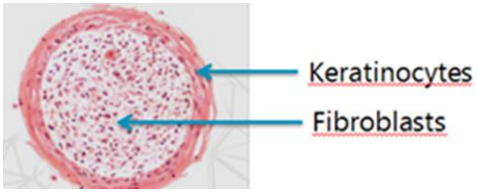
- Anti-inflammation
- Anti-ageing
- Skin hydration
- Anti-pollution
- Skin lightening
- Sun-protection
- Wound healing

Label claims

Alternative skin models for safety and efficacy testing

- 2D Cell culture derivative from skin
  - Keratinocytes
  - Fibroblasts
  - Melanocytes
  - Dendritic and Langerhans cells

- 3D Skin spheroids



- 3D Human skin equivalent
  - Reconstructed human epidermis (RHE)
  - Full thickness (Keratinocytes + Fibroblasts)
  - + Melanocytes; + Langerhans cells

- Excised Human skin
  - Gold standard

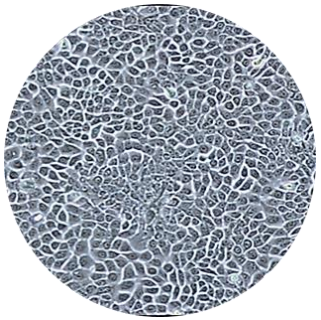
Use of *in vitro* skin models in R&D process

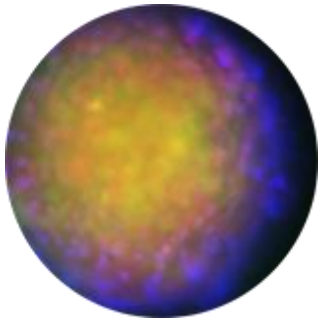
Early stage
Active ingredient

Late stage
Finished product

2D skin cell culture

- Anti-inflammatory properties
- Anti-ageing properties
- Pigmentation,
- Wound healing
- Sensitization





3D Human skin equivalent

- Anti-inflammatory
- Skin irritation
- Pigmentation
- Sensitization

Ex vivo Human skin

- Dermal absorption
- Skin metabolism
- Drug transporters

Example of safety and efficacy testing using *in vitro* alternative skin models

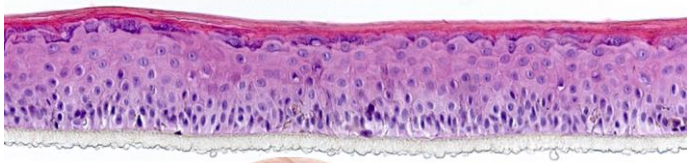
Irritation (RHE)

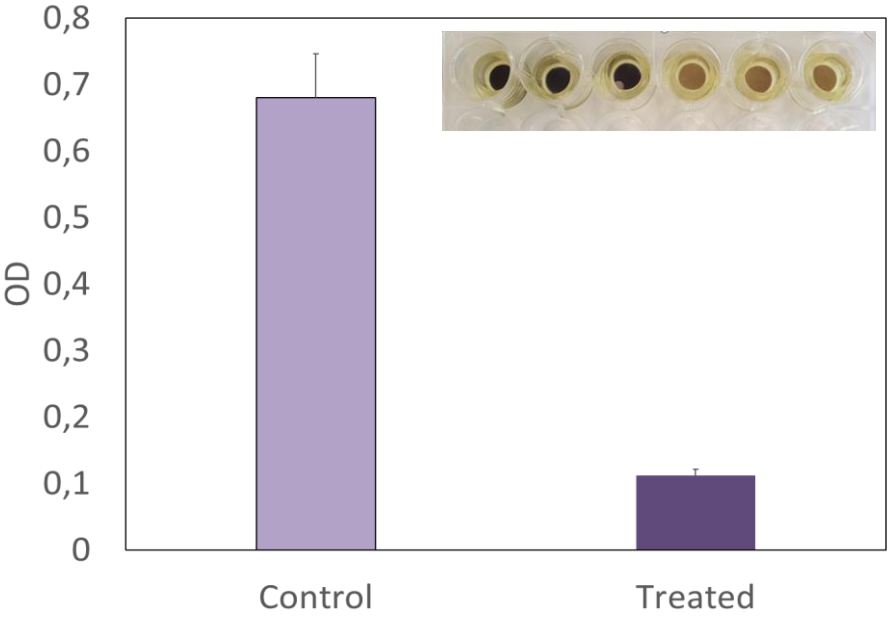
Inflammation

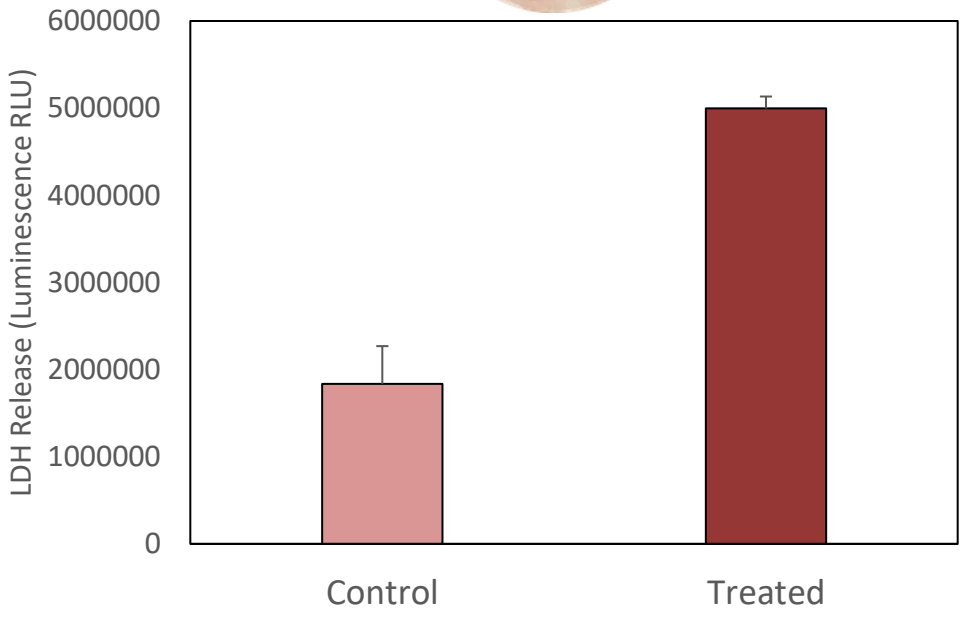
Dermal absorption

In vitro skin irritation assay

- 3D Human Reconstructed Epidermis
  - Active ingredients and finished products
  - Negative control and positive control
  - Measurement of cell viability (MTT, LDH)
  - OECD 439

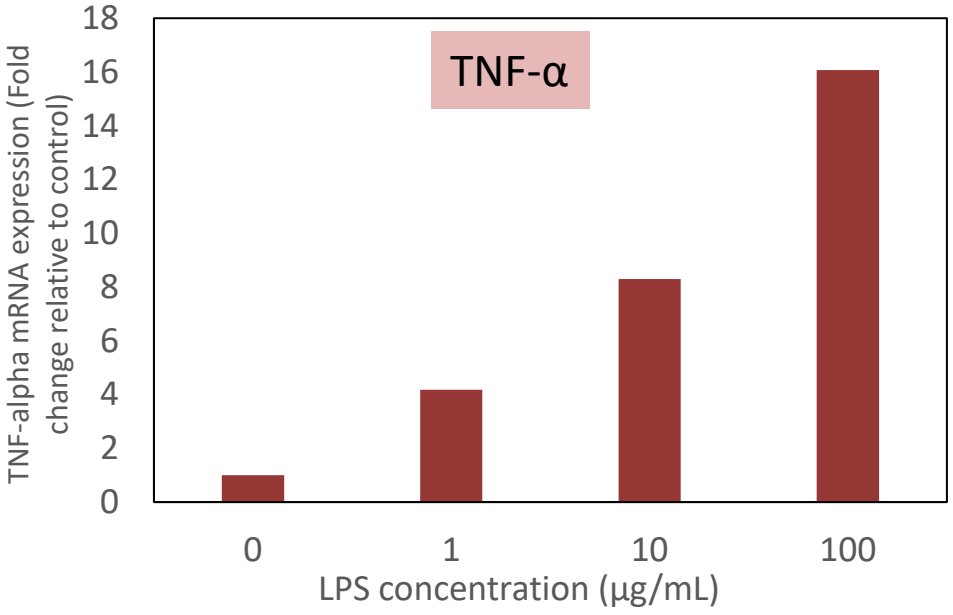


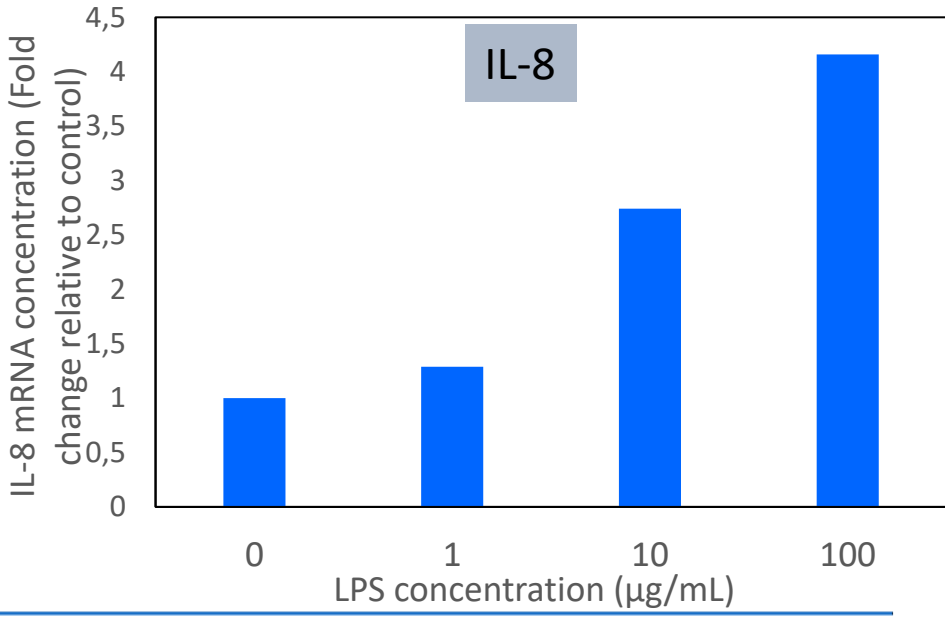




In vitro skin inflammation assay

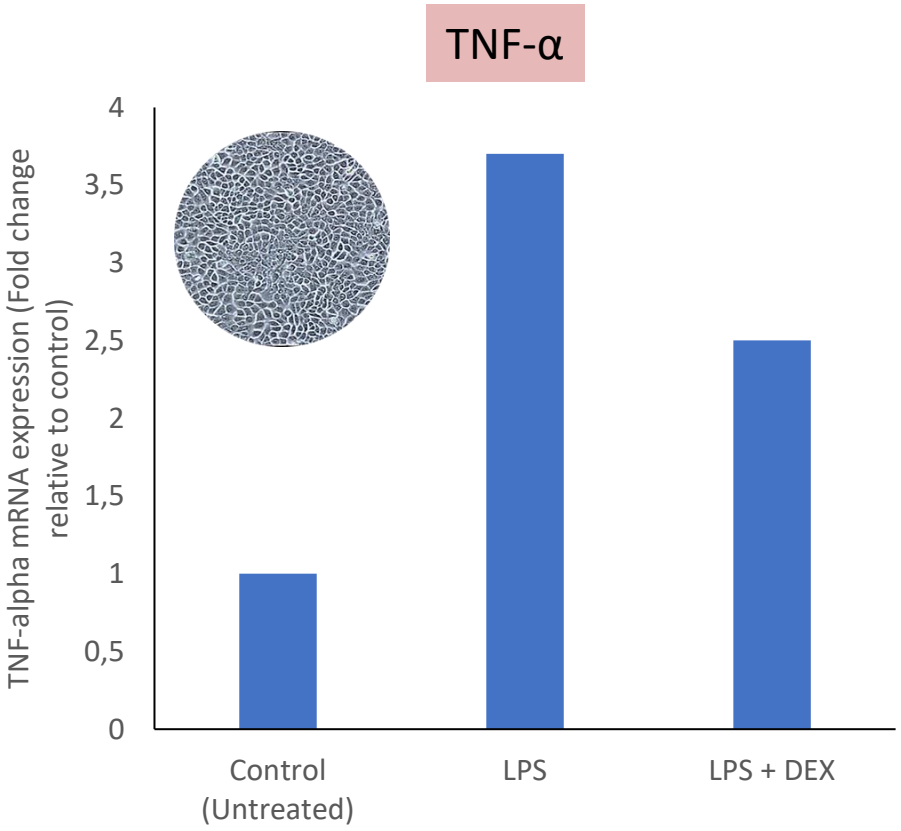
- 2D keratinocytes
  - Soluble active ingredients
- 3D Human Reconstructed Epidermis
  - Finished products (cream, onguent, …)
  - Measurement of mRNA expression by q-RT-PCR

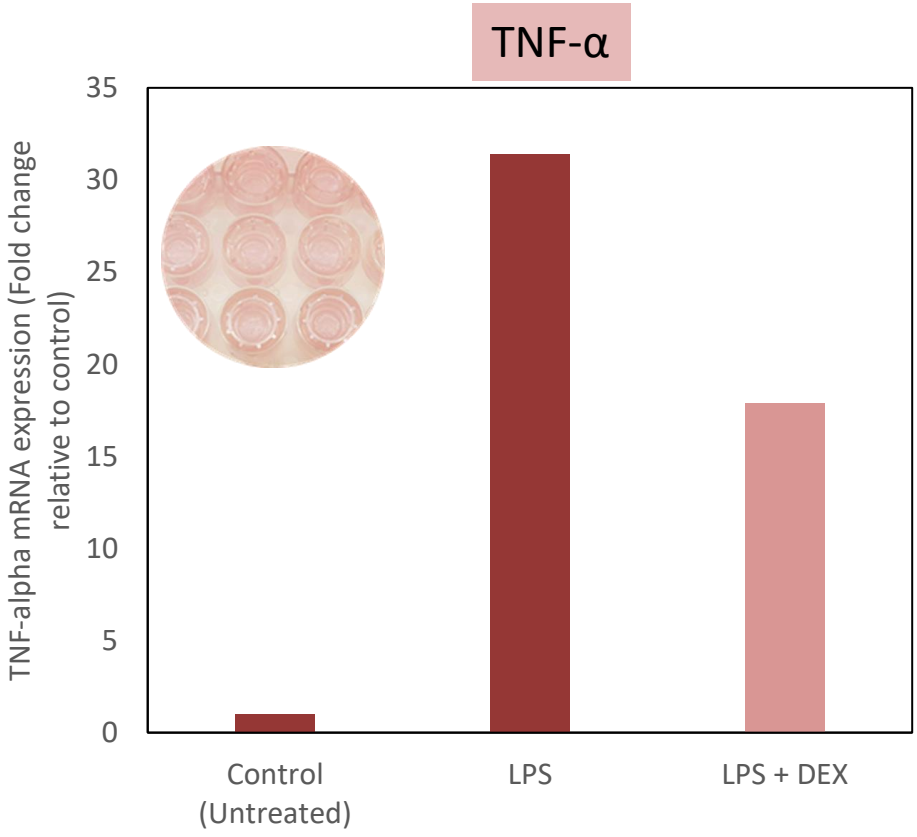




In vitro skin inflammation assay

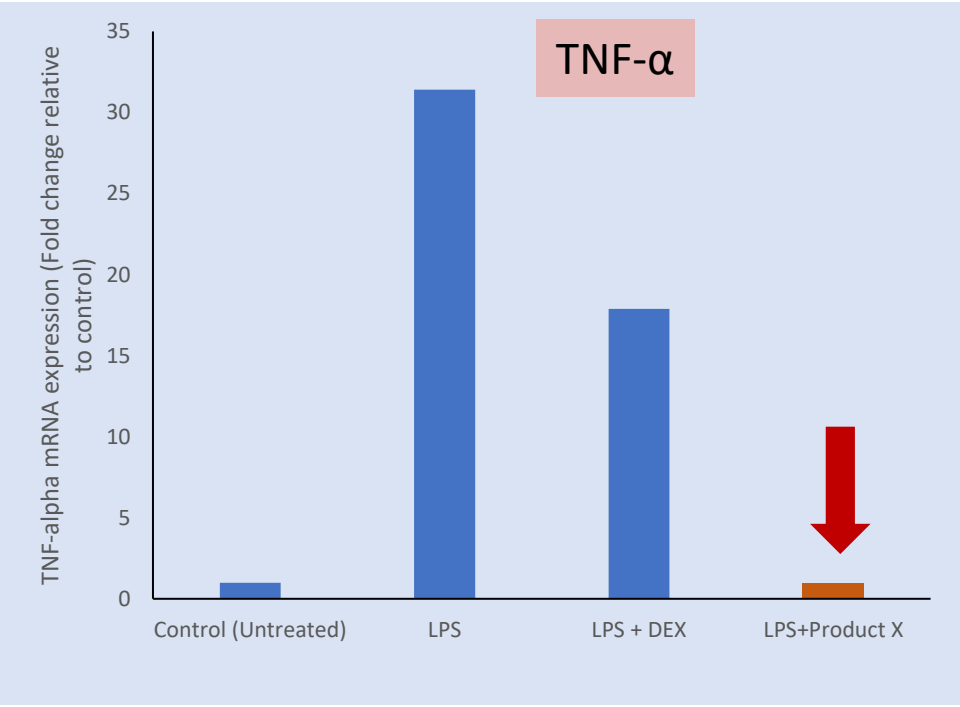
- Comparison 2D keratinocytes & 3D RHE
  - Dexamethasone as anti-inflammation agent

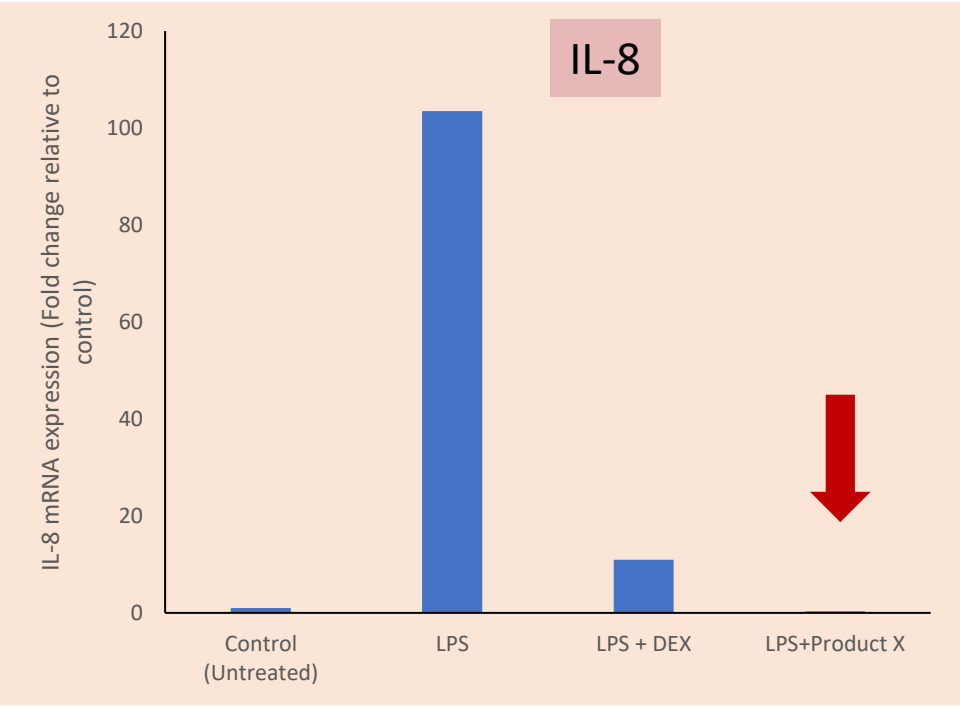




In vitro skin inflammation assay

- Example of a skin care product

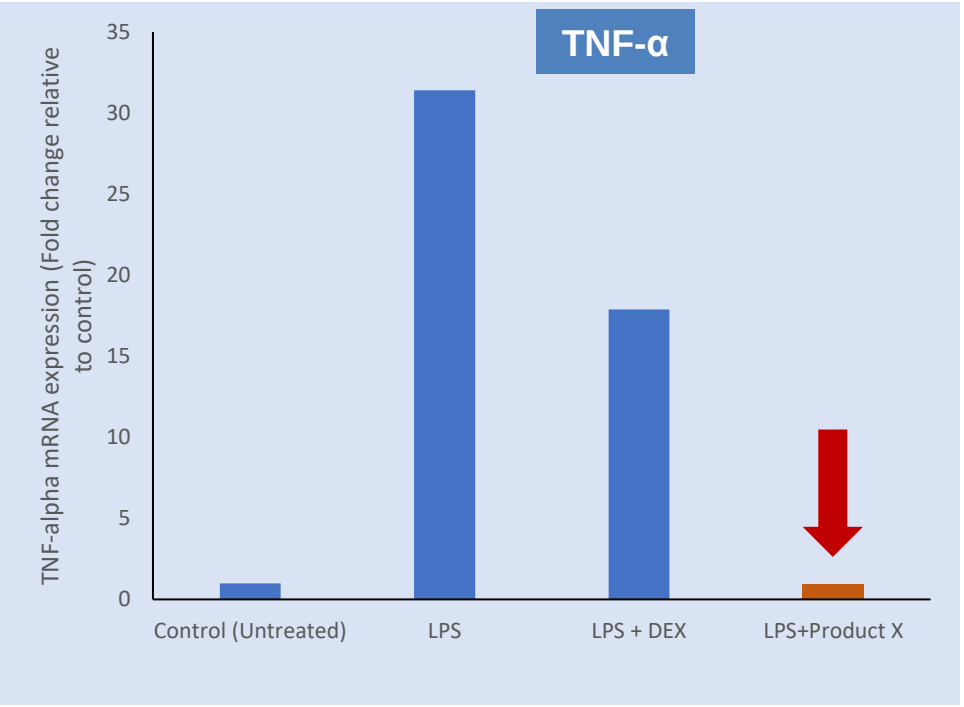


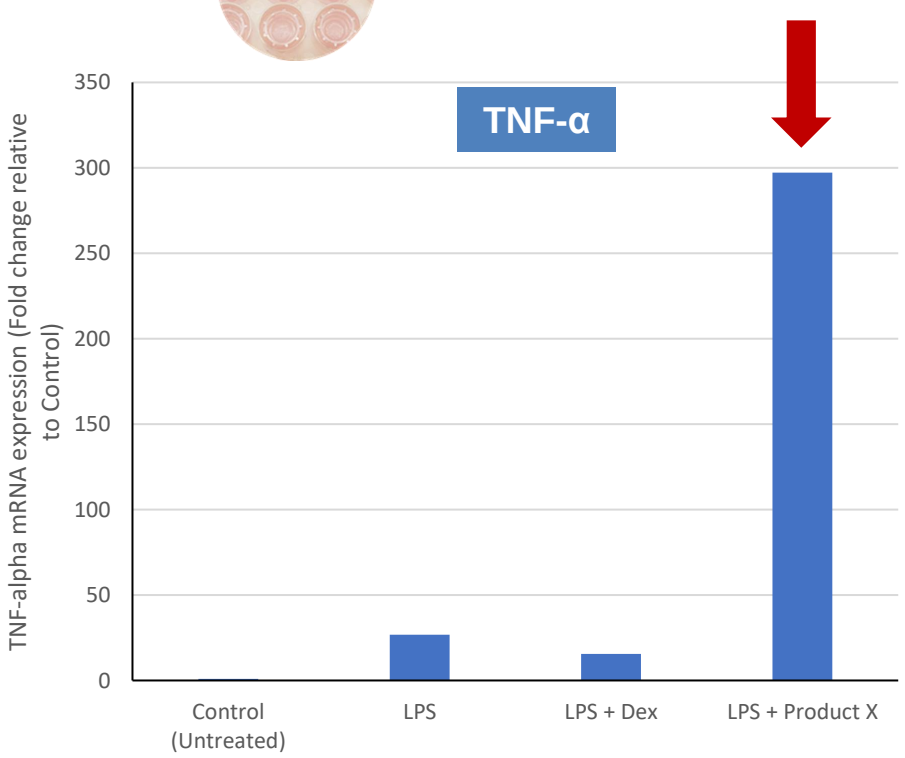


- Product X has a strong anti-inflammatory properties

In vitro skin inflammation assay

- Example of a skin care product

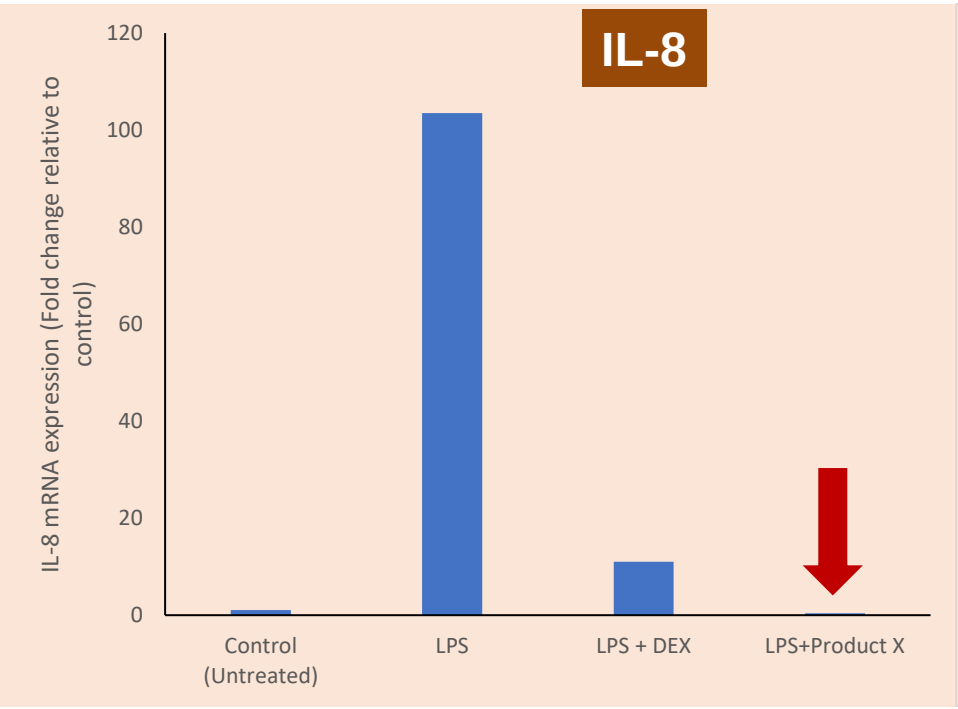


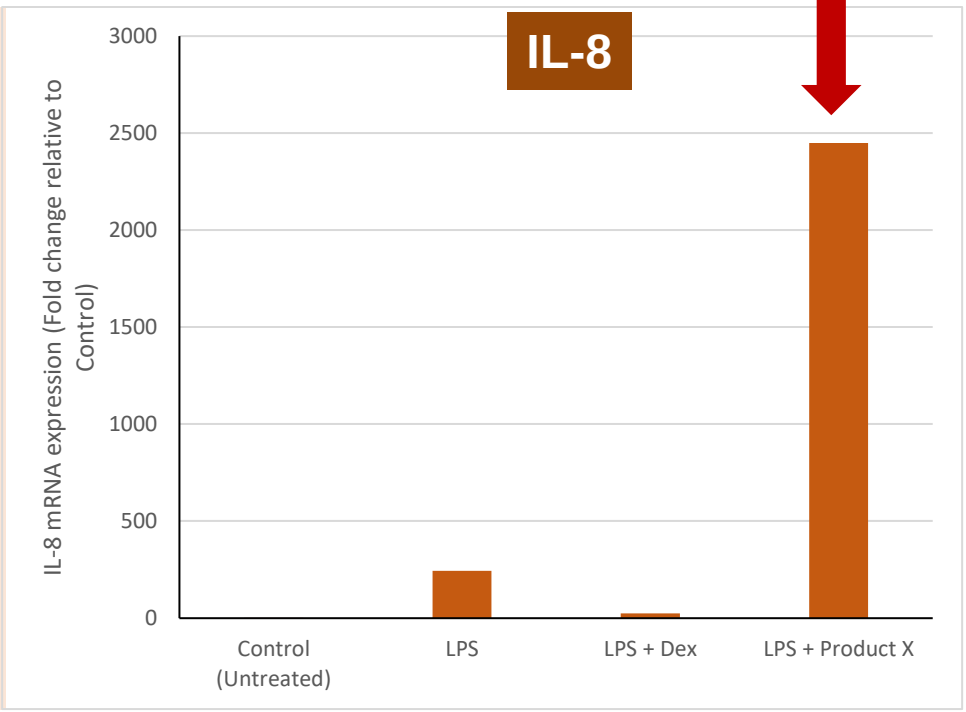


- The same product can have different effects according to active ingredient sourcing

In vitro skin inflammation assay

- Example of a skin care product



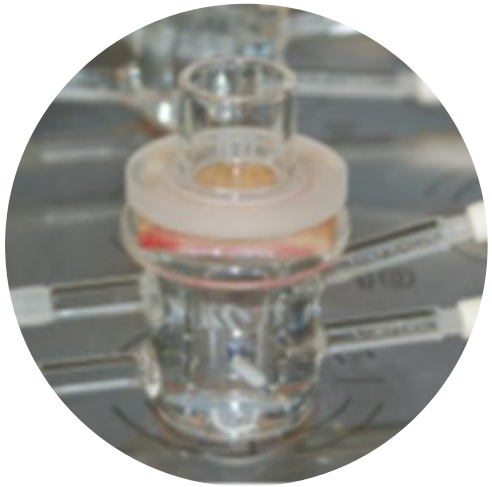


- The same product can have different effects according to active ingredient sourcing

PKDerm Innovative solutions

In vitro Dermal absorption - Overview

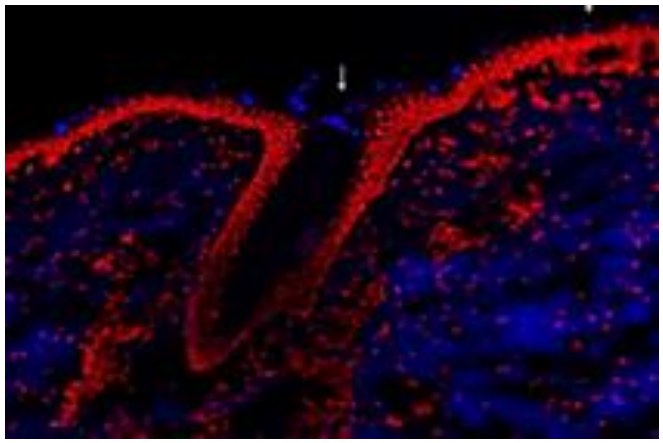
- Excised Human skin (Gold standard model)
  - Evaluation of distribution profile and dermal absorption to support safety and efficacy profile
  - Dermal absorption performed on diffusion cells (Franz cells) or on Transwell

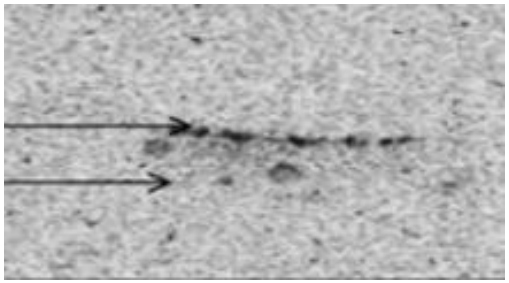


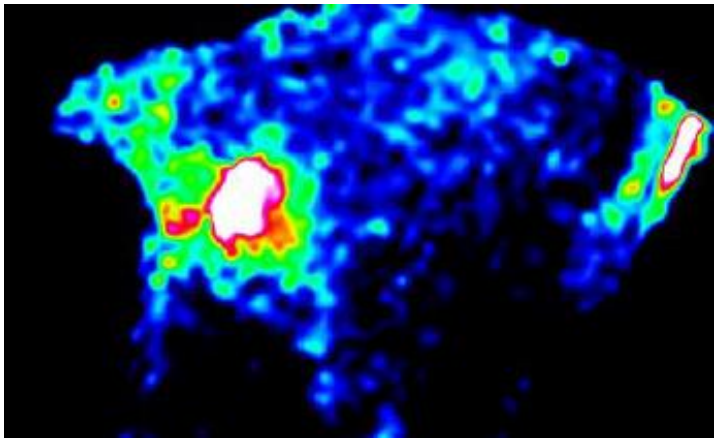
  - Frozen skin or fresh skin
  - Treatment time: according to use conditions

In vitro Dermal absorption - Overview

- Analysis methods
  - LC-MS/MS; LC-UV; LC-Fluo; LSC
    - Receptor liquid
    - Dermis
    - Epidermis
    - *Stratum corneum*
  - Imaging : Fluorescence microscopy; Autoradiography; MALDI-MSI, …







Example of *In vitro* Dermal absorption study

In vitro Dermal absorption: Comparison of 3 formulations

- Objective of the study
  - To measure dermal absorption of a cosmetic ingredient in three different formulations on excised human skin mounted on Franz type diffusion cells

In vitro Dermal absorption: Comparison of 3 formulations

- Human Skin samples
  - Full thickness human skin
  - 3 different donors
  - Each condition performed in 3 replicates on each donor (N = 9)
- Skin quality
  - Measurement of skin thickness
  - TEWL measurement before application

In vitro Dermal absorption – Comparison of 3 formulations

- **Diffusion cells**
  - Surface area: 2 cm²
  - Volume of receptor compartment: 3 mL
  - Receptor liquid:
    - PBS pH 7.2 + 1% Tween® 80

In vitro Dermal absorption – Comparison of 3 formulations

- **Treatment**
  - Static conditions
  - Application: 10 mg/cm²
  - Treatment duration: 24 hours
  - Temperature: 32°C

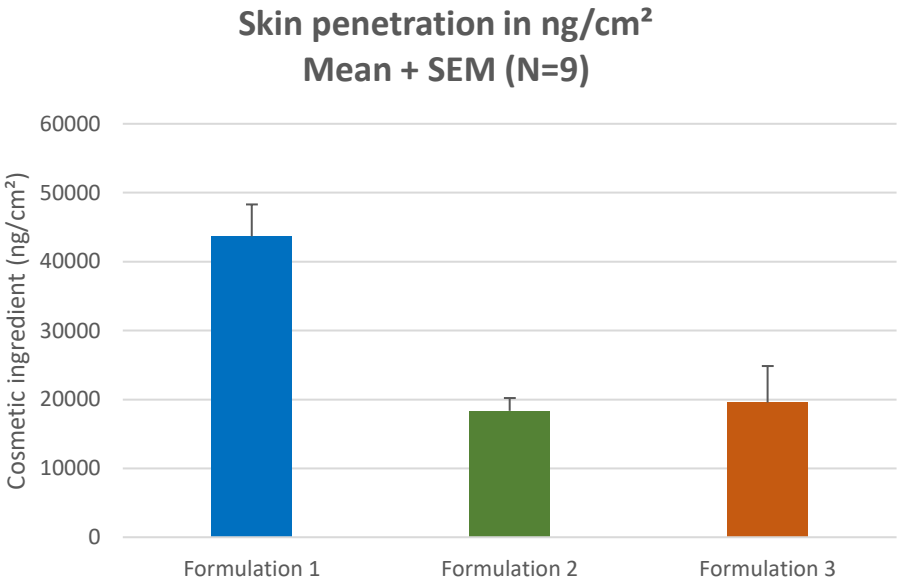
In vitro Dermal absorption – Comparison of 3 formulations

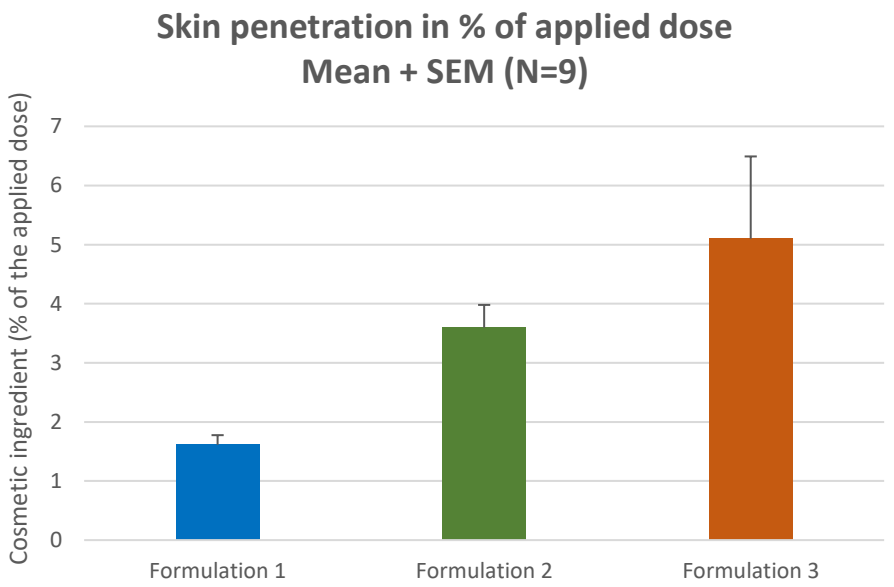
- **Sample analysis**
  - Treated area of skin collected and crushed in organic solvent.
  - Analysis performed using LC-MS/MS method

Results

Results: Skin penetration

Comparison of 3 products





- Concentration of cosmetic ingredient penetrated the skin is proportional to the concentration in the formulation
- Dermal absorption data are used in toxicological risk assessment to extrapolate human exposure

How to increase dermal absorption ?

- Different physical methods can increase dermal absorption:
  - Increase efficacy

How to increase dermal absorption ?

- Different physical methods :
  - Microneedle
  - Skin preparation pad
  - Tape stripping (reference)

Microneedling

Dermaroller®

Skin preparation pad

Tape stripping

Adhesive tapes

How to increase dermal absorption ?

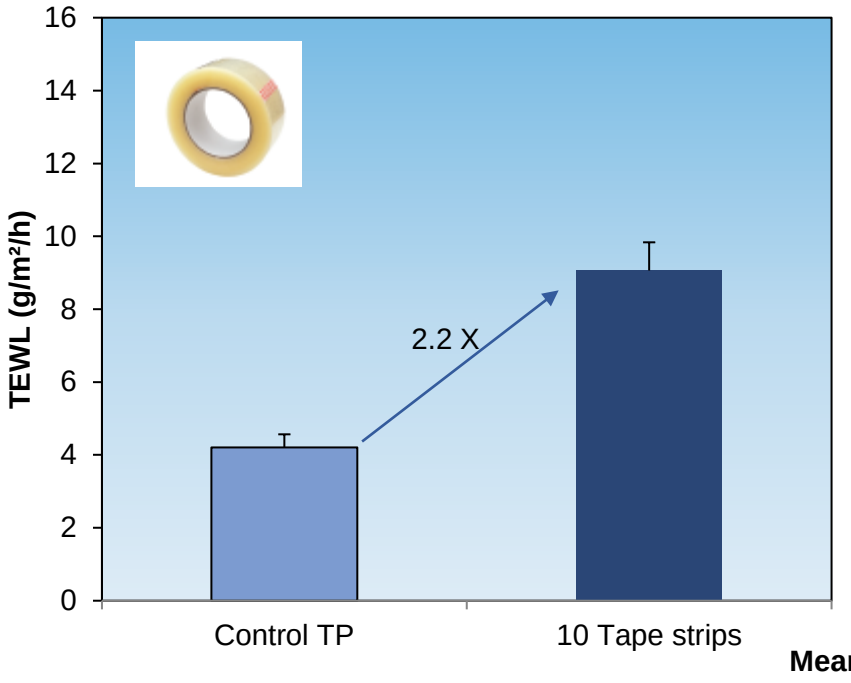
Experimental procedure

TEWL Measurement

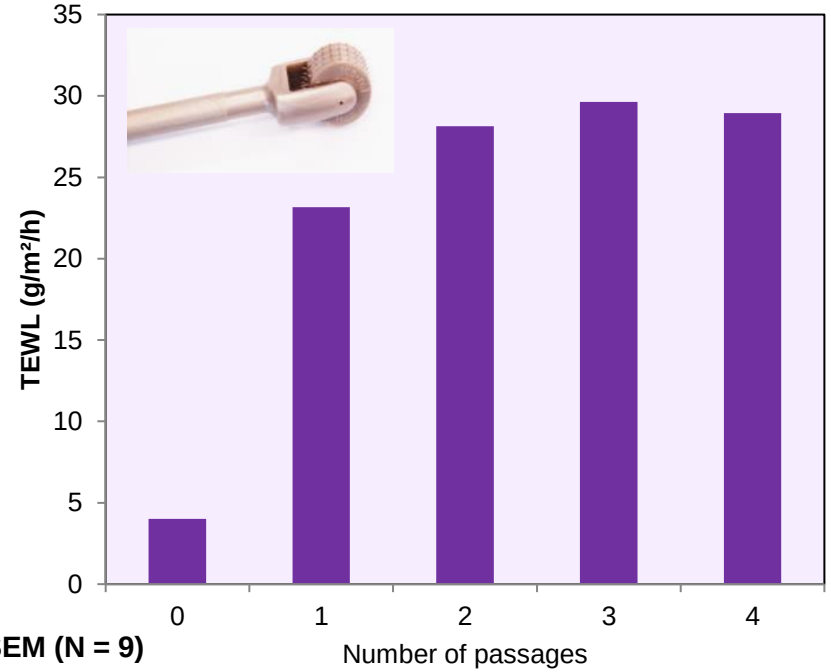
Tewameter

Before and after skin preparation

How to increase dermal absorption ?



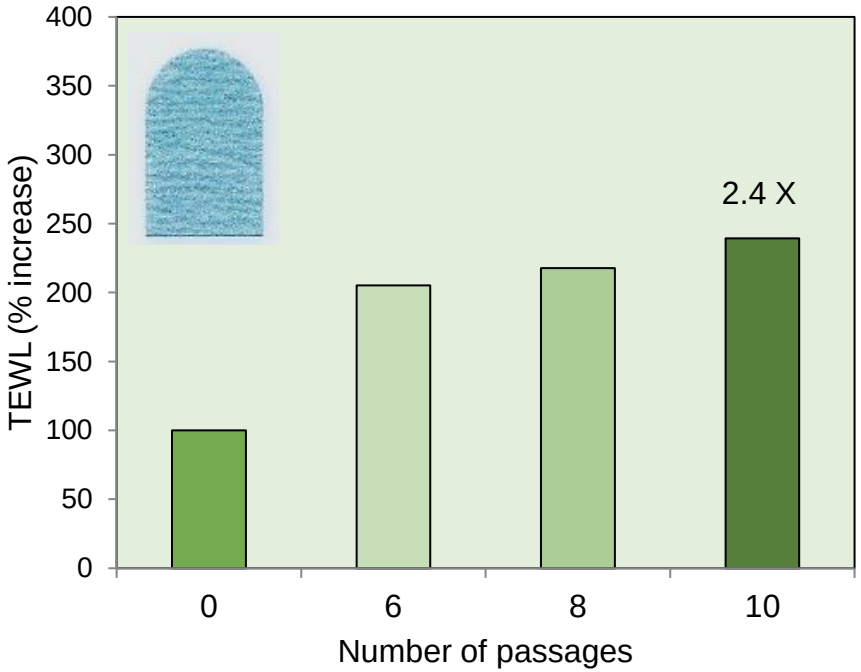
Mean + SEM (N = 9)

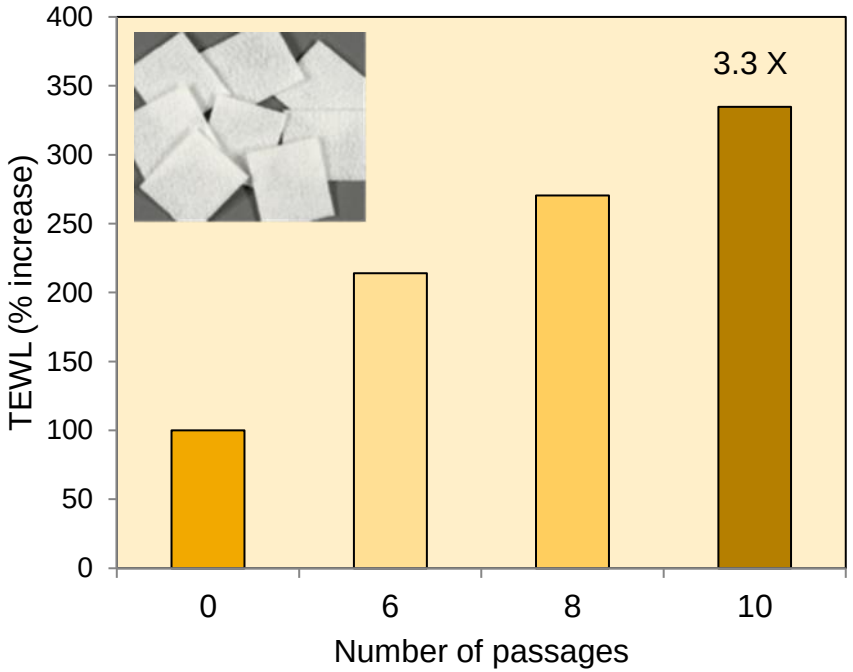


- TEWL increased after tape stripping and microneedling
- Skin barrier function impaired

Osman-Ponchet et al., 2017, Photodiagn. Photodyn. Ther

How to increase dermal absorption ?

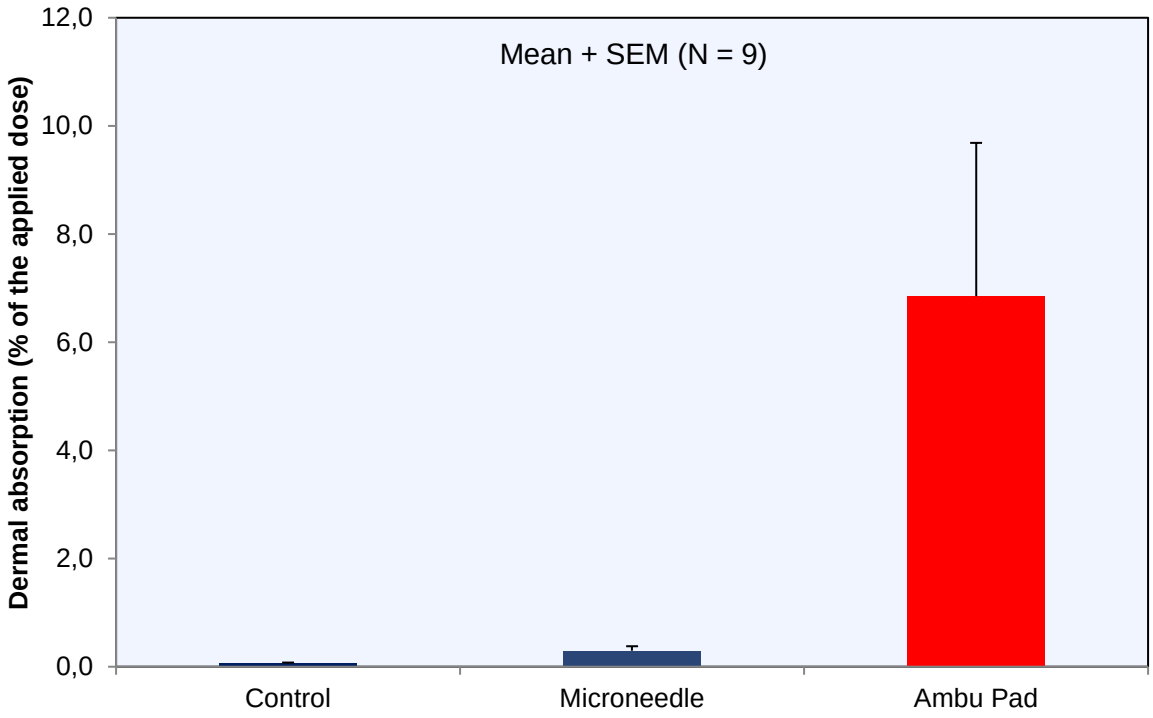


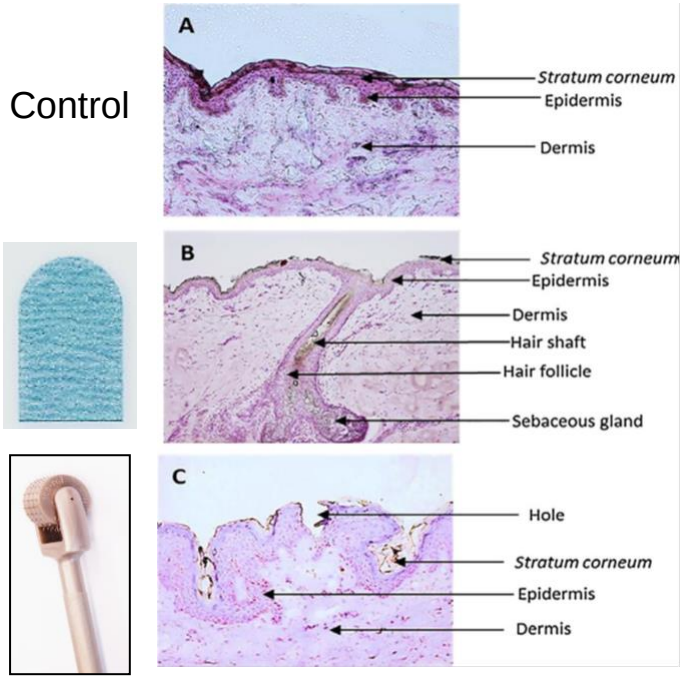


– TEWL increased with increasing number of skin pad passages

Osman-Ponchet et al., 2017, Dermatol Ther (Heidelb)

How to increase dermal absorption ?





– Dermal absorption increased by 4 times after microneedling and by 100 times after skin preparation pad

Osman-Ponchet et al., 2017, Photodiagn. Photodyn. Ther

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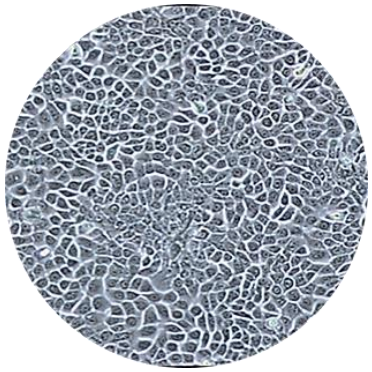
Conclusion

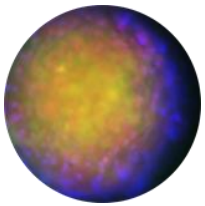
- Different *in vitro* skin models exist for safety and efficacy evaluation
- Each model has advantage and inconvenient
- Choose the right model at the right time

Early stage
Active ingredient

Late stage
Finished product

2D skin cell culture





3D Human skin equivalent

Ex vivo Human skin


ThanK You!

In vitro Skin sensitization assay

- GARD™ skin assay (SenzaGen) – Dendritic cells
  - Uses genomics and machine learning tools to identify skin sensitizers

GARD™skin
A robust *in vitro* assay to test candidate ingredients or formulations and identify potential chemical skin sensitizers with over 90% prediction accuracy.

GARD™potency
An add-on *in vitro* test to GARD™skin for potency classification according to GHS/CLP (1A or 1B).

GARD™skin Medical Device
A robust and accurate *in vitro* assay to test for skin sensitizers in Medical Device extracts according to ISO 10993-10: 2012.

GARD™air
The first *in vitro* assay capable of identifying chemical respiratory sensitizers. Can be used alone or in combination with GARD™skin to discriminate between respiratory and skin sensitizers.

More information on: www.senzagen.com

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for safer products

In vitro skin and respiratory sensitization testing

- High accuracy
- Short turnaround time
- Broad applicability - "difficult-to-test samples"