# Use of alternative skin models in safety and efficacy testing of cosmetic products

1 author:

Hanan Osman-Ponchet
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**22** PUBLICATIONS **36** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Drug transporters in the skin View project

Conference " Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies" View project

# Use of alternative skin models in safety and efficacy testing of cosmetic products

Hanan Osman-Ponchet, PhD

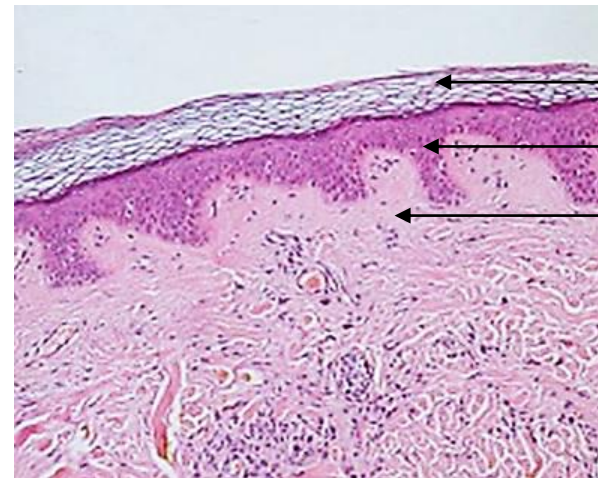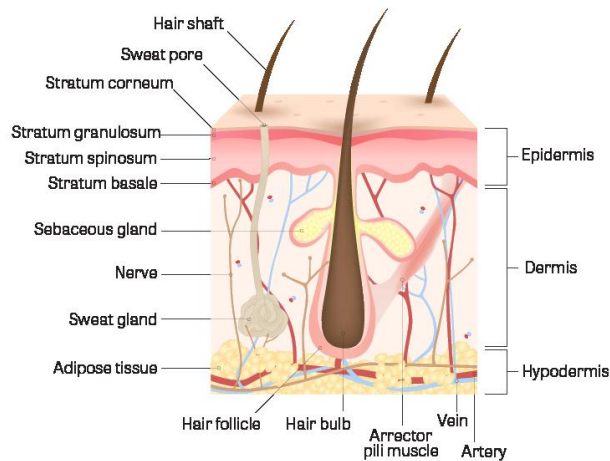Skin Models in Cosmetic Science: Bridging Established Methods and Novel Technologies

3th December 2019, Tours - France



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# Human skin

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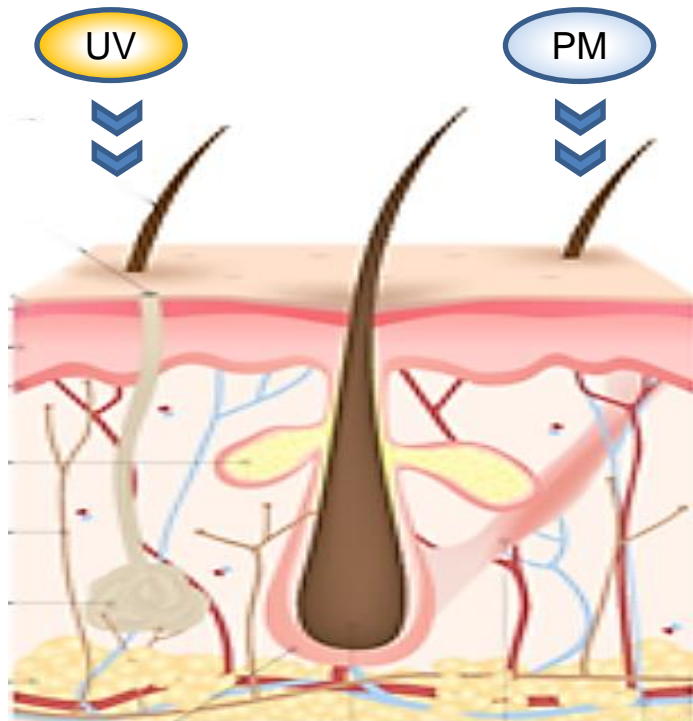- Skin is the boundary between the environment and the organism, plays a crucial role in body protection



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

# Human skin as major target for environmenal attacks



- **Skin is the major target for environmental attacks (Pollution, UVs, Ozone)**

- **Alteration of skin barrier**
- **Oxidative stress**
- **Inflammation (IL1, IL6, TNF-α, …)**
- **Activation of AhR**
- **Collagen degradation**

- **Premature aging**
- **Atopic dermatitis, Psoriasis**
- **Skin pigmentation**

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# Cosmetic strategies for skin 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
    - Face care, Sun care, Body Care, Hair care, Cosmetics (make up)

- Different claims:
    - Anti-inflammation
    - Anti-pollution
    - Anti-ageing
    - Skin lightening
    - Sun protection
    - …



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# Cosmetic products European regulations

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

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

# Cosmetic product criteria

- When developing a cosmetic product, we should ensure:



**Safety**

**Efficacy**

**Market trend**

# What kind of safety testing?

Dermal absorption

Dermal irritation

Eye irritation

Skin sensitization

Photoirritation

Photosensitization

Skin metabolism & drug transporters



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# What kind of label claims?

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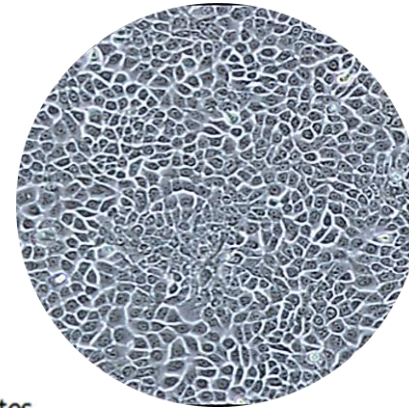- Skin hydration
- Anti-pollution
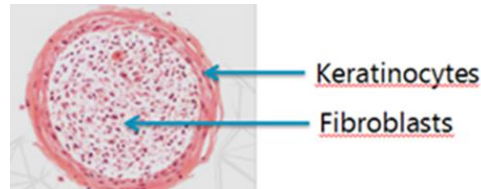- Skin lightening
- Sun-protection
- Wound healing

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# Alternative skin models for safety and efficacy testing

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

- **3D Skin spheroids**



Keratinocytes
Fibroblasts

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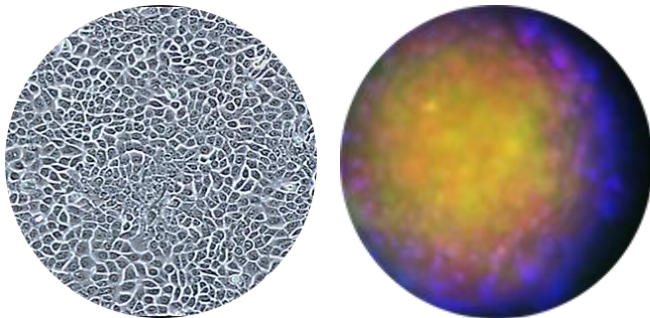
- **Excised Human skin**
  - Gold standard

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# 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



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

Irritation (RHE)

Inflammation

Sensitization

Dermal absorption
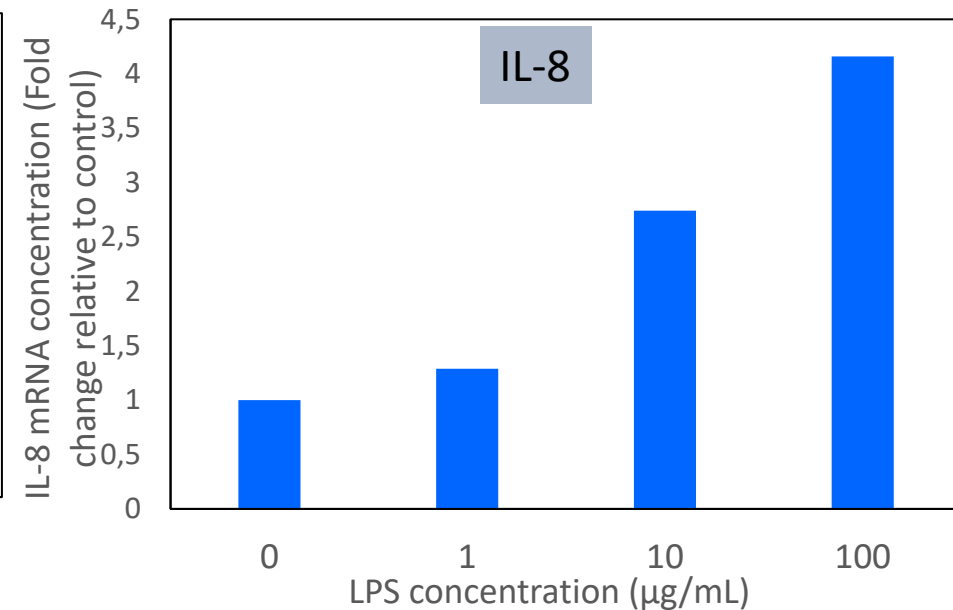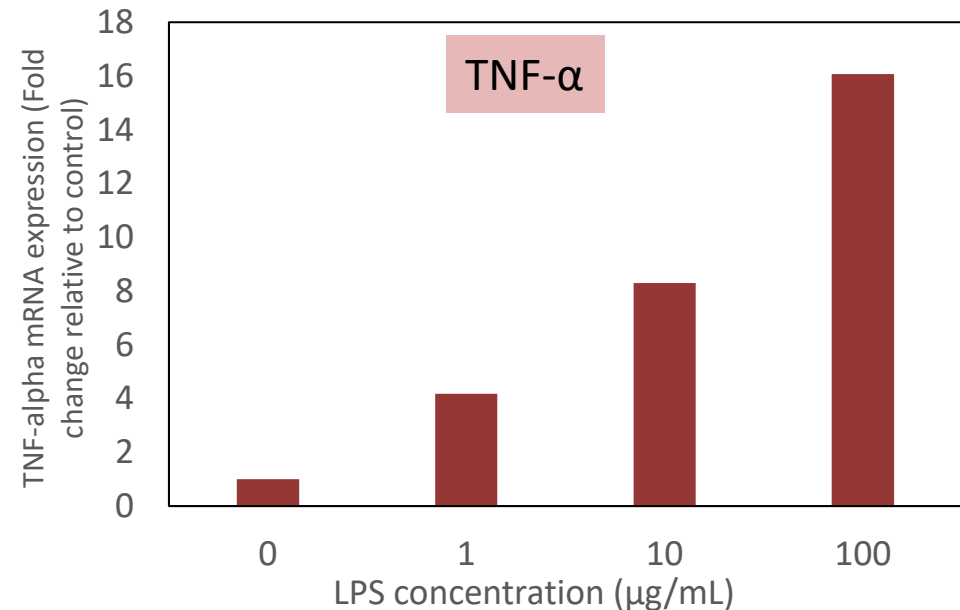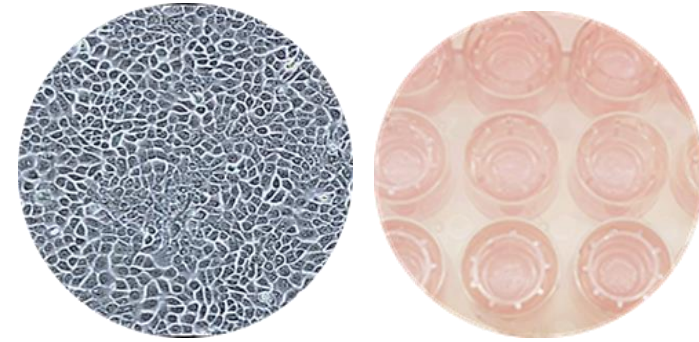
# *In vitro* skin irritation assay



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

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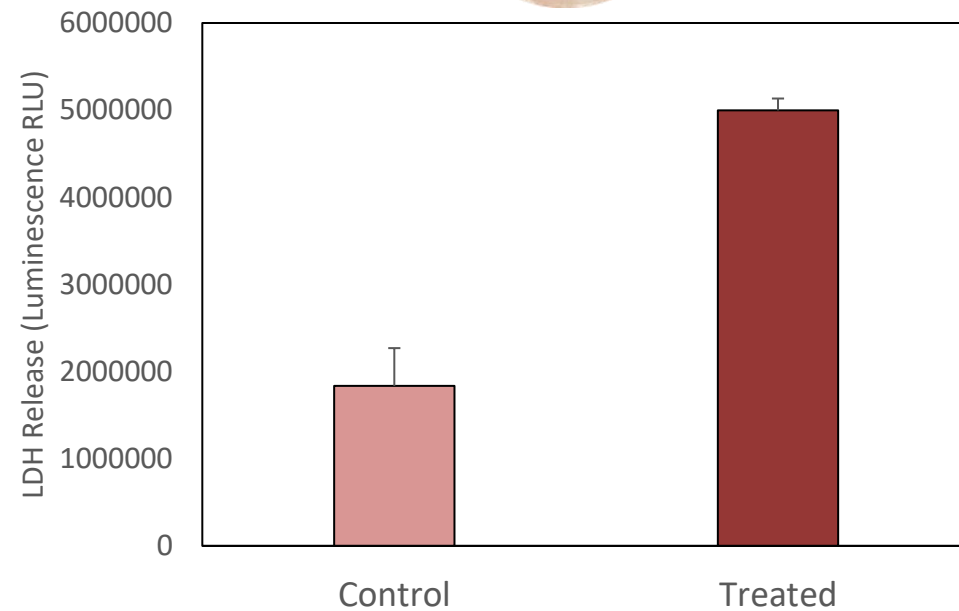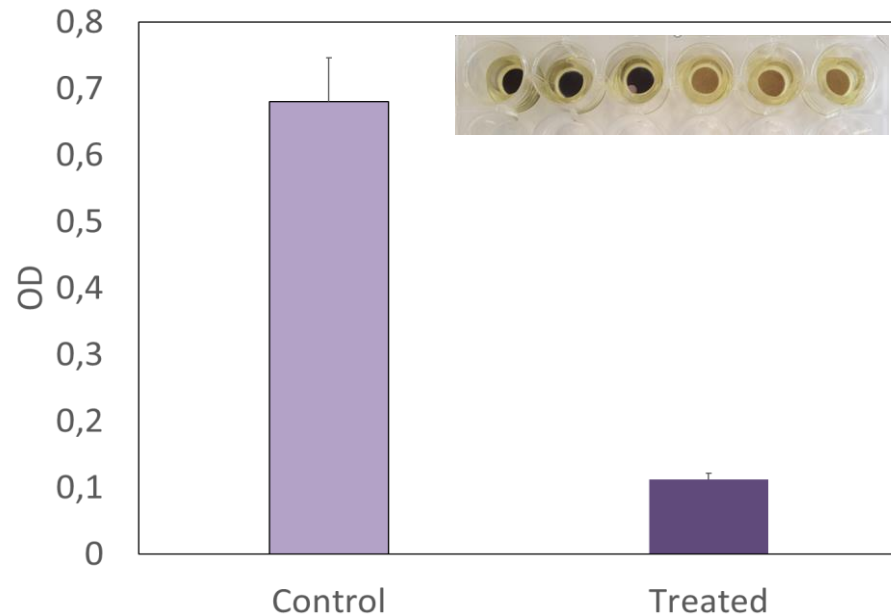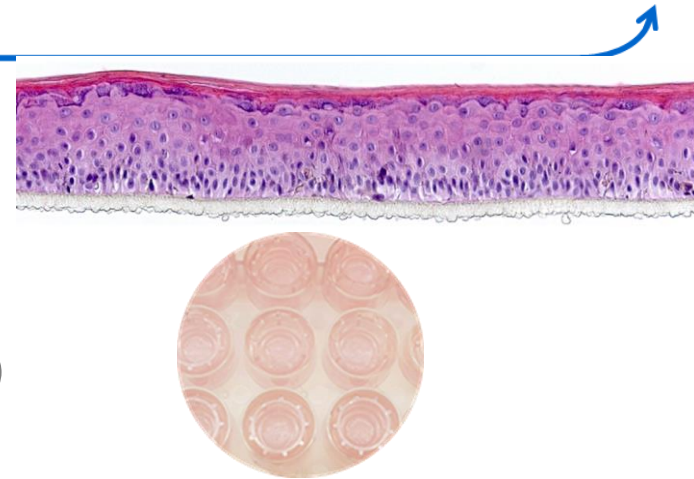
# *In vitro* skin inflammation assay

- ## 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
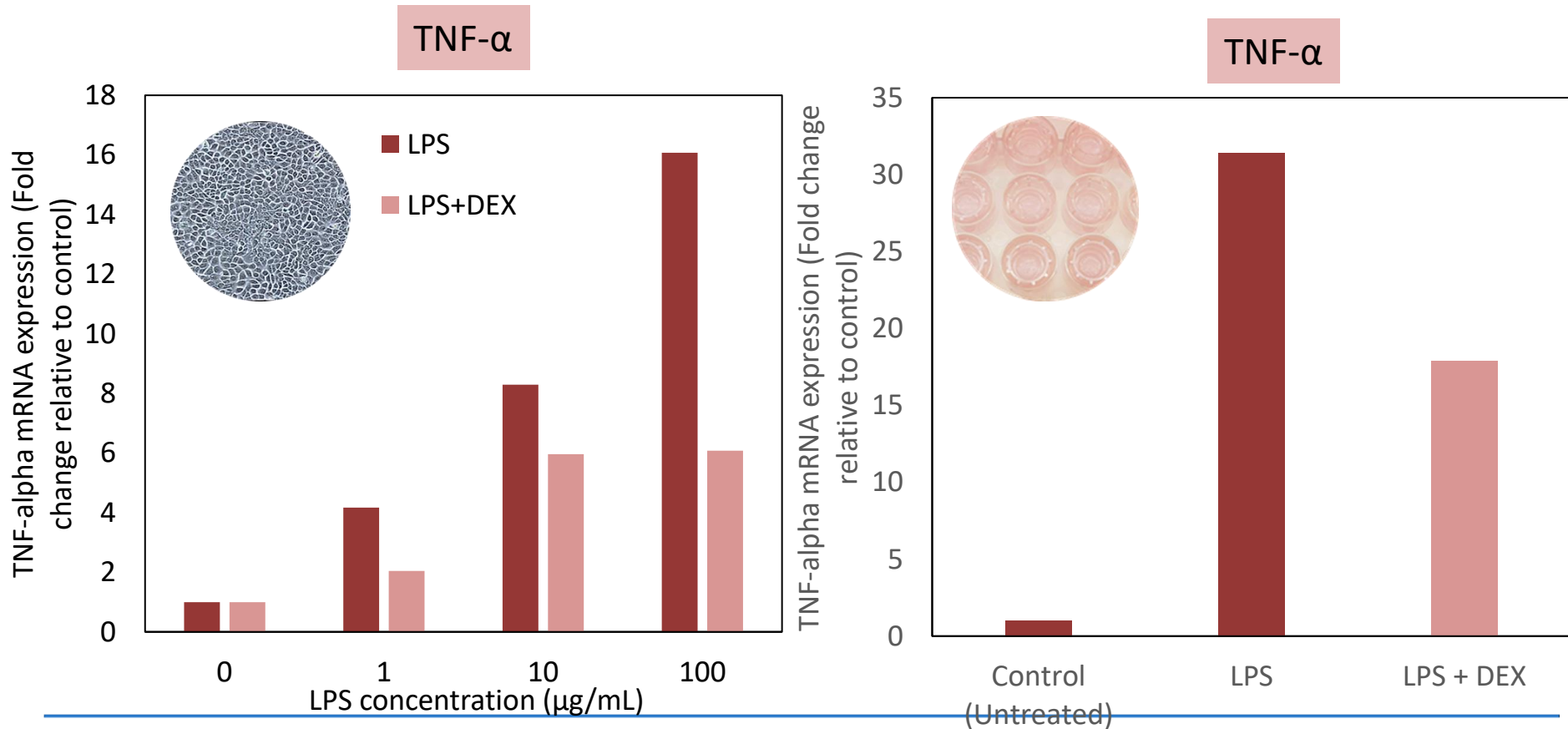
# *In vitro* Skin sensitization assay

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

**Dr. Andy Forreryd**
**Wednesday 4th December**

SENZA GEN

**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

# GARD™
## for safer products

*In vitro* skin and respiratory sensitization testing

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

Genomics
Machine learning
Pattern recognition

# *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
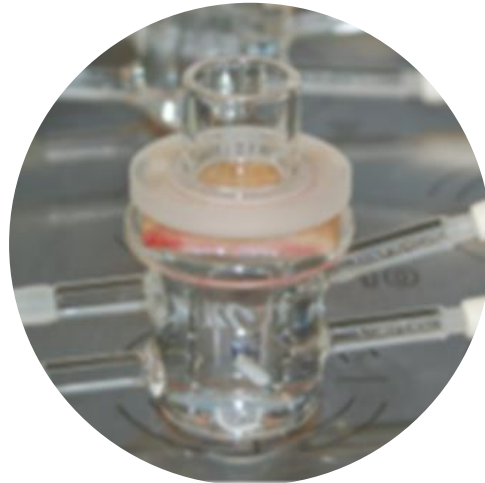


  - Frozen skin or fresh skin
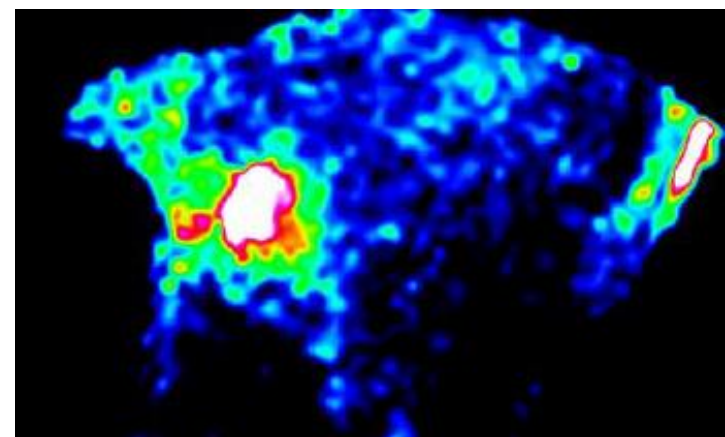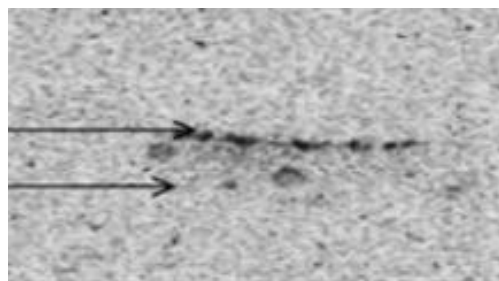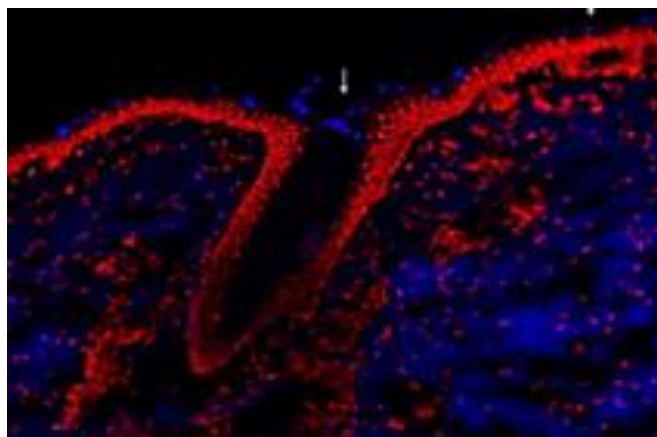  - Treatment time: according to use conditions

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# *In vitro* Dermal absorption - Overview

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

# *In vitro* Dermal absorption – Comparison of 2 formulations



- *Ex vivo* skin samples
  - Full thickness human skin
  - 6 human donors
  - Each condition performed in 4 replicates (N = 24)
- Diffusion cells
  - Surface area: 2 cm²
  - Volume of receptor compartment: 3 mL
  - Receptor fluid: PBS pH 7.2 + 0.25% Tween® 80
- TEWL measurement
  - Before application
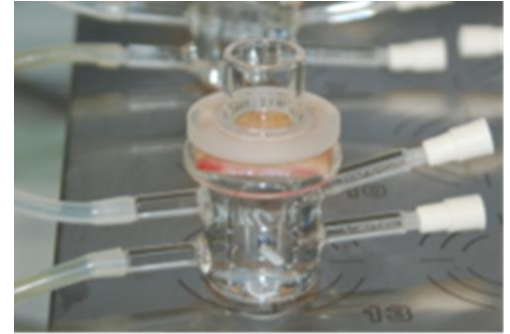
# *In vitro* Dermal absorption – Comparison of 2 formulations

- Treatment conditions
  - Static conditions
  - Application: 5 mg/cm²
  - Treatment duration: 5 minutes
  - Washing
  - Incubation for 24 hours at 32°C
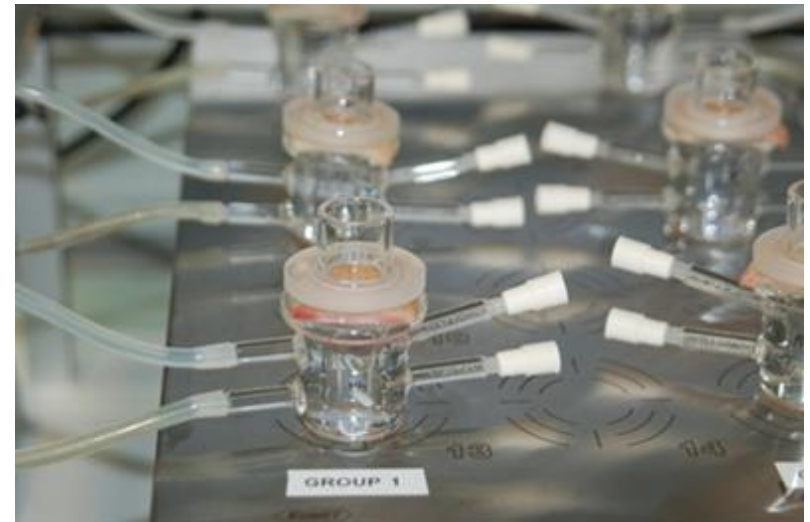
# *In vitro* Dermal absorption – Comparison of 2 formulations



- Sample analysis
  - Formulation excess:
    – Washing
    – Cotton swab
    – 1 tape strip
  – Total skin (including *stratum corneum*, epidermis and dermis)
    – Crushed in 5 mL ethanol/water (50/50, v/v)
  – Receptor liquid samples
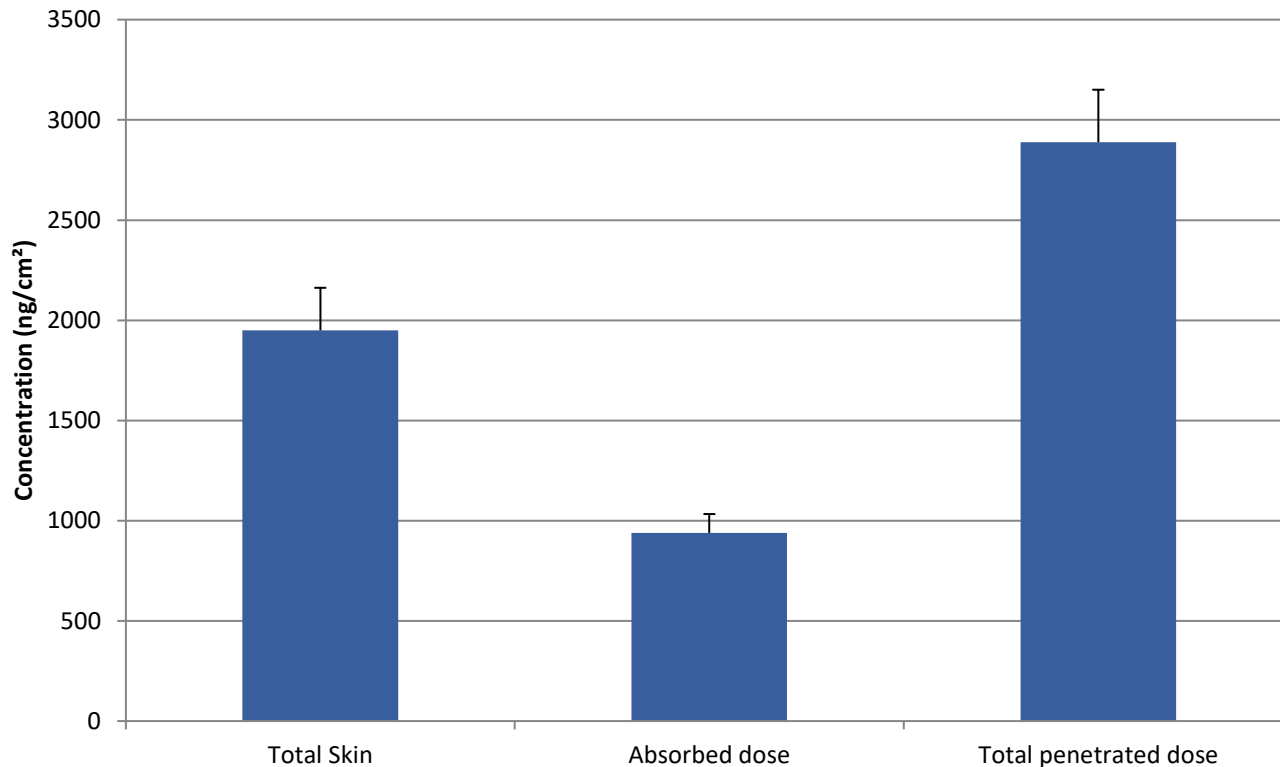- analysis performed using validated HPLC method with UV detection

# *In vitro* Dermal absorption – Comparison of 2 formulations

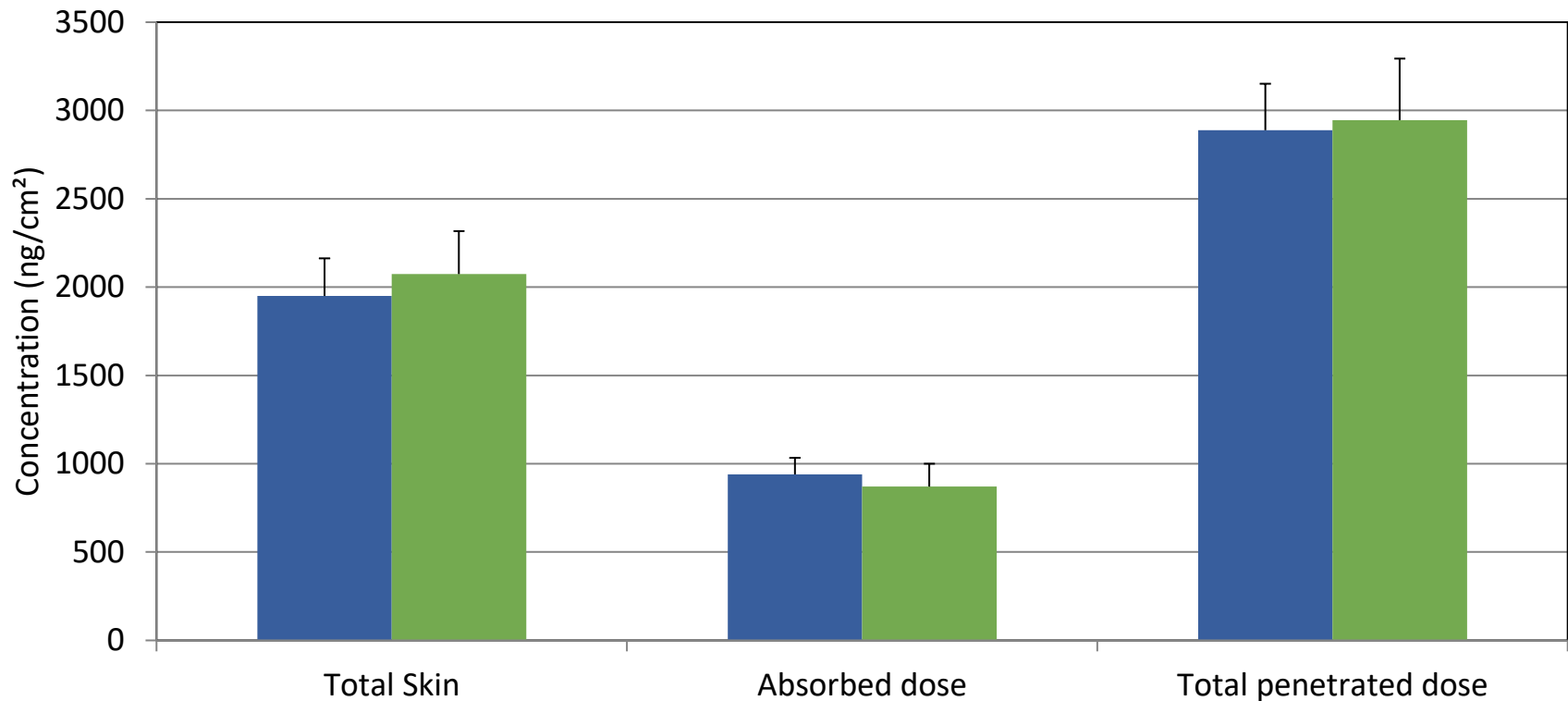Mass balance : 100 +/- 20%
Mean and SEM, N = 24
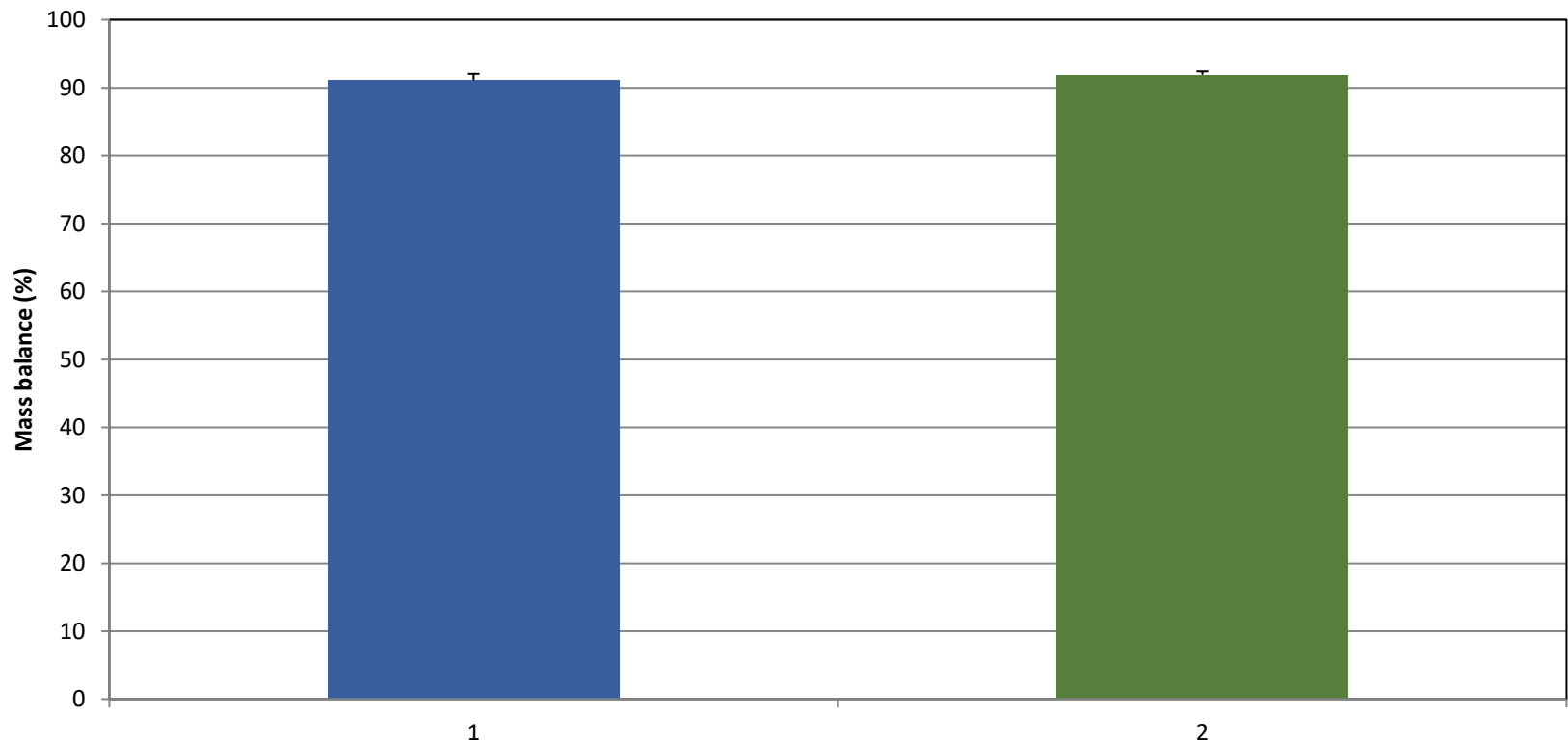
# *In vitro* Dermal absorption – Comparison of 2 formulations

## Distribution profile of formulation 1
## Mean and SEM, N = 24

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# *In vitro* Dermal absorption – Comparison of 2 formulations



Comparison of 2 formulations
Mean and SEM, N = 24

# In vitro Dermal absorption – Comparison of 2 formulations

- Both formulations, the current marketed formulation (1) and the new one (2) are considered similar

- Both formulations are <u>equivalent</u> in:
  - Total penetrated dose, absorbed dose and total skin. Similar efficacy and tolerance at action site with similar safety profile

- Overall, the new formulation should be as safe as the marketed one in terms of systemic absorption with the same efficacy

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# How to increase dermal absorption ?

- Different physical methods can increase dermal absorption:

  - Increase efficacy

  - Case of MAL : Treatment of AK

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# How to increase dermal absorption ?

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

| Microneedling | Skin preparation pad | Tape stripping |
|---|---|---|
|  |  |  |
| Dermaroller® Model 902 200 µm needle length | Gauze pads PREPSTER™ Ambu® Unilect™ | Adhesive tapes of 19 mm width |

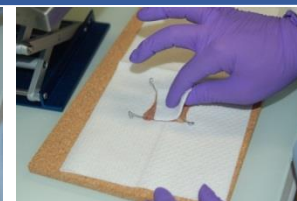# How to increase dermal absorption ?

## Experimental procedure

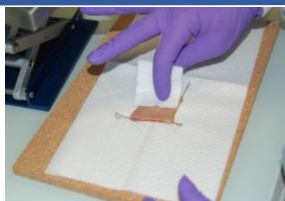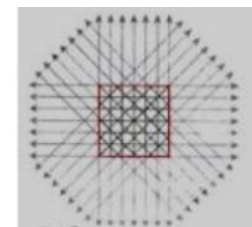| | | | | | |
|---|---|---|---|---|---|
| **Tape stripping** |  | | | | 10 Tape strips |
| **Skin preparation pad Ambu® Unilect™** |  | | | | 6, 8, 10 passages |
| **Skin preparation pad PREPSTER™** |  | | | | 6, 8, 10 passages |
| **Microneedling Dermaroller®** |  | | | | |
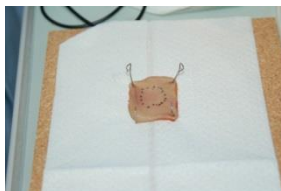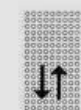
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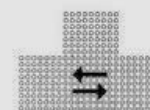
# How to increase dermal absorption ?

## Experimental procedure

### TEWL Measurement



Tewameter



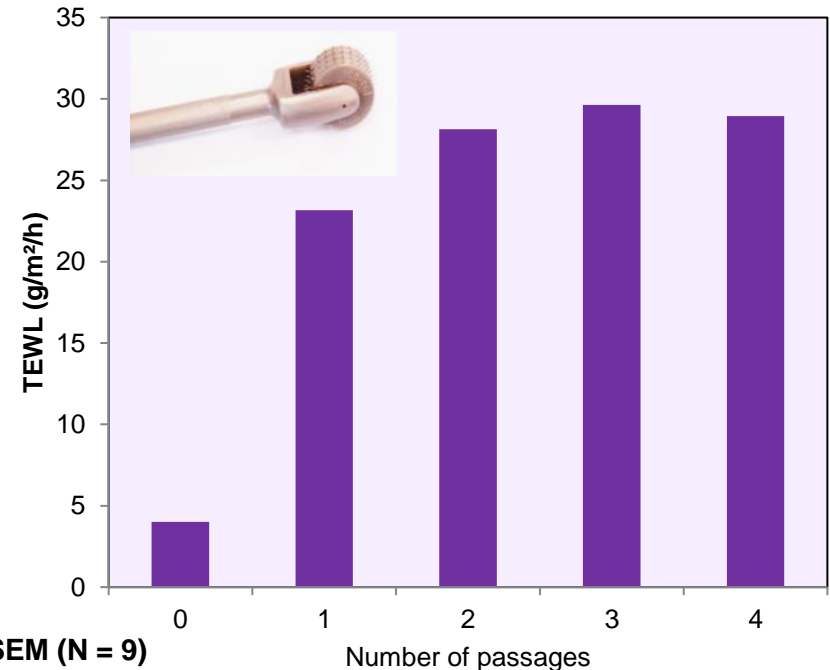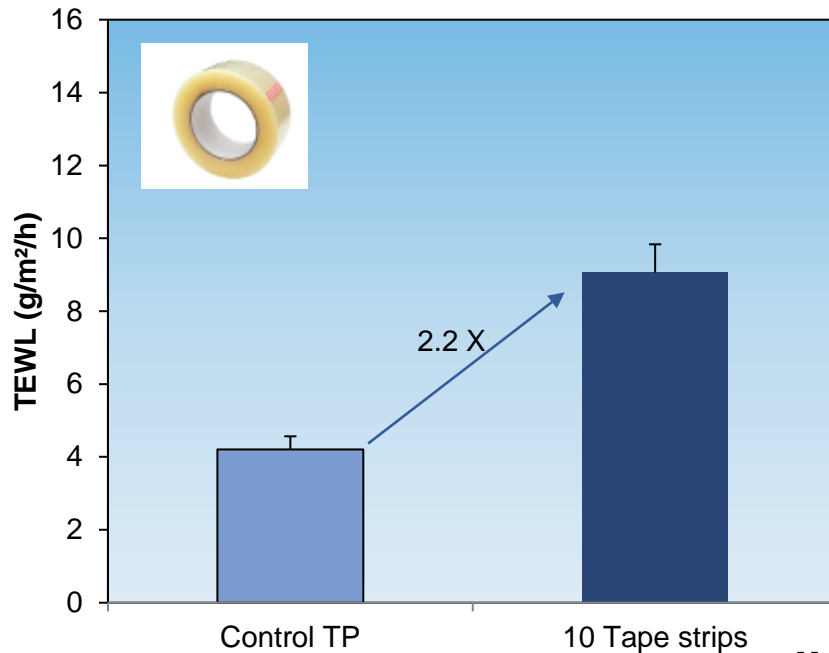Before and after skin preparation

### Dermal absorption - MAL



[$^{14}$C]-MAL

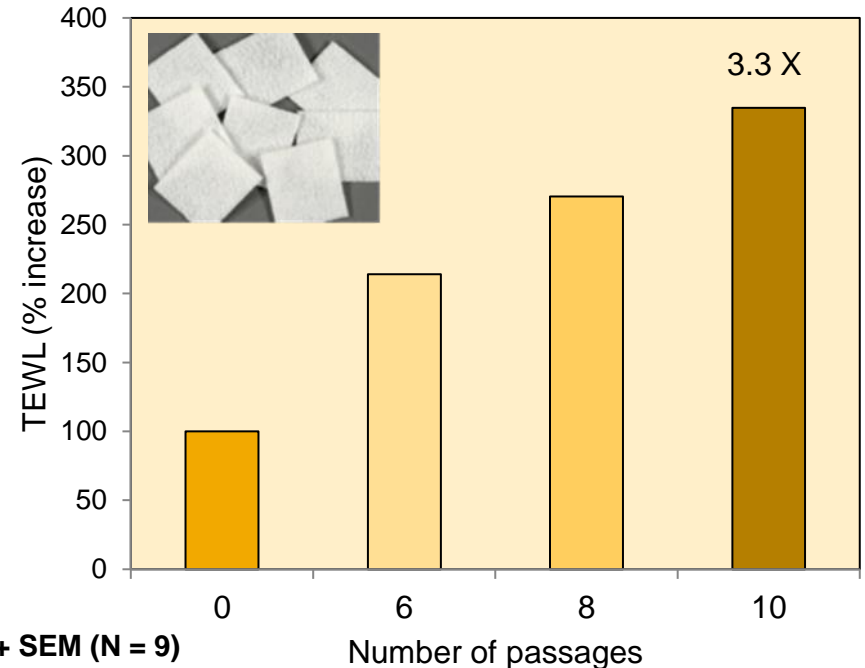Metvix cream



100 mg/cm², 2.5 h, N = 3
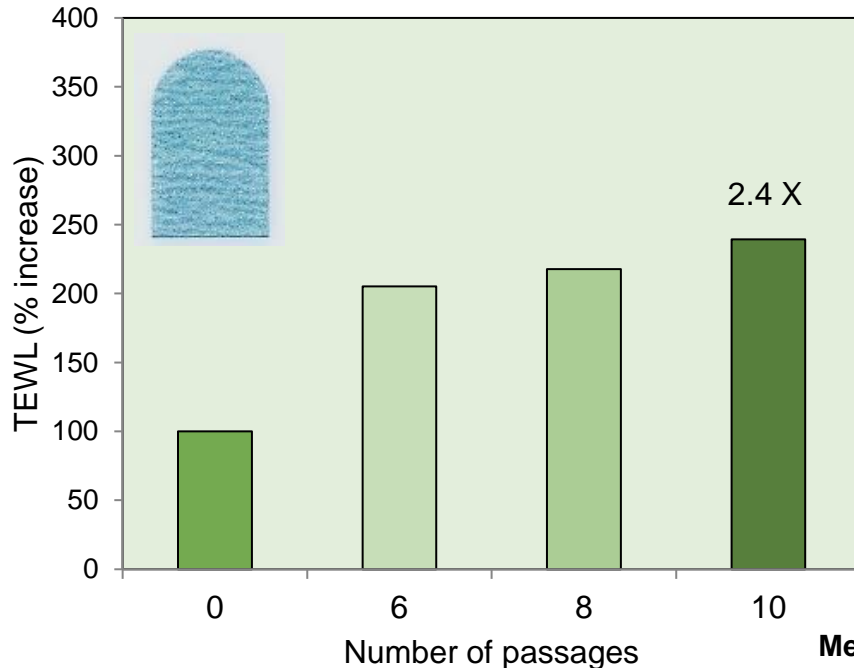
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# How to increase dermal absorption ?



Mean + SEM (N = 9)

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

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

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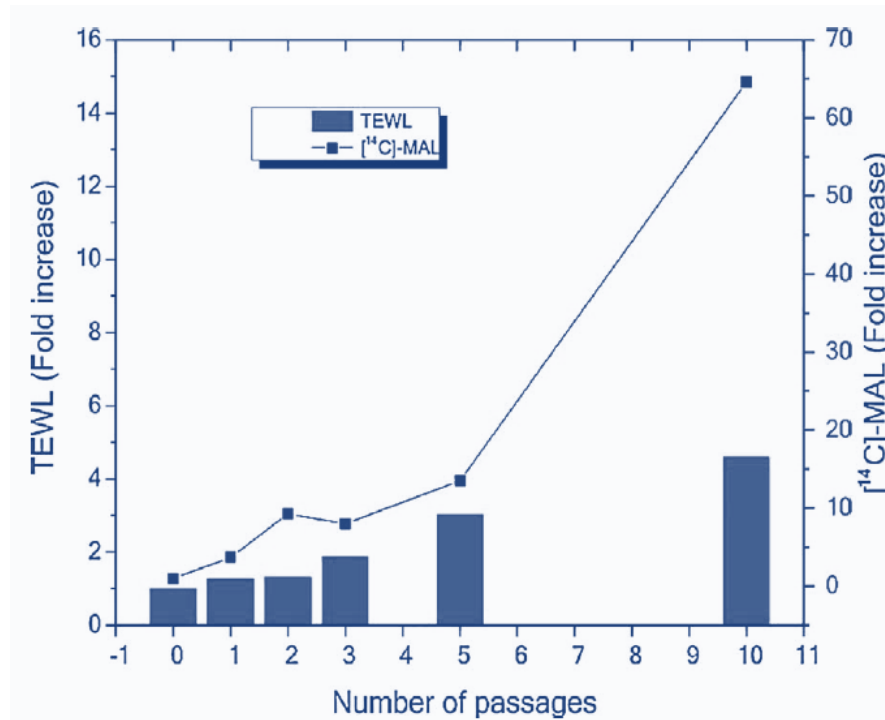# How to increase dermal absorption ?



Mean + SEM (N = 9)

– TEWL increased with increasing number of pad passages

– PREPSTER™ > Ambu®

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

# How to increase dermal absorption ?
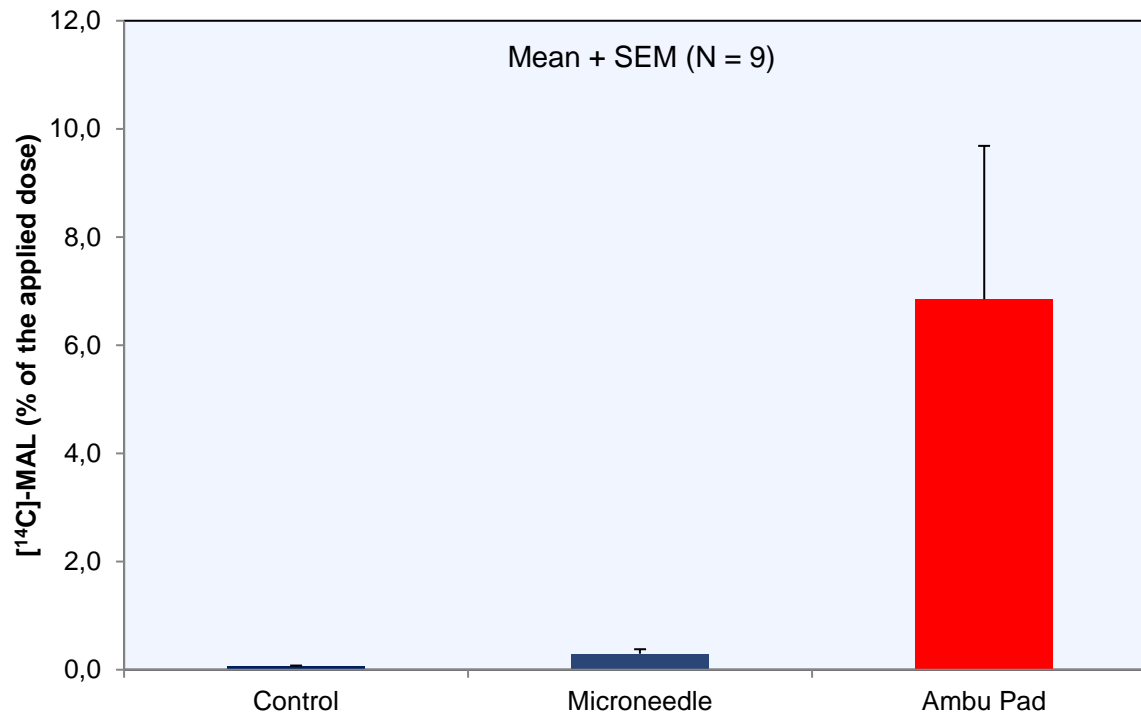


Ambu® Unilect™
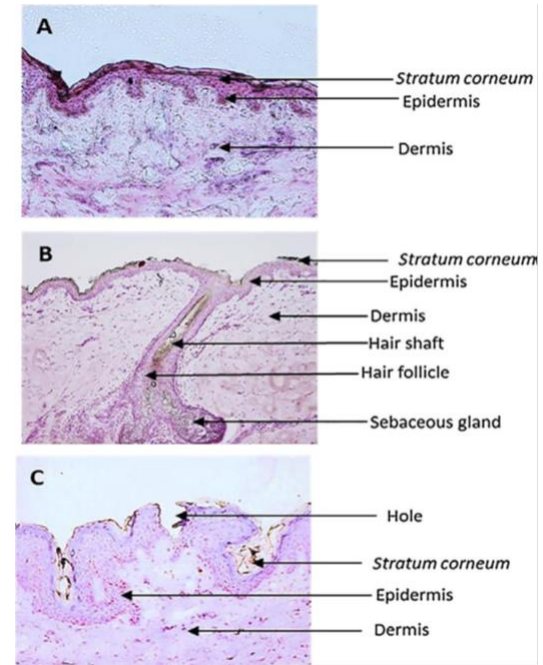
*Osman-Ponchet et al., 2017,*
*Photodiagn. Photodyn. Ther*

– Dermal absorption of MAL increased by increasing number of pad passages
– Good correlation between TEWL and MAL absorption

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# How to increase dermal absorption ?



Mean + SEM (N = 9)

[14C]-MAL (% of the applied dose)

Control    Microneedle    Ambu Pad

Control

A
Stratum corneum
Epidermis
Dermis

B
Stratum corneum
Epidermis
Dermis
Hair shaft
Hair follicle
Sebaceous gland

C
Hole
Stratum corneum
Epidermis
Dermis

– Dermal absorption of MAL increased by 4 times after microneedling and by 100 times after Ambu 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

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# ThanK You!