

Introducing SSbD concept to Cosmetics:

Advancing Human Skin *Ex-Vivo* Models: A Promising Platform for Studying Skin Irritation, Skin Allergy, and UVB-Induced Damage

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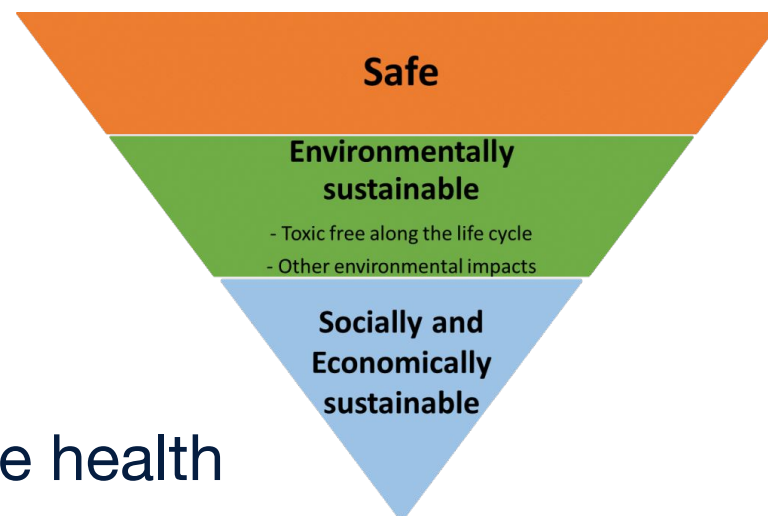
AHAVA – Dead Sea Laboratories



SSbD4Chem

SSbD - The triple challenge:

How to measure? & How to improve?



1. **S**afety – No harm to People health
2. **S**ustainable – No harm to Environment - Air, Soil, Water
3. **b**y **D**esign - Economically sustainable

Taking decisions before technology on a large scale

NOAEL - a Key Factor for Safety Evaluation in Cosmetics

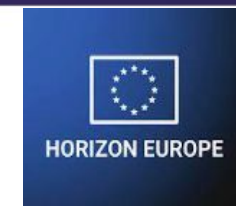
Safety Assessors Calculate MoS value

To distinguish between safe and not safe cosmetic product.

MoS – Margin of Safety – if above 100 the product is safe

$$\text{MoS} = \text{NOAEL} / \text{SED}$$

- **NOAEL** – No Observed Adverse Effect in mg/kg body weight –
related to **chemical/ingredient** specific toxicity
- **SED** – Systemic Exposure Dosage – in mg/kg body weight/day
related to **skin parameters** (age, area and mode of application)



***Ex-vivo* skin as promising NAMs (New Approach Methods):**

Skin pieces, preserve the native structure, cellular complexity, and extracellular matrix of human skin.

Comprise of keratinocytes, fibroblasts, Langerhans cells, and melanocytes, that closely replicates in vivo human skin.

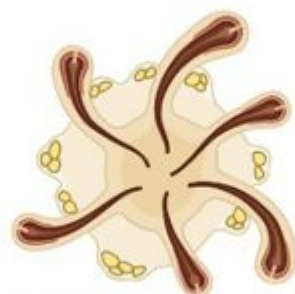
Aligned with the EU Cosmetic regulation of NO animal testing, ex-vivo skin models could serve as a next-generation tool for risk assessment.



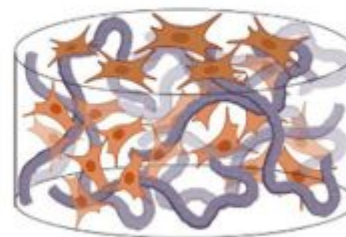
Advancing Human Skin *ex-Vivo* Models: A Promising Platform for Studying Skin Irritation, Skin Allergy, and UVB-Induced Damage



2D Cultures



Organoids



3D Cultures



***Ex-vivo* Human
skin model**

**Preparation
Simplicity**

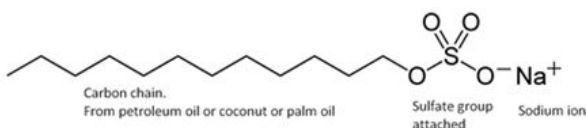
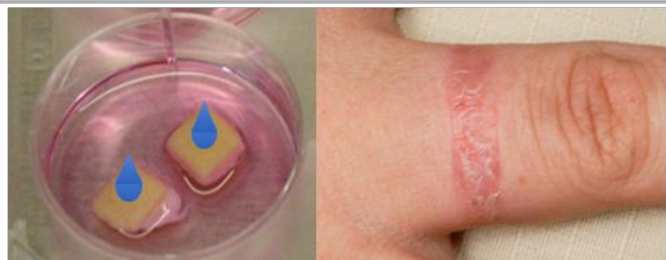


Safety evaluation methods for human epidermal and dermal topical exposure human skin *ex-vivo* and in vitro models

AHAVA's *ex-vivo* skin models (3 kinds of stressors)

Irritant Contact Dermatitis (ICD)

Induced by Sodium Lauryl Sulfate (SLS)



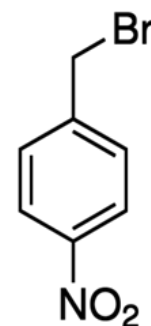
UV Damage

Induced by UVB lamp



Allergen Contact Dermatitis (ACD)

Induced by Nitrobenzobenzene (NBB)



1. Cell viability (MTT assay), **2. Apoptosis** (Caspase 3 activity) **3. Exposure time** (24, 48, 72 and 96 Hrs.)



AHAVA's *ex-vivo* human skin model biomarkers:

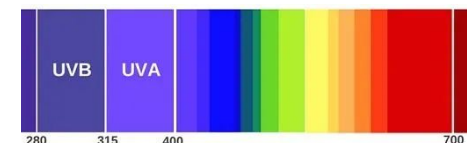
Parameters

Methods / Stressor

Read out

UVB damage
(anti-aging properties)

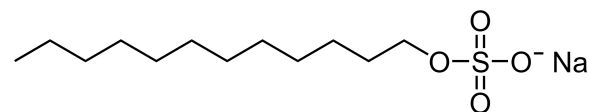
UVB light (200 mJ/cm²)



Apoptosis
Viability
Cytokines secretion:
TNFα, IL-6, IL-8, IL-10, IL-36

Irritation contact dermatitis

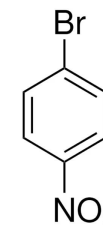
Sodium Lauryl Sulfate (SLS/SDS)



Apoptosis
Viability
Cytokines secretion:
IL-1α, IL-1β, IL-6, IL-8, IL-18,
IL-36

Allergic contact dermatitis

Nitrobromobenzen (NBB)



Apoptosis
Viability
Cytokines secretion:
IL-1α, IL-1β, IL-18, IL-36



AHAVA

Safety evaluation methods for human epidermal and dermal topical exposure: human skin *ex-vivo* and *in vitro* models

Paper in preparation:

"Advancing Human Skin *ex-vivo* Models: A Promising Platform for Studying Irritation, Allergy, and UVB-Induced Damage

Toward Future Validation"

		Validation Ex-vivo human skin organ model (without any topical application)			
Stressor/ Cytokine	Untreated				
Hours	24	48	72	96	
IL-1 α					
IL-1 β					
IL-6					
IL-8					
IL-18					
IL-36					
TNF α					
IL-10					

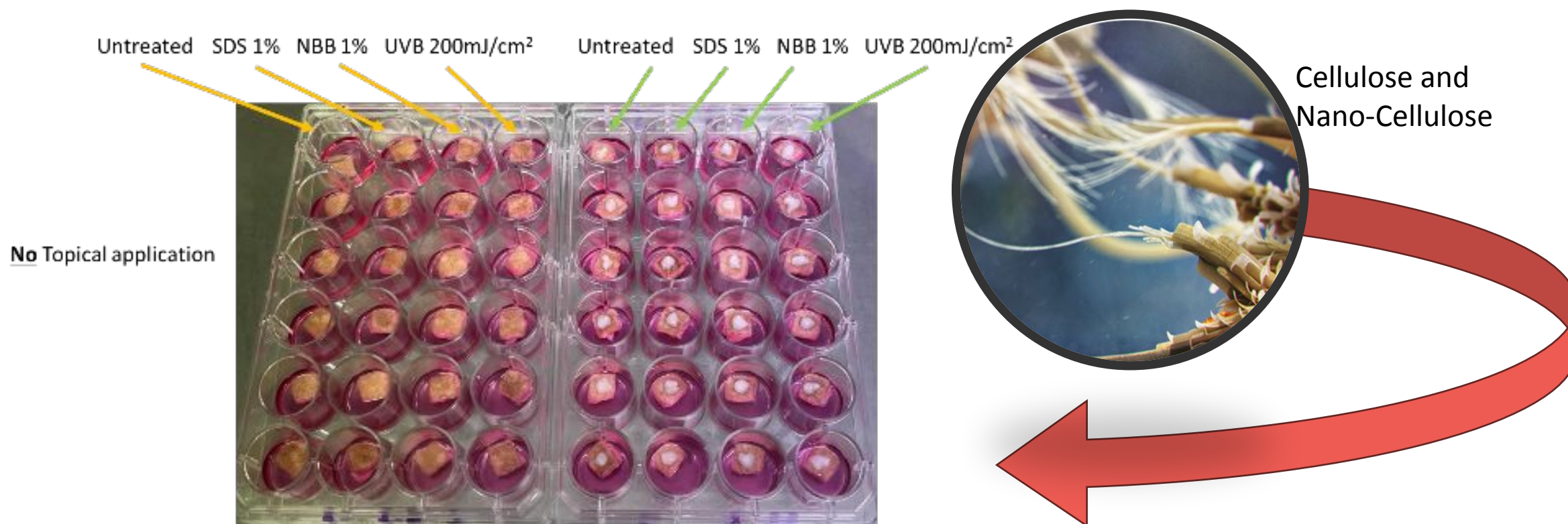
Heat map: All results obtained (so far, by 4 skin donors) for the Validation of the *ex vivo* model

Viability tests and apoptosis assays were performed – data not shown



Safety evaluation methods for human epidermal and dermal topical exposure of human skin *ex-vivo* and *in vitro* models.

Commercial nano-cellulose was topically tested on the *ex-vivo* human skin model as case study





Safety evaluation methods for human epidermal and dermal topical exposure –human skin *ex-vivo* and *in vitro* models

Results of each stressor (up) and Topical application of nano-cellulose (down)

	Validation Ex-vivo Human Skin Model (without any topical application)															
Stressor/ Cytokine	Untreated															
Hours	24	48	72	96												
IL-1 α																
IL-1 β																
IL-6																
IL-8																
IL-18																
TNF α																
IL-10																
	Ex-vivo Human Skin Model (+ topical application with nano cosmetic cellulose case study)															
Stressor/ Cytokine																
Hours																
IL-1 α																
IL-1 β																
IL-6																
IL-8																
IL-18																
TNF α																
IL-10																

Summarizing remarks:

***Ex-vivo* Human Skin Organ Validation**

1. The *ex-vivo* model demonstrates a robust tool as the actual human skin response.
2. Each stressor was found to :
 - Shows a specific cytokine fingerprint
 - Different stressors activate distinct cytokine sets
 - *Ex-vivo* responses mirror in vivo skin behavior (literature-supported)

Conclusions:

- *Ex-vivo* human skin systems represent a reliable and scientifically validated platform for advancing cosmetic, dermatological and pharmaceutical product development, ensuring good predictability, regulatory compliance and public safety.
- MoS could be evaluated, if **NOAEL** would be replaced by an alternative reliable protocol, based on *ex-vivo* tests results, combined with chemical in silico data.
- The concept of **Safe and Sustainable by Design** may offer exciting opportunities for the growing cosmetics business and could serve as a practical approach to advance cosmetic products, delivering hope for healthier life to the people and the planet.

Masada Skin Biotechnology Lab. At the Dead Sea-Arava Science Center (DSASC)



Project partners



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