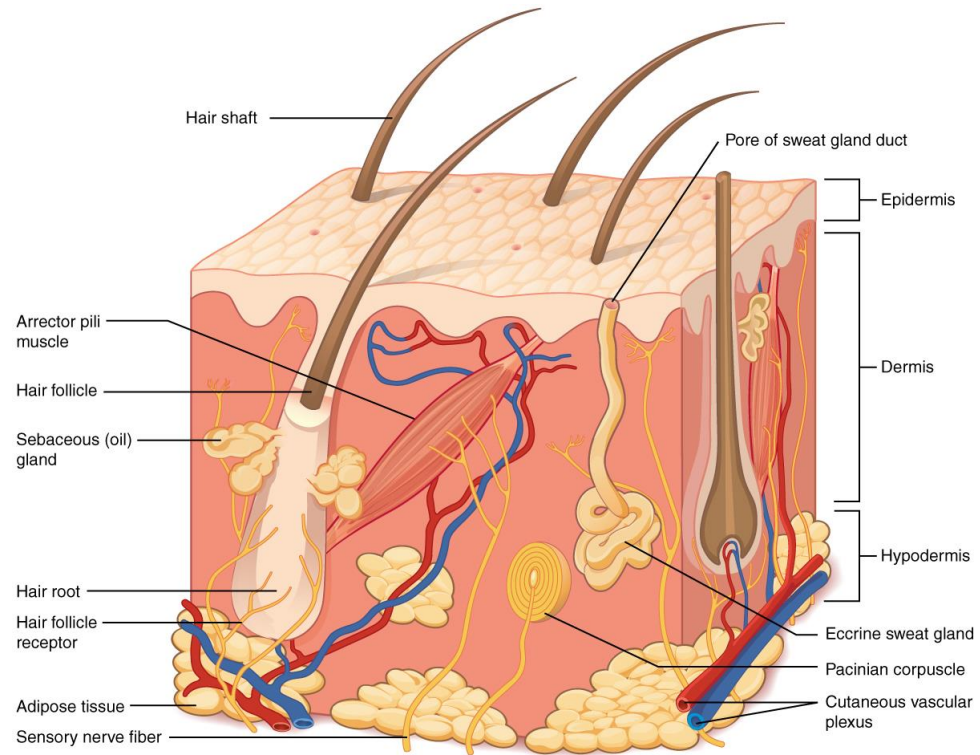


# Unveiling Efficacy: Harnessing Human *Ex Vivo* Skin Models for Active Ingredients and Cosmeceutical Products

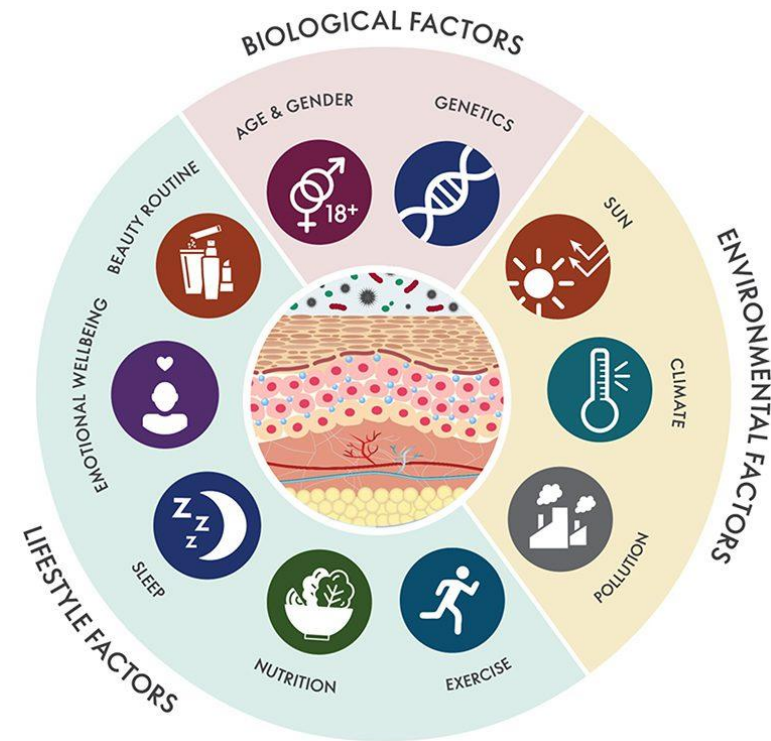
**Wannita Klinngam, Ph.D.**

Researcher at National Nanotechnology Center (NANOTEC)  
National Science and Technology Development Agency (NSTDA)

# Understanding Skin Biology

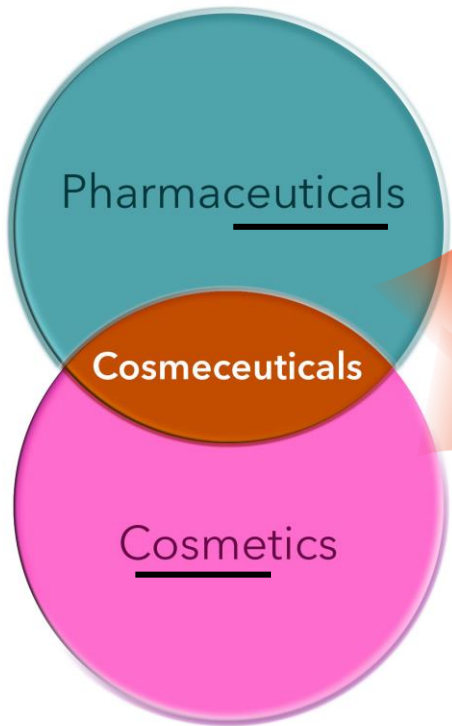


Skin is the largest organ of the body

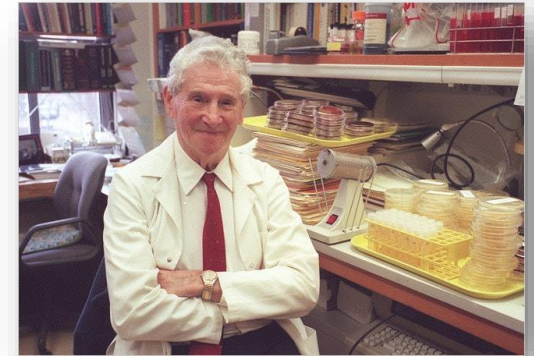


Dynamic environment influenced by genetics, aging, and environmental stressors

# "Cosmeceuticals": The New Medicine of Beauty

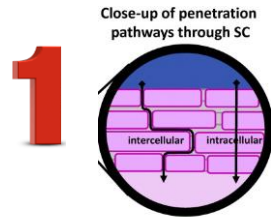


Cosmeceuticals is  
"a topical preparation that is sold as a cosmetic but has performance characteristics that suggest pharmaceutical action."



Dr. Albert Kligman  
The father of cosmeceuticals

"Three crucial questions" for cosmeceutical product



Can the active ingredient **penetrate to stratum corneum** with sufficient concentration that can show consistent mechanism of action?

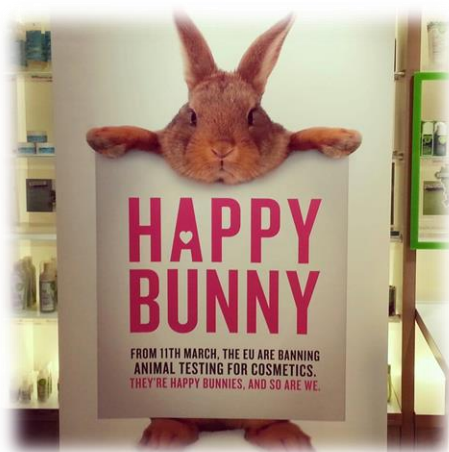


Does the active ingredient have a known **specific biochemical mechanism of action** in the cells/human skin tissues?



Are there published, double-blind, peer-reviewed, placebo-controlled, **statistically significant, clinical trials** to substantiate the efficacy claims?

# The Ban on Cosmetic Testing on Animals



**11.09.2004**

Prohibition of the testing of finished products on animals

**11.03.2009**

Prohibition of the testing of ingredients or combination of ingredients on animals

**11.03.2009**

Marketing ban for all human health effects with the exception of repeated-dose toxicity, reproductive toxicity and toxicokinetics.



**11.03.2013**

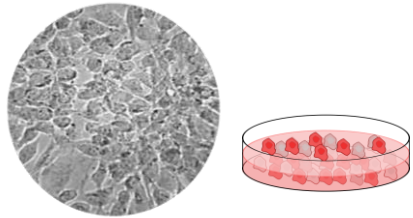
Total marketing ban, irrespective of the availability of alternative non-animal tests

Sources:

EU Regulation 1223/2009/EC

<http://www.youtube.com/watch?v=U9lww0Gg5rQ>

# Pros and Cons of Efficacy Testing in 2D Cell Culture



**2D Cell Culture**



Well-established & fast for primary assessment



Economical



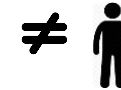
Easy & convenient for analysis and cell maintenance



High throughput capacity



Reveals the mechanism of action of tested articles



Unable to mimic real human skin



No physical skin barrier

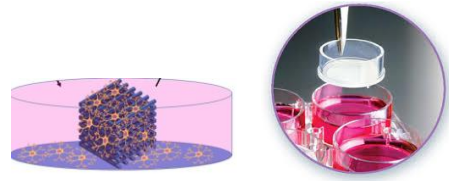


Lack of some essential cells/proteins



Enables testing of active ingredients

# Pros and Cons of Efficacy Testing in 3D Skin Models



**3D Skin Model**



Closer Resemblance to Human Skin Than 2D Culture



Validated models available commercially



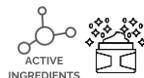
Facilitates early screening and regulatory toxicity testing



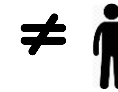
Long term life span



Reveals the mechanism of action of tested articles



Enables testing of active ingredients & finished products



Unable to mimic real human skin



Impaired physical skin barrier function



Lack of some essential cells/proteins



Lack of skin appendages



Expensive

# Pros and Cons of Efficacy Testing in Clinical Trials



## Clinical Trials



Study in human



Complete of physical skin barrier function



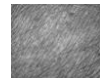
Full of essential cells/proteins



Regulatory compliance



Consumer Confidence



Providing results based on skin health and appearance parameters



Ethical considerations, particularly with invasive procedures



Expensive



Time consuming



Invasive method for investigating testing article's mechanism of action

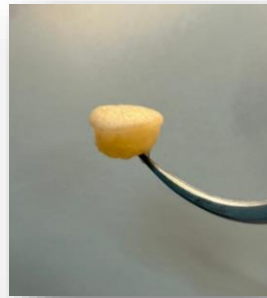


Individual variation

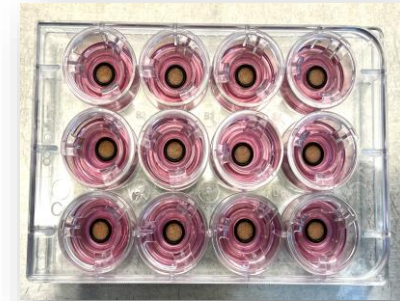
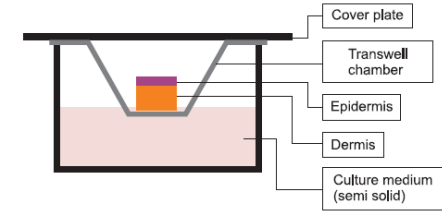
# ***Ex Vivo* Skin Models: Bridging the Gap Between *In Vitro* and Clinical Studies**



**Skin from  
Abdominal Surgery**



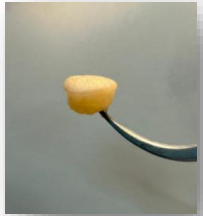
**Cut into small pieces**



**Air-liquid interface  
culture condition**




# Pros and Cons of Efficacy Testing in *Ex Vivo* skin Model







Human *Ex Vivo* Skin



-  Closely resembling clinical testing
-  Enhanced predictivity for clinical outcomes
-  Customizability for specific research needs
-  Capability to select donor age and skin type
-  Reveals the mechanism of action of tested articles
-  Rapid observation of product effects
-  Enables testing of active ingredients & finished products



-  Lack of blood circulation
-  Ethic consideration
-  Short lifespan
-  Donor variation

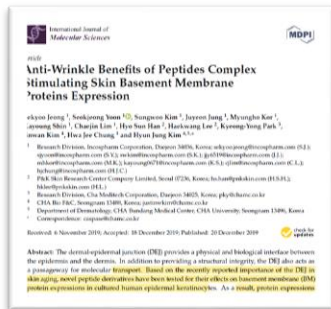
# Bridging Connections: *Ex Vivo* and Clinical Trial Results



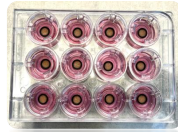
Peptide complex  
conjugated with vitamin C



**incospharm**



Jeong, S., et al. *Int.J.Mol.Sci*, 2020, 21,73

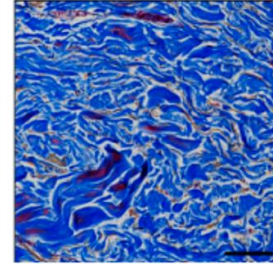
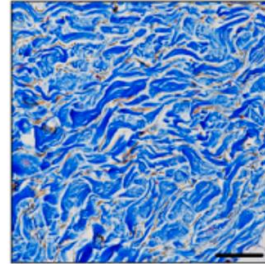


*Ex Vivo* Skin  
2 days

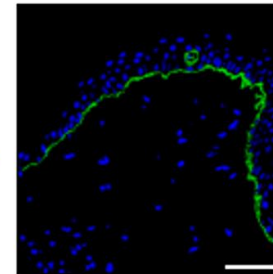
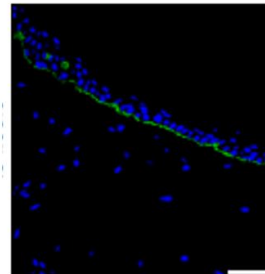
Vehicle

Peptide complex

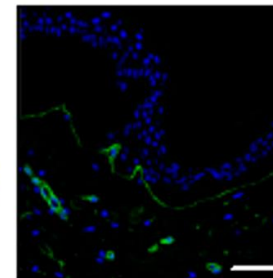
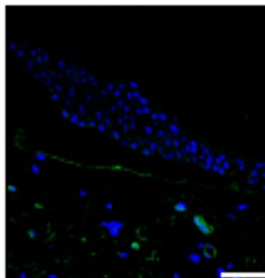
Collagen



Collagen XVII



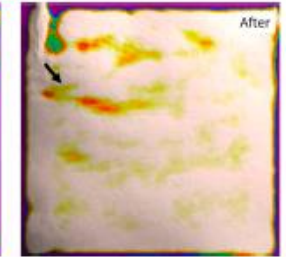
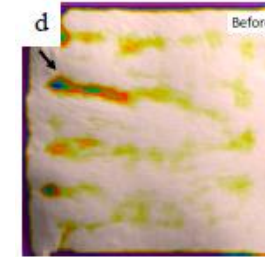
Laminin



Facial wrinkle  
8 weeks

Before

After



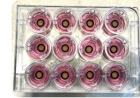
# Bridging Connections: *Ex Vivo* and Clinical Trial Results



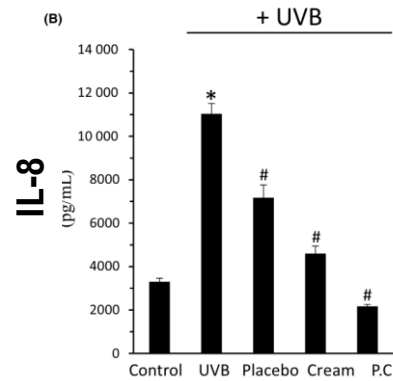
**Gel containing  
CBD and EPA**



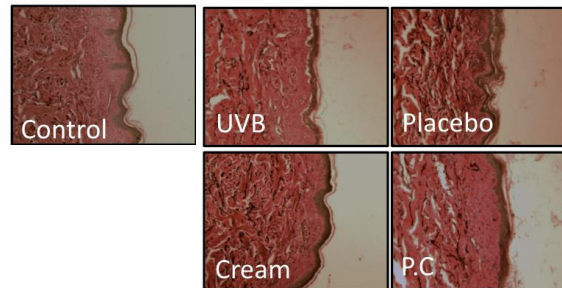
Cohen G, et al. *J Cosmet Dermatol.* 2023; 22: 3047-3057



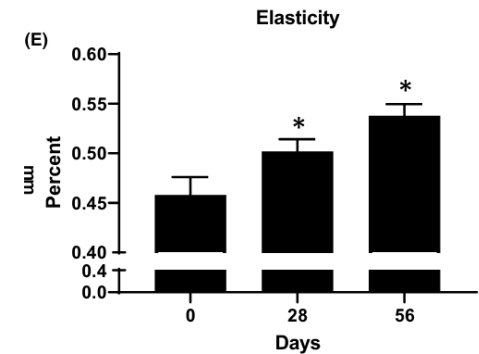
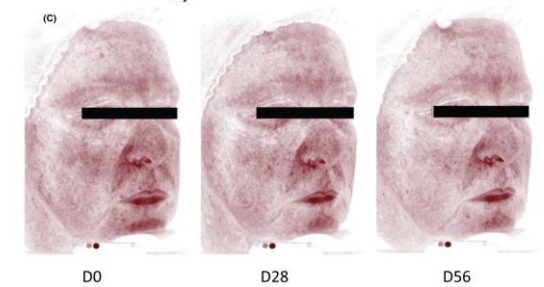
**Ex Vivo Skin  
1 day**



**Collagen & Elastin**



**Red Spot & Skin Elasticity  
8 weeks**



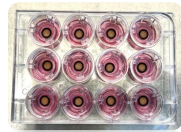
# Bridging Connections: *Ex Vivo* and Clinical Trial Results



**Peptan® F**  
collagen peptides  
supplement



**Rousselot**  
Health & Nutrition



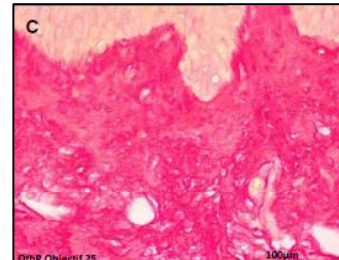
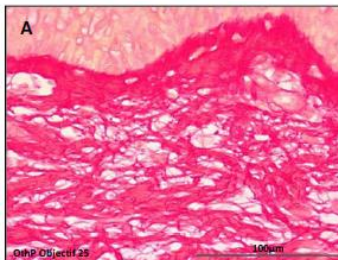
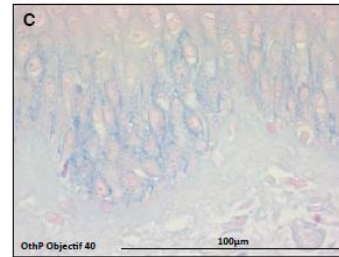
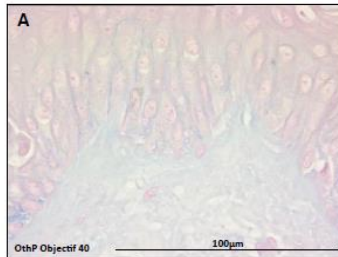
**Ex Vivo Skin**  
9 days

Glycoaminoglycan

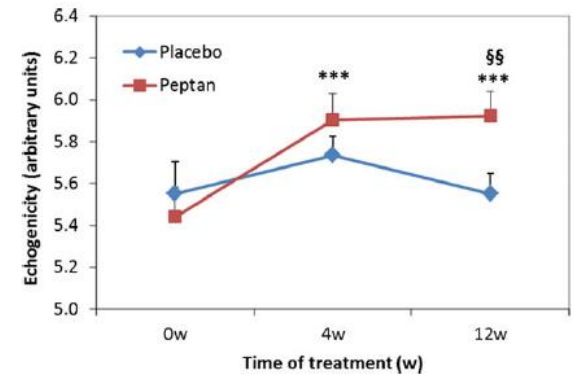
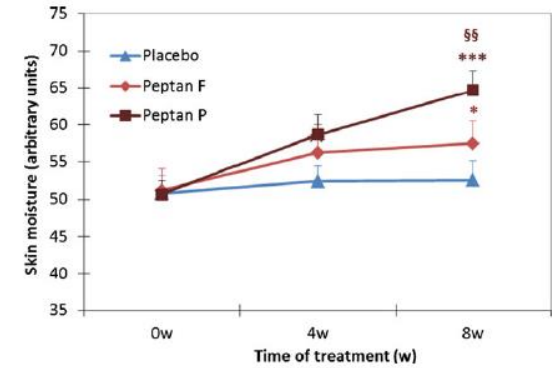
Collagen

**Vehicle**

**Peptan®**



**Skin Hydration &  
Collagen Density**  
8 weeks





# Efficacy & Safety Testing of Active Ingredients & Products



**3D tissue**



**Ex vivo skin**



**Clinical study  
(Our partners)**








# MOLECULAR BEAUTY TEAM





## OUR CUSTOMERS

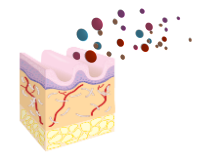
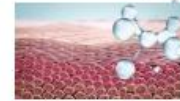


# Evaluating Anti-Aging Effects with Human *Ex Vivo* Skin Models

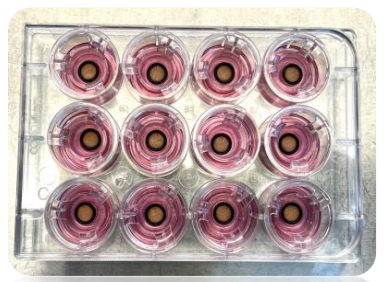
 Water/Vehicle control  
 Customer's active ingredient /product  
 **Active/Product benchmarks**

---

 2% EtOH + 1% Tween 20  
 0.5% Retinol



Hyaluronic acid stimulation	Collagen stimulation	Anti-inflammation
<ul style="list-style-type: none"> <li>➤ Hyaluronic acid staining/secretion</li> </ul>	<ul style="list-style-type: none"> <li>➤ Collagen type I staining</li> <li>➤ MMP-1 (Collagenase-1)</li> </ul>	<ul style="list-style-type: none"> <li>➤ IL-6</li> </ul>



**Topical administration daily, for 5 days**

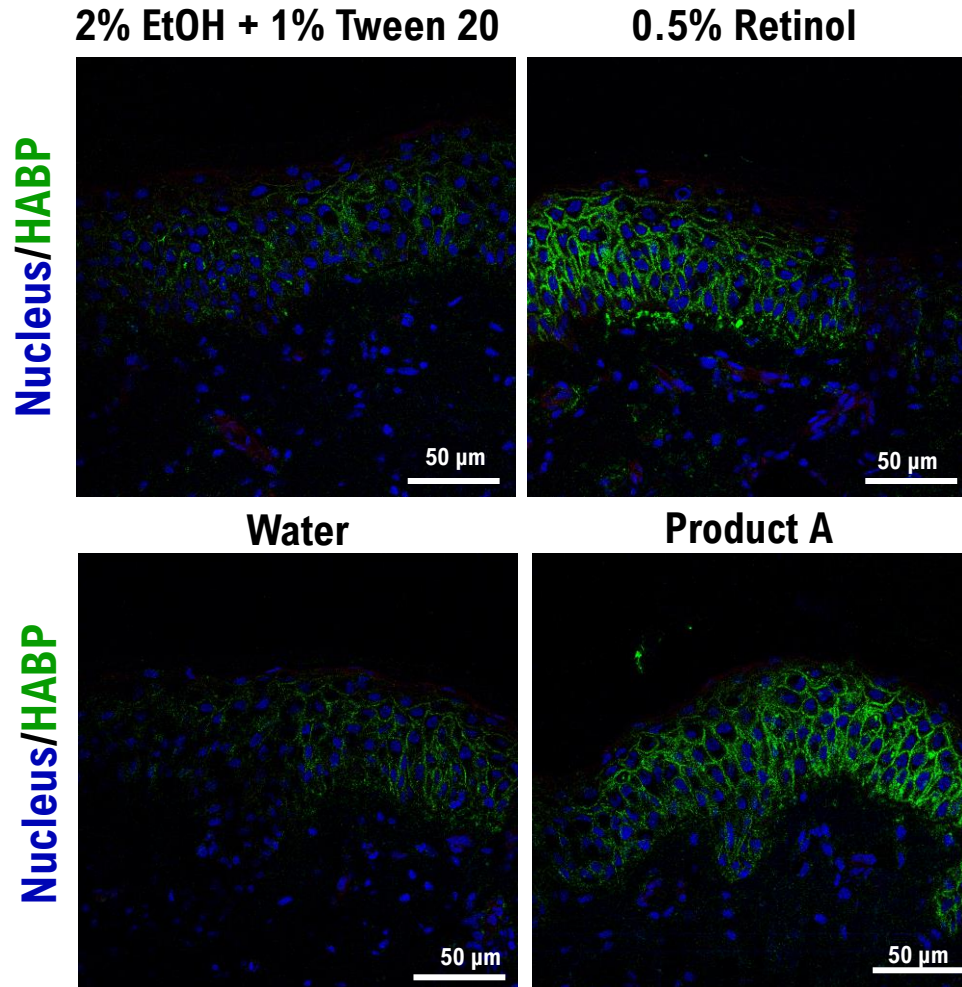


**Chronologically aged *ex vivo* skin model**

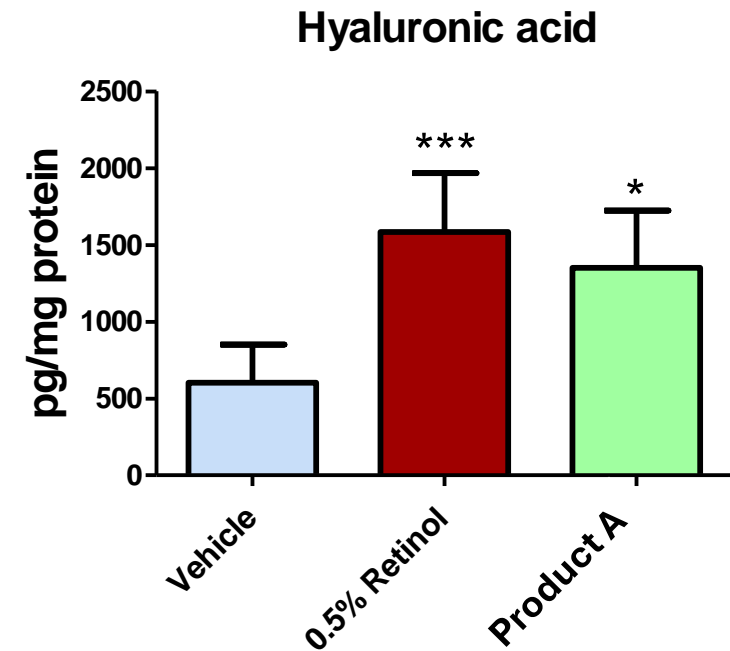


# HYA-Stimulating Effect in an Aged *Ex Vivo* Skin Model

## Hyaluronic acid staining



## Hyaluronic acid secretion

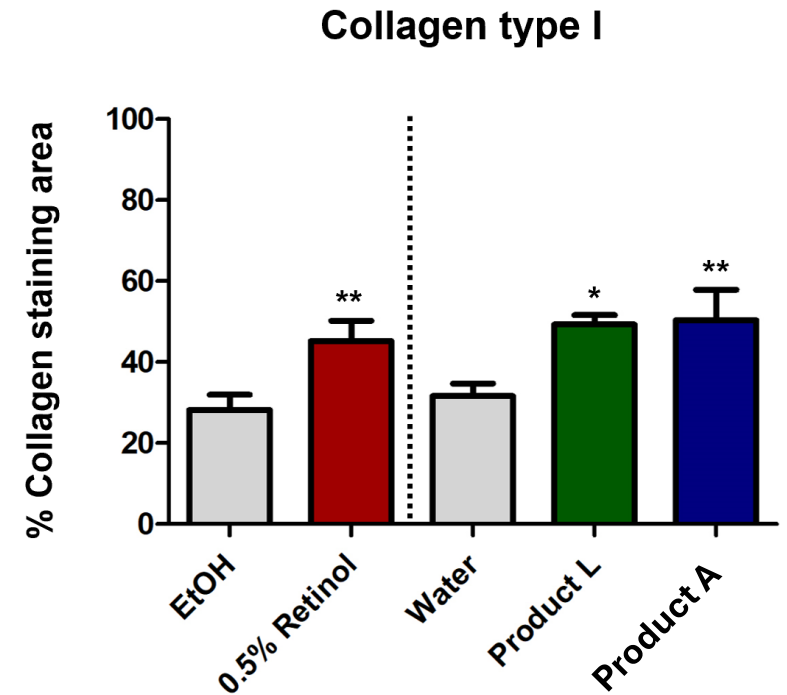
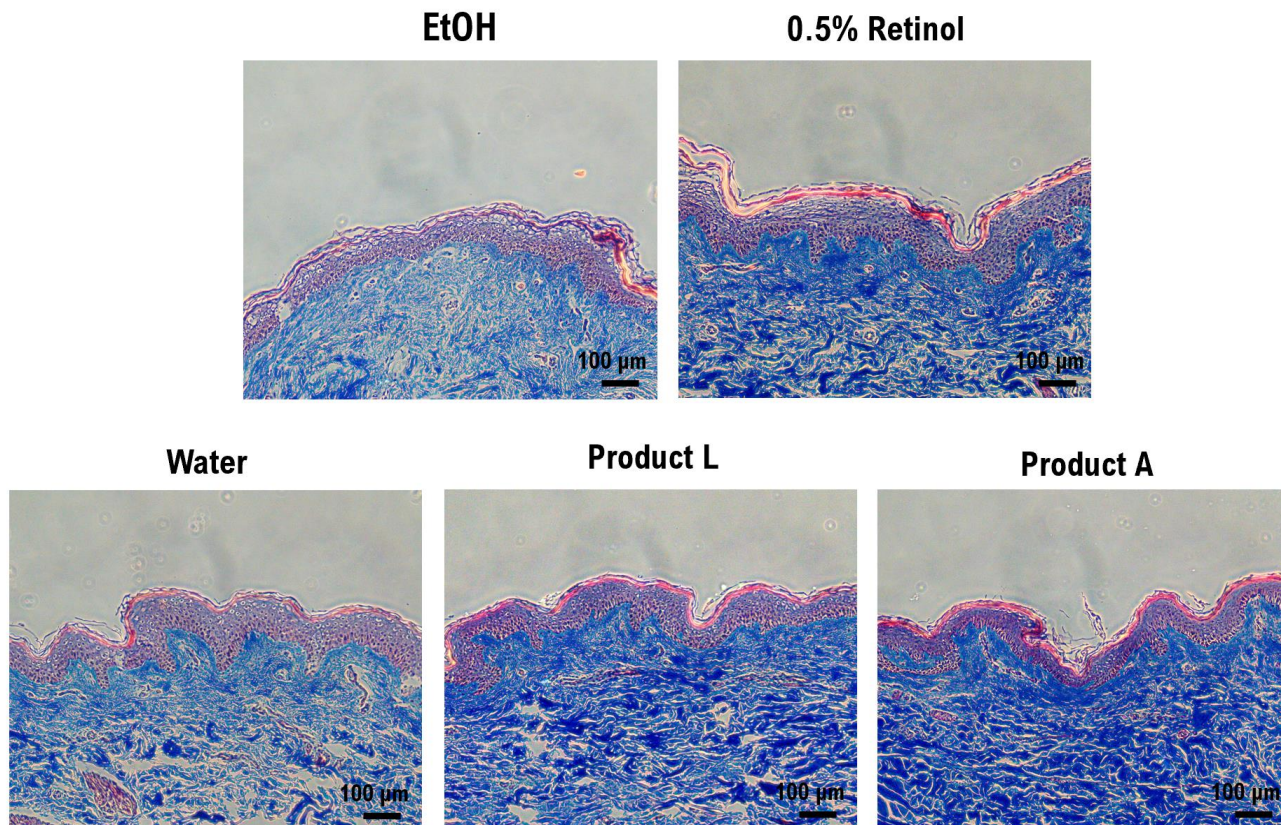






# Collagen-Stimulating Effect in an Aged *Ex Vivo* Skin Model

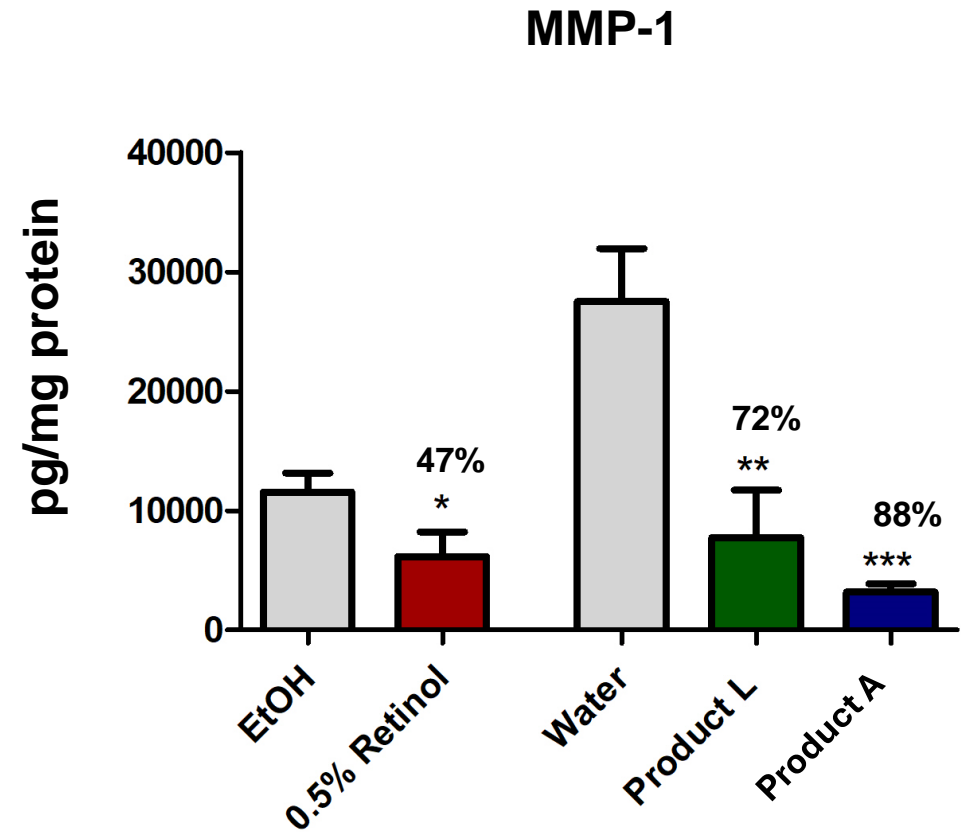
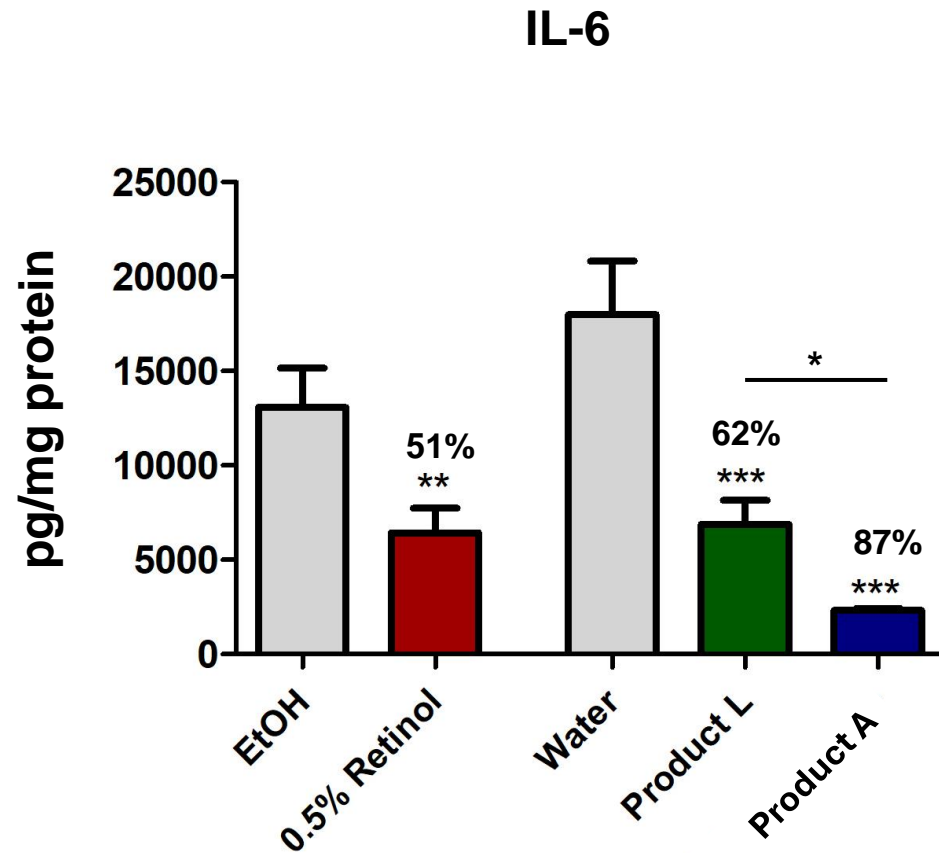
## Collagen type I staining



Product L = Product benchmark






# Anti-inflammatory Effect in an Aged *Ex Vivo* Skin Model





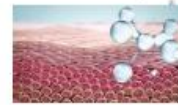
Product L = Product benchmark

# Evaluating Anti-Pollution Effects with Human *Ex Vivo* Skin Models

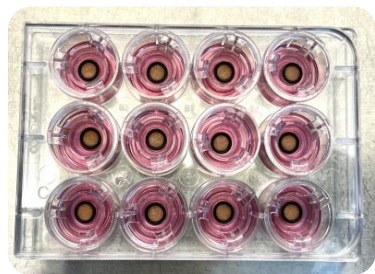
 Water/Vehicle control  
 Customer's active ingredient /product  
 Active/Product benchmarks

---

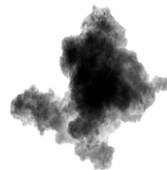
 1% EtOH + 1% Tween 20  
 0.25% Sytenol A



Hyaluronic acid stimulation	Collagen stimulation	Anti-oxidant
➤ Hyaluronic acid staining/secretion	➤ Collagen type I staining	➤ Reactive oxygen species (ROS)



**PM-Exposed Ex Vivo Skin Model**



**PM2.5**

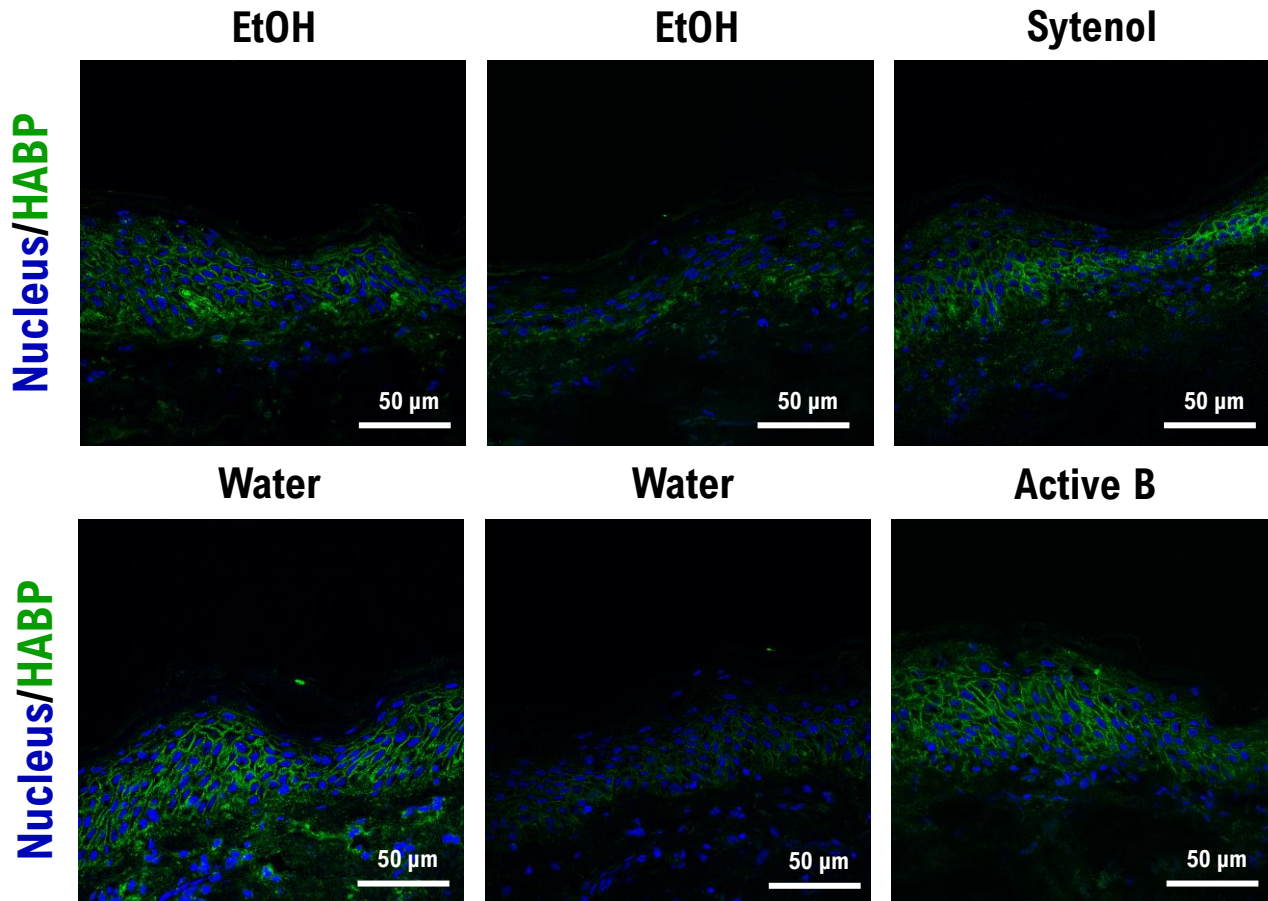


**Topical administration daily, for 5 days**

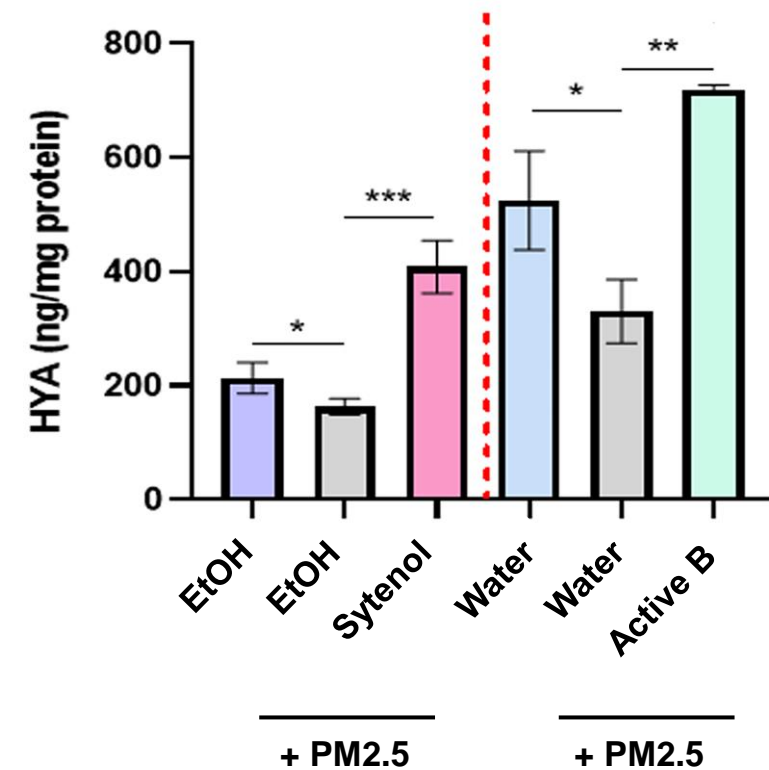
# HYA-Stimulating Effect in a PM-Exposed *Ex Vivo* Skin Model

## Hyaluronic acid staining

+ PM2.5



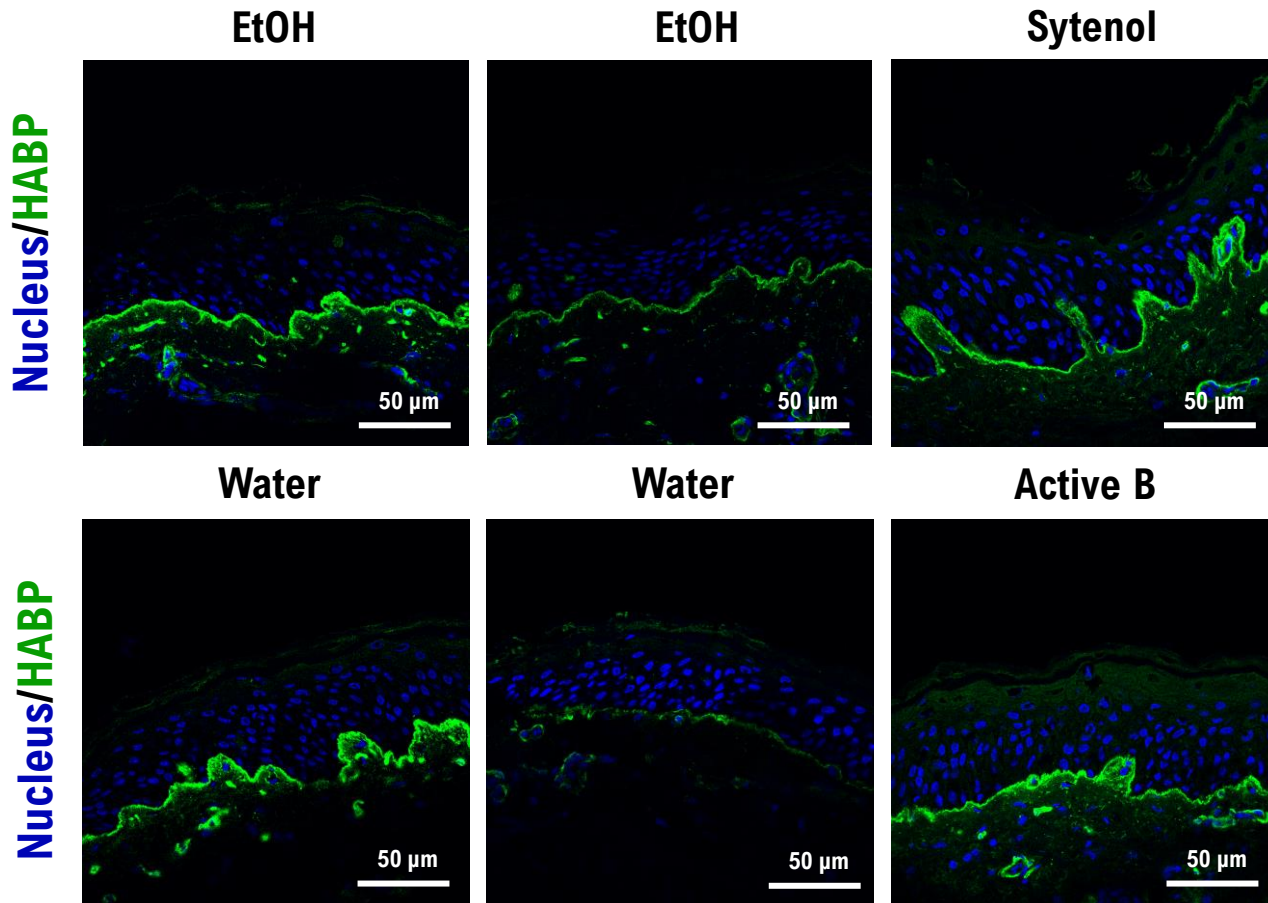
## Hyaluronic acid secretion



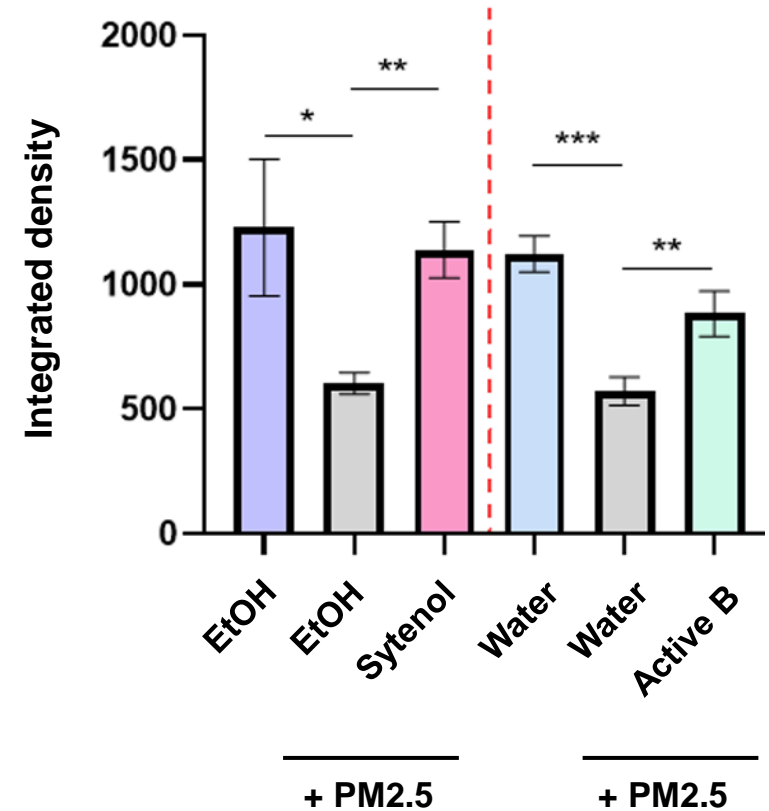
# Collagen-Stimulating Effect in a PM-Exposed *Ex Vivo* Skin Model

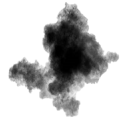
## Collagen type I staining

+ PM2.5

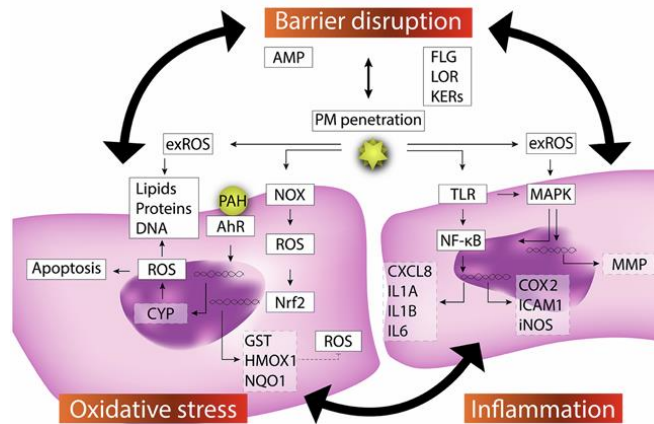
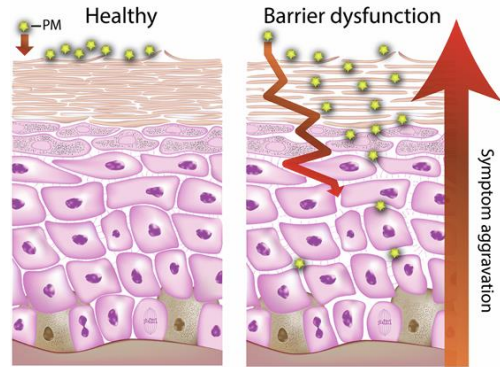


## Collagen type I

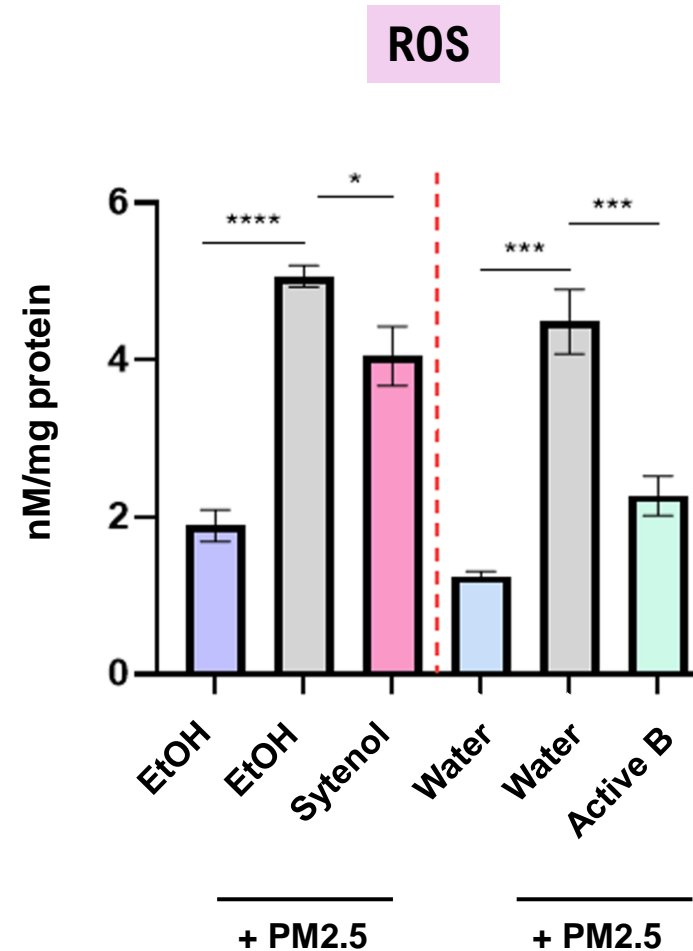




# Anti-Oxidant Effect in a PM-Exposed *Ex Vivo* Skin Model



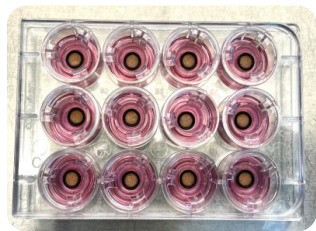
Dijkhoff IM, et al. Part Fibre Toxicol. 2020 Jul 25;17(1):35.



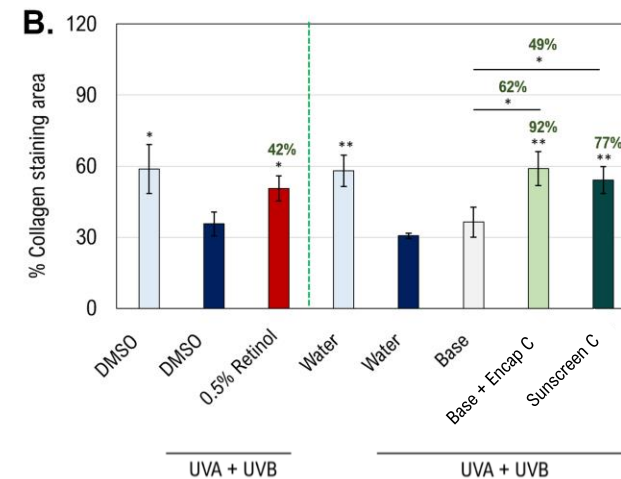
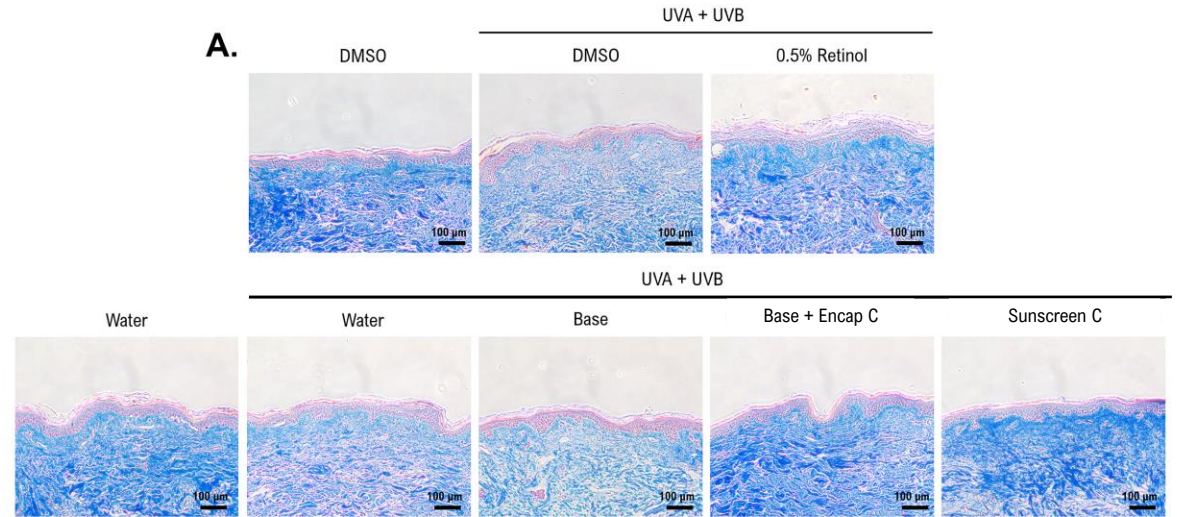
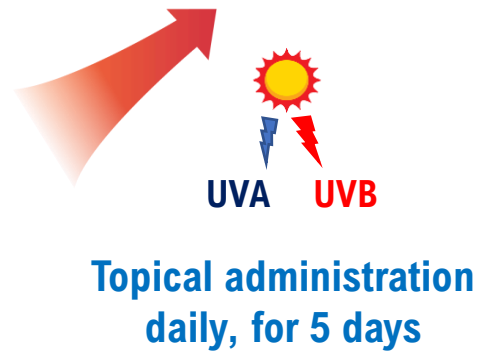


# Using a Photo-Aged Ex Vivo Skin Model for Herbal Cosmeceutical Health Claims (Collagen-Stimulating Effect)

- Base
  - Base + Encap C
  - Sunscreen C
  - Water
- 
- 0.5% Retinol
  - 0.5% DMSO



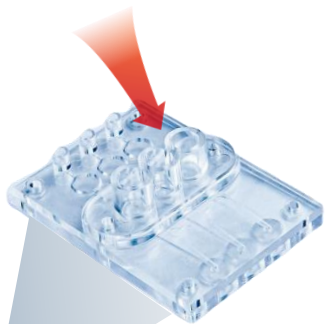
**UV-Exposed  
Ex Vivo Skin Model**



# Transforming *Ex Vivo* Skin Testing: A Glimpse into the Future



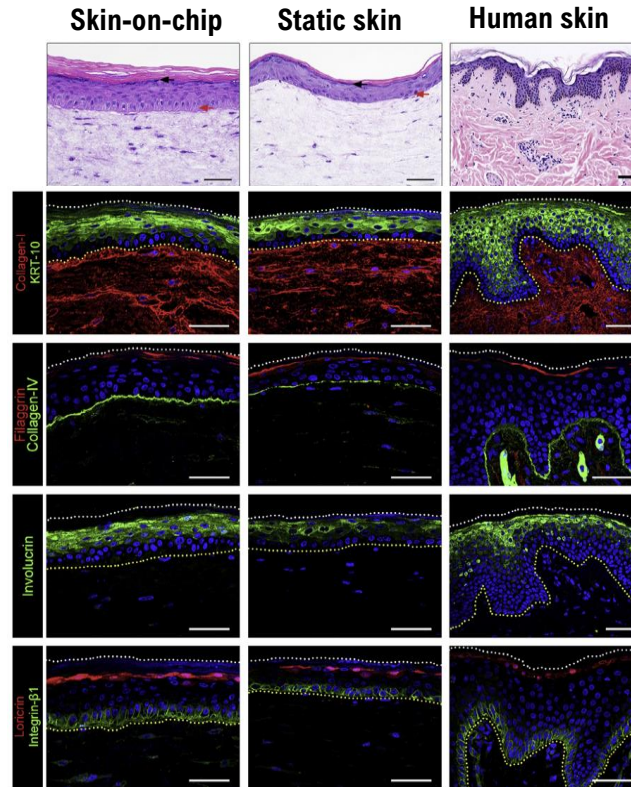
Human skin biopsy



Skin-on-chip device mimicking blood flow



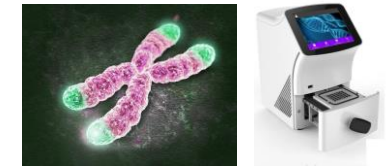
4D tissue culture platform



4D skin culture platform mimicking blood flow can **prolong tissue viability** and **enhance similarity** to real human skin



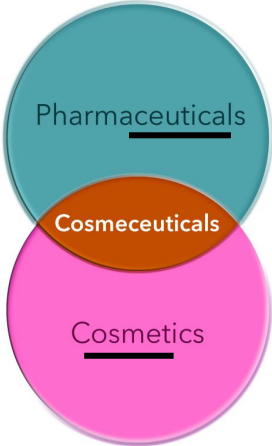
Aging clock



Telomere length



# Paradigm Shift in Skincare Research: Harnessing Human *Ex Vivo* Skin Models



**Forbes**

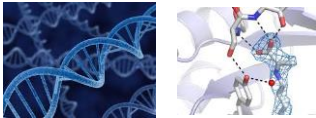
What Successful Beauty Startups Are Focusing On Now

Richard Kestenbaum Contributor  
Retail



Chella Lifestyle COURTESY CHELLA

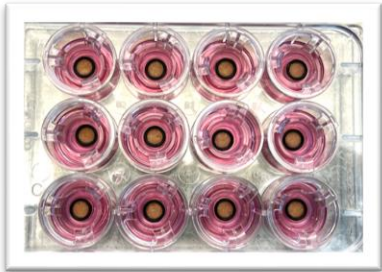
One of the “Three crucial questions”  
for cosmeceutical product



A known specific biochemical  
mechanism of action  
in the cells/human skin tissues?



Human *ex vivo* skin model



- Mimics real human skin
- Reveals mechanism of action of tested articles
- Suited for testing both active ingredients and finished products

“when you say **science**, it’s about performance...**ingredients must deliver immediate, tangible results**”

# THANK YOU & WE LOOK FORWARD TO COLLABORATING WITH YOU



## Molecular Beauty: The Paragon of Efficacy & Safety Testing Services

Active Ingredients  
Cosmeceuticals  
Nutraceuticals

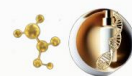


### REAL HUMAN SKIN (*Ex vivo* skin)

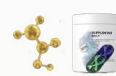


### ADMINISTRATION

#### TOPICAL



#### SYSTEMIC



### INDUCERS



### ASSAYS



STARTING FROM 4,900 USD  
(175,000 THB)



More details on  
our efficacy testing services



Contact: Wannita.kli@nanotec.or.th  
Tawin@nanotec.or.th

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