GENENCARE® OSMS for skin care

From Nature to Nurture™

5/17/2019

DuPont Nutrition & Biosciences

GENENCARE® OSMS skin care master presentation

Content

Range overview - high level and detailed level

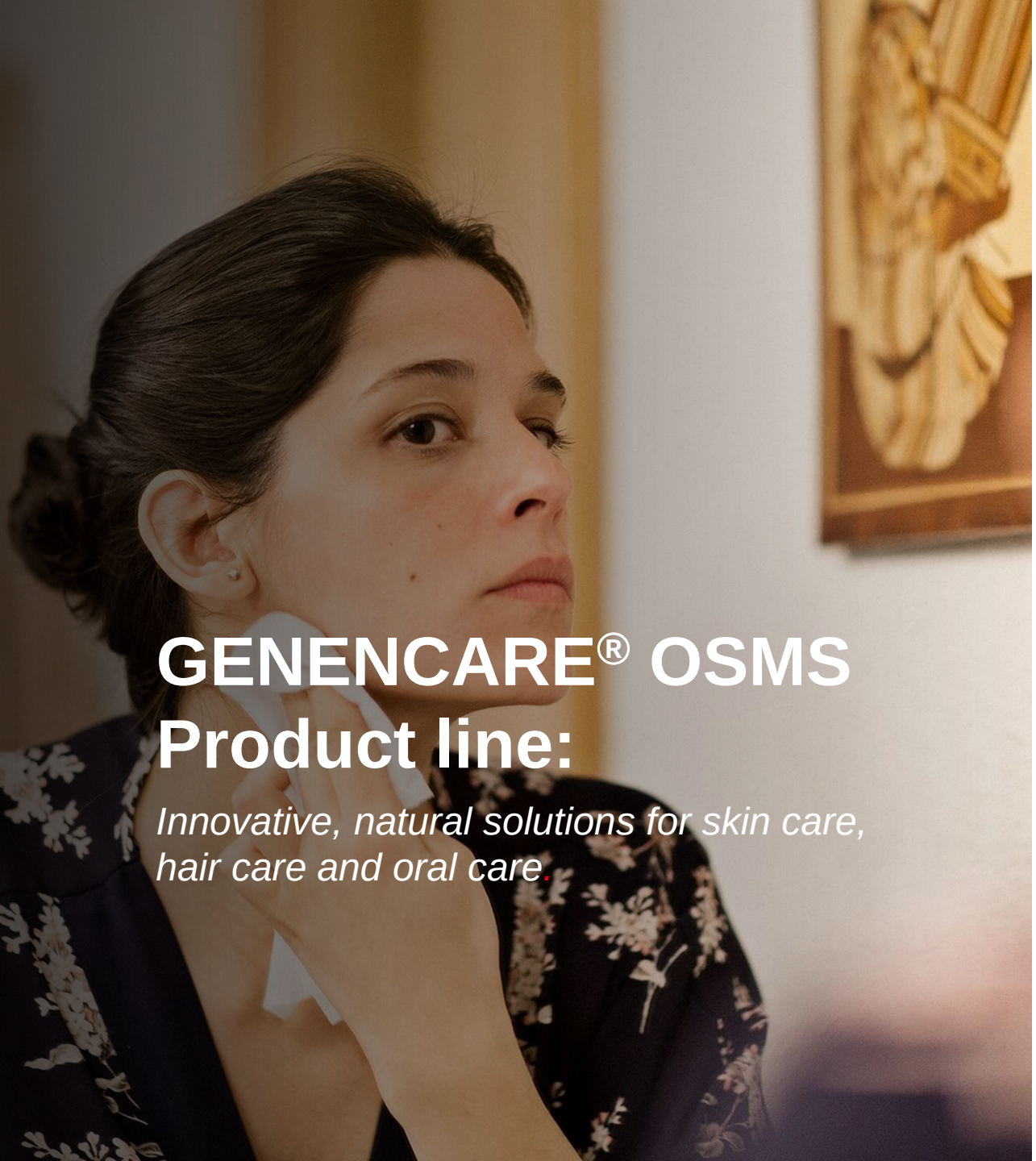
Skin science primer

Osmolytes – The science behind osmolytes

GENENCARE® OSMS BA
- Product overview
- Benefit drill-downs: Moisturization, Protection, Sensory

GENENCARE® OSMS MI
- Product overview
- Benefit drill-downs: Water, Oxygen, Energy

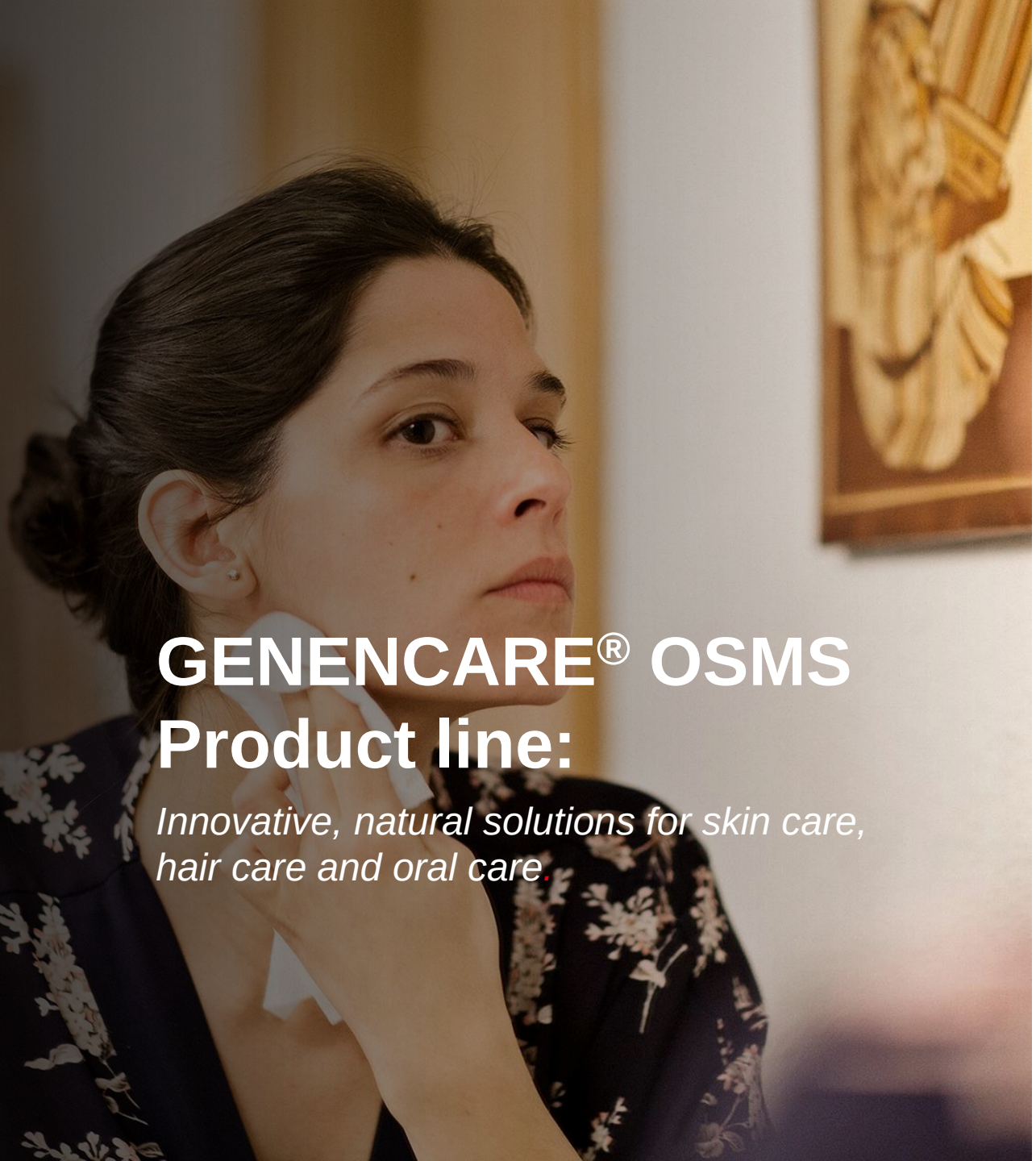
GENENCARE® OSMS PRO
- Product overview
- Benefit drill-downs: Antiox, Detox, Skin barrier



GENENCARE® OSMS Product line:

Innovative, natural solutions for skin care, hair care and oral care.

1. **GENENCARE® OSMS BA**
   Betaine
2. **GENENCARE® OSMS CC**
   Betaine and Tricalcium Phosphate
3. **GENENCARE® OSMS MI**
   Inositol
4. **GENENCARE® OSMS PRO**
   Water, Betaine, Proline, Serine, Inositol



GENENCARE® OSMS Product line:

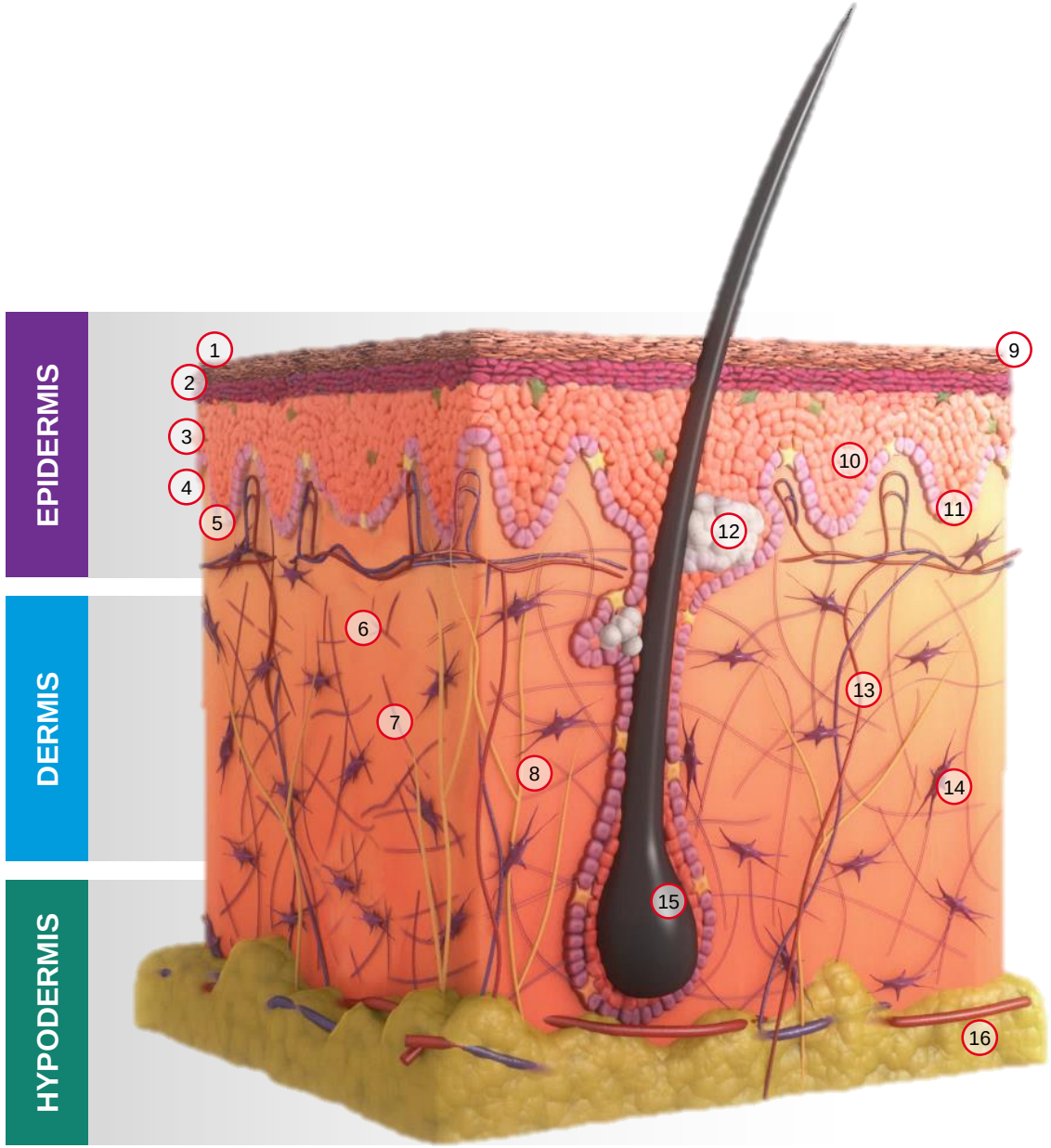
Innovative, natural solutions for skin care, hair care and oral care.

1. **GENENCARE® OSMS BA**
   Moisturizing osmolyte
2. **GENENCARE® OSMS CC**
   Moisturizing osmolyte for anhydrous formulas
3. **GENENCARE® OSMS MI**
   Invigorating osmolyte
4. **GENENCARE® OSMS PRO**
   Detox osmolyte complex

Skin science primer

Basics

Skin structure

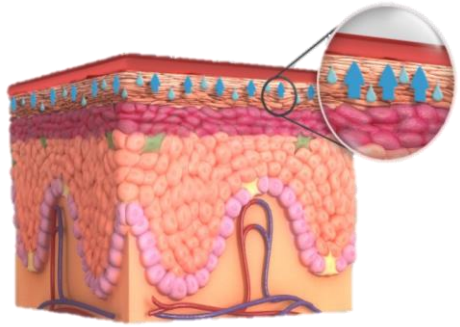


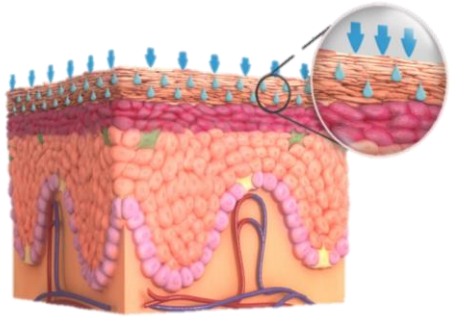
1. Stratum corneum = Horny layer
2. Stratum granulosum
3. Stratum spinosum
4. Basal layer
5. EDJ
6. Elastin
7. Collagen
8. Glycosaminogylcans (GAG)
9. Corneocytes
10. Keratinocytes
11. Melanocytes
12. Sebaceous gland
13. Blood vessels
14. Fibroblasts
15. Hair
16. Adipocytes

Skin moisturization strategies

SHORT TERM

Where?





What?

OCCLUSION

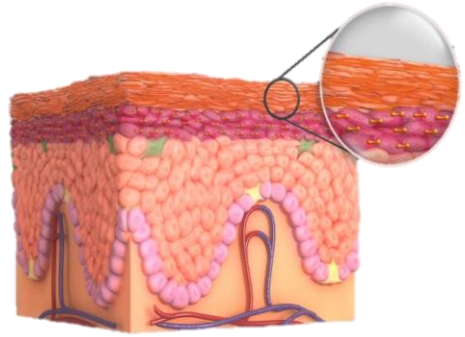
- Create a film at the surface
- Stop / Limit TEWL and dehydration

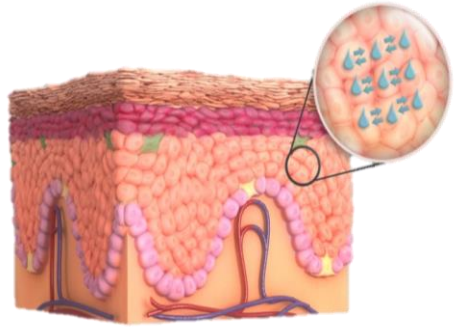
HUMECTANCY

Attract and retain water in the Stratum Corneum

LONG TERM

Where?





What?

STRENGTHEN SKIN BARRIER

- Quality and cohesion of Stratum Corneum
- Strengthening Tight Junctions in Stratum Granulosum → Limit TEWL

OSMOPROTECTION

- Improve water flow
- Restore cell water balance

Anti-ageing for skin 101

	Hydration	Barrier	Matrix Proteins	Inflammation & Antioxidant	Pigmentation	Shielding
Ingredients' Mode of Action	• Reduces transepidermal water loss; • Attracts and retains water into stratum corneum; • Modulates water homeostasis and exchanges	• Lipids synthesis, reinforce lipidic barier and composition • Desquamation /exfoliation; • Modulates keratino differentiation and proliferation; • Enhances cell-cell junctions	• Stimulates fibroblast metabolism for collagen/elastin/GAGs synthesis; • Reduces /prevent dermal matrix proteins breakdown (elastosis); • Anti-glycation	• Neutralizes free radicals (ROS); heavy metals • Modulates inflammation & oxidative stress pathways; • Detoxification of xenobiotics;	• Modulates (up/down regulate) melanin synthesis; • Modulates pigments distribution • Improves microcirculation /angiogenesis	• Forms invisible films on skin to prevent pollutants from entering skin • Repels insects • Reflects/scatters UV, IR, Blue light radiation
Consumer Product Claims	• Improves skin feel, softer skin, moisturizing, hydrating, improves skin surface, microrelief, nourishing	• Restore lipidic barrier integrity, protecting, nourishing, skin defense, moisturization, improve skin feel	• Lifting, firming, elasticity, tonicity, suppleness, regenerating, anti-wrinkle, reduces signs of aging, anti-sagging, re-draw the face oval	• Antioxidant, anti-inflammatory, skin defense, reduces free-radical damage, detox, anti-acne	• Even skin tone, reduce age-related dark spots, pigment normalization, whitening, lightening, brightening	• Protection against UV, (SPF, UVA+), pollution, pollen, toxins, dust, heavy metals, insects, and other harmful substances; detoxifying.
Ingredients / market	• **GENENCARE® OSMS BA & MI** • All osmolytes • Pyrrolidone Carboxylic Acid (PCA, Na PCA)	• **GENENCARE® OSMS BA** • **Mevalonolactone** • Vegetal oils & butters • Ceramides	• **GENENCARE®** • **OSMS MI** • **Mevalonolactone** • Peptides (Palmitoyl-Pentapeptide 3) • Retinol/retinoic acid • Hyaluronic acid	• **GENENCARE® OSMS PRO** (amino acid blend) • Vitamine E • Glycolphenols	• Kojic acid • Niacinamide	• UV filters • TiO2 • Biosacharide gum • Polyglucosides • Plant extracts…

Mode of action of Active in skin health/antiaging can be defined by six pillars

Skin anti-aging modes of action

1

Hydration

- Reduces transepidermal water loss;
- Attracts and retains water into stratum corneum;
- Modulates water homeostasis;
- Faciliate water exchanges;
- Facilitate skin surface enzymes activity

2

Barrier

- Lipid synthesis: reinforce lipidic barrier, improve lipids composition
- Desquamation (exfoliation);
- Modulates keratinocytes differentiation and proliferation;
- Enhances DEJ
- Reinforce cell cell junctions
- Enhance keratinocytes communication
- Regulation of cell defense and survival in response to stress

3

Matrix Proteins

- Improve fibroblast metabolism
- Stimulates structure protein s (collagen/elastin) , GAGs and other matrix elements synthesis;
- Reduces collagen breakdown or elastosis (anti-MMPs);
- Enhances fibroblast communication;
- Anti-glycation
- DNA repair

4

Shielding

- Forms invisible films on skin to prevent pollutants from entering skin
- Repels insects
- Reflects/scatters UV, IR, Blue light radiation

5

Pigmentation

- Modulates (up/down regulate) melanin synthesis;
- Modulates pigments distribution
- Improve microcirculation /angiogenesis

6

Antinflammation & Antioxidant

- Neutralizes free radicals (ROS);
- Modulates inflammation & oxidative stress pathways;
- Detoxification of xenobiotics;
- Chelates heavy metals

…which each drive different possible consumer claims.

Hydration

Improves skin feel, emolliency, softer skin, moisturizing, hydrating, improves skin surface, microrelief and texture, nourishinig, reduce waterloss, maintain skin moisture

Barrier

Restore lipidic barrier integrity, protecting, nourishing, skin defense, moisturization, improve skin feel

Matrix Proteins

Lifting, firming, elasticity, tonicity, suppleness, skin resiliency, regenerating, anti-wrinkle, visibly reduces signs of aging, fight against sagging, re-draw the face oval, stretch marks reduction

Inflammation & Antioxidant

Antioxidant, anti-inflammatory, skin defense, reduces free-radical damage, detox, anti-acne

Pigmentation

Even skin tone, reduce age-related dark spots, pigment normalization, whitening, lightening, brightening

Shielding

Protection against UV, IR, ROS, Blue light, anti-pollution, pollen, dust, heavy metals, toxins, insects, and other harmful substances; detoxifying,

DUPONT™

Osmolytes

The science behind osmolytes

GENENCARE® OSMS - THE SCIENCE OF OSMOLYTES

What are Osmolytes?

- Naturally occur in the cells of living organisms
- Osmolytes are small organic molecules involved in osmosis
- 3 categories :
  - ***Amino acid derivatives***: Glycine betaine, taurine
  - ***Polyols***: sorbitol, inositol, trehalose
  - ***Amine oxides***: trimethylamine oxide (TMAO)

Osmolytes functionalities

Involved in osmosis

- Spontaneous net movement of solvent molecules (water) through a semi-permeable membrane into a region of higher solute concentration
- Driving force by which water is transported
- Process which controls water balance in living organisms and cells

Protection of cells and organisms from osmotic stress

- Bind and transport water
- Actively used by cells to regulate water traffic

Protection of proteins from denaturation

- Stabilize the native structure of proteins

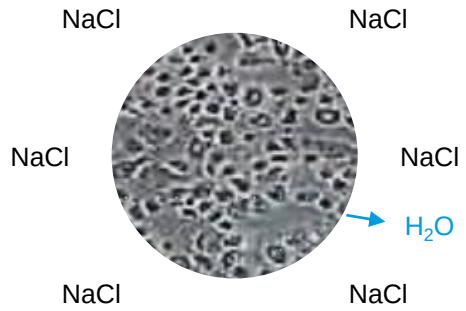
Osmolytes: Control of water balance in keratinocytes

- Osmolytes are molecules with the ability to manage water balance in cells.
- GENENCARE® OSMS protects keratinocytes from hyperosmotic stress.

WITHOUT OSMOLYTES

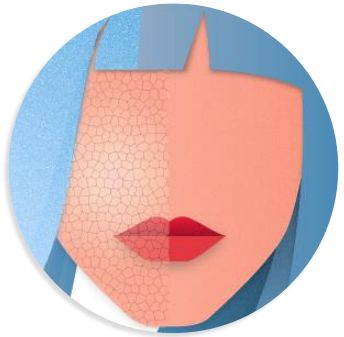
Shrunken

Water leakage



HYPER-OSMOTIC STRESS

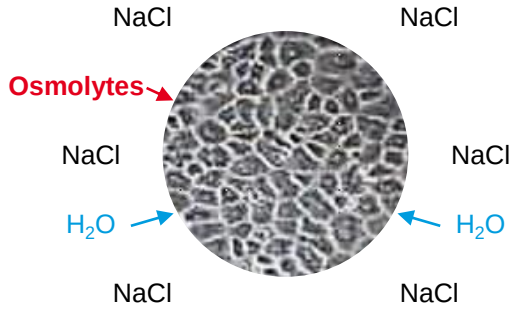
High solute (salt) content outside cells



WITH OSMOLYTES

Normal

Water balance



Osmolytes: Control of water balance in keratinocytes

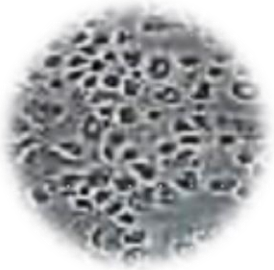
Osmolytes are molecules with the ability to manage water balance in cells.

GENENCARE® OSMS protect keratinocytes from hypersosmotic stress.

WITHOUT OSMOLYTES

NaCl NaCl NaCl NaCl NaCl NaCl

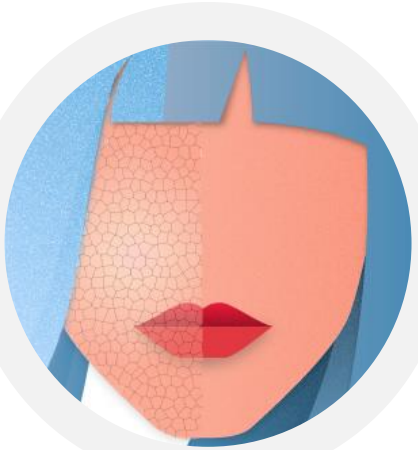
H_2O



Shrunken

Water leakage

HYPER-OSMOTIC STRESS



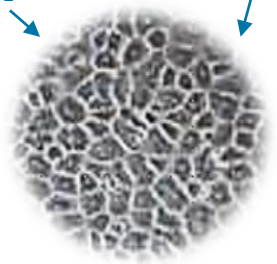
High solute (salt) content outside cells

WITH OSMOLYTES

NaCl NaCl NaCl NaCl

Osmolytes

H_2O H_2O



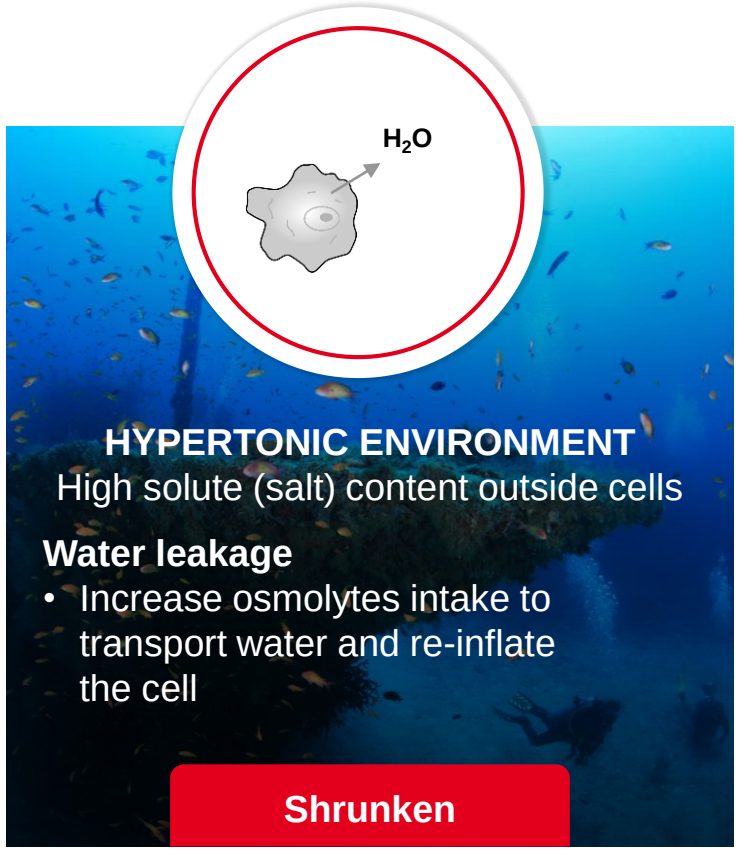
Normal

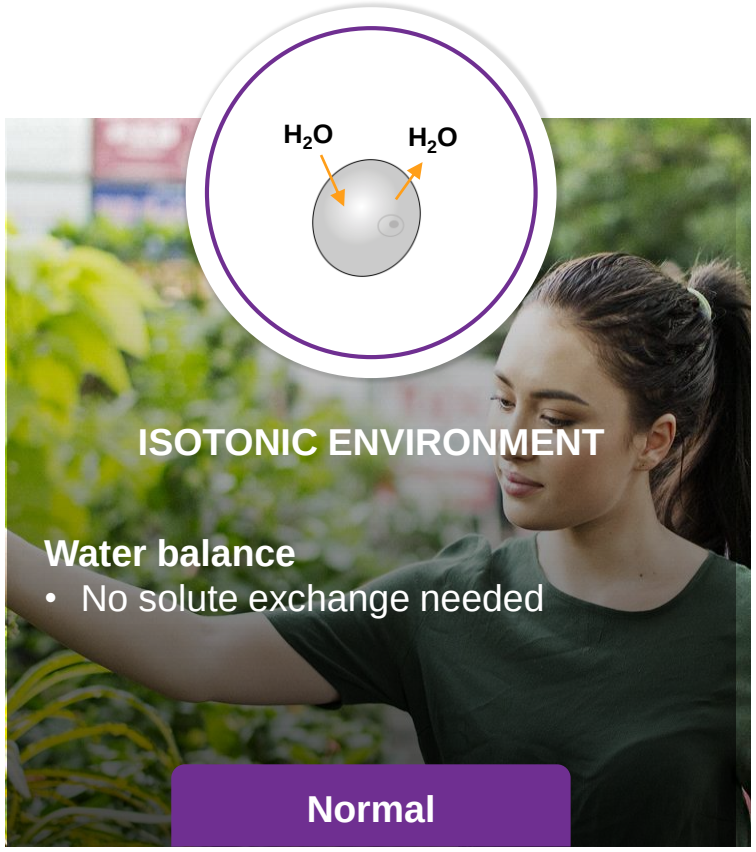
Water balance

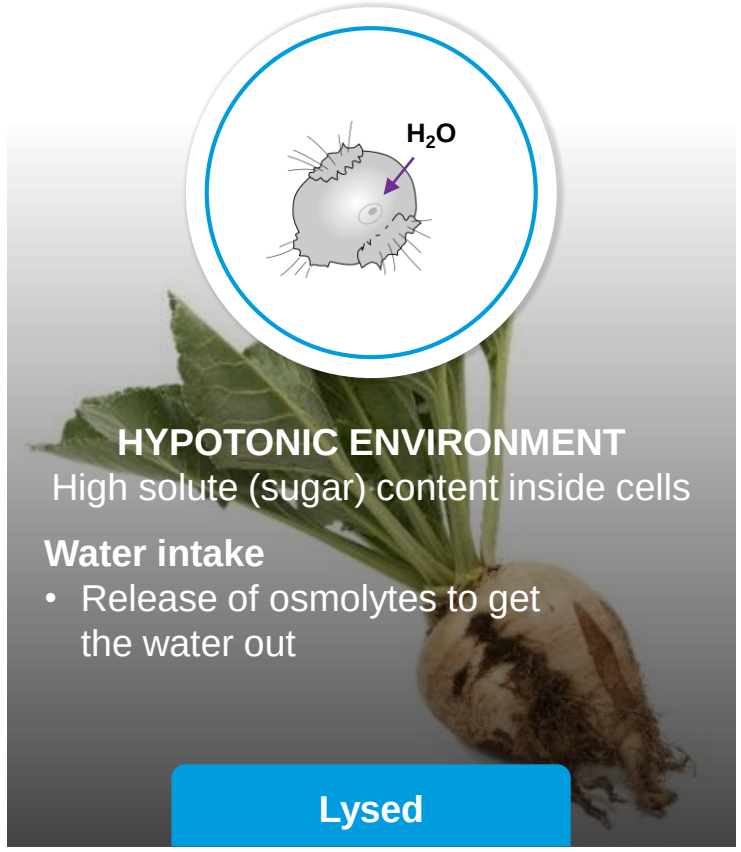
Copyright © 2019 DuPont. All rights reserved

The Science of Osmolytes

Osmolytes: Control of water balance in living cells







GENENCARE® OSMS BA - Moisturizing Osmolyte

- Moisturization
- Protection
- Sensory

GENENCARE® OSMS MI - Invigorating Osmolyte

- Energy
- Oxygen
- Water

GENENCARE® OSMS PRO - Detox Osmolyte complex

- Anti oxidation
- Detoxification
- Skin barrier function improvement

DUPONT™

Osmolytes are involved in Keratinocytes' and Fibroblasts' strategy to survive environmental stress (UV + heat)

Control of water balance in keratinocytes and fibroblasts

1 Hyperosmotic stress
Dry environment [1]
Or
Oxidative stress caused by UVA and UVB radiation[2], [3]

2 Water and K+ efflux

Cell shrinkage

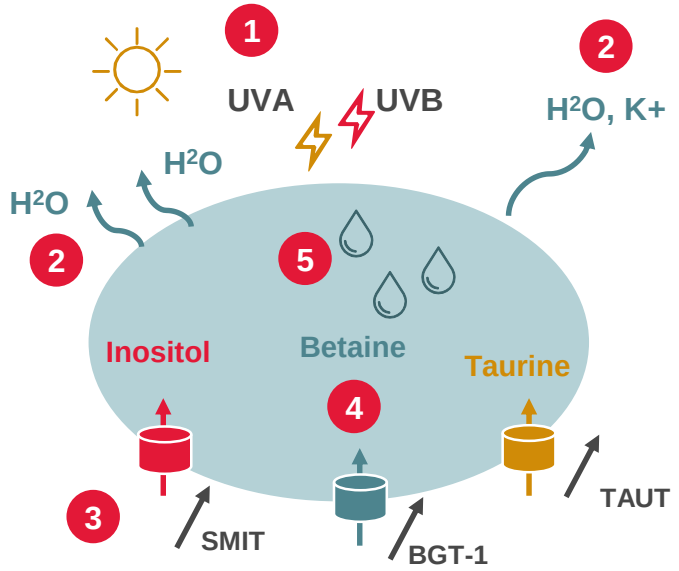
3 **Increased expression of osmolyte transporters** specific for myo-inositol, betaine and taurine

4 **Increased uptake of osmolytes**

5 **Cell hydration retained**

Normal cell shape and size

Keratinocytes and fibroblasts



1. Warskulat D., A. Reinen, S. Grether-Beck, J. Krutmann and D. Häussinger.Journal of Investigative Dermatology (2004) 123, 516–521
2. Warskulat, D., Brookmann S, Reinen A, Häussinger D. Biol Chem. (2007) 388(12):1345-52.
3. Warskulat, U. et al., "UV-A induces transport of compatible organic osmolytes in dermal fibroblasts", Exp. Dermatol. 17, 1031-1036 (2008)

Protection

Osmolytes & Proteins are naturally present in cells

Osmolytes

- Osmolytes = “chemical chaperone”
- Osmolytes are typically accumulated in the intracellular environment
- Osmolytes are compatible in the intracellular environment at high concentrations without perturbing cellular processes

Proteins

- Proteins need to maintain their natively folded structures for proper functions under physiological conditions
- Proteins are sensitive to change in cellular and environmental conditions (stress) :
  - Temperature
  - Pressure
  - Presence of salts and other solutes (hypertonicity)
  - When instability leads to denaturation, adaptation strategies are required

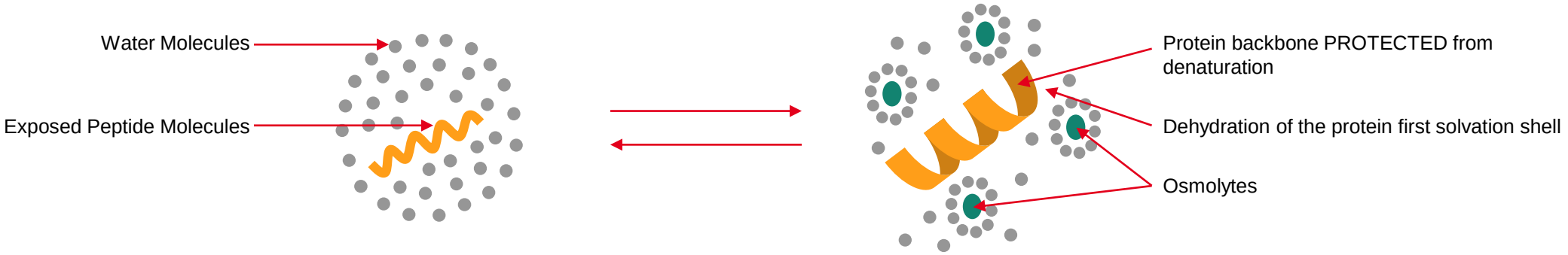
Protection

Osmolytes:
Protein stabilizing effect

Osmophobic effect: Protein backbone is “osmophobic”. Osmolytes are not in direct contact with the protein.

Indirect mechanism: Osmolytes are active on the bulk solvent, surrounding the proteins. Water molecules are excluded from the protein surface and less available for denaturation.

Increased stability: Osmolytes increase thermodynamic stability of the native folded state, functional 3D conformation, of the proteins ($\Delta G<0$).



Protein in the Unfolded State
Less stable

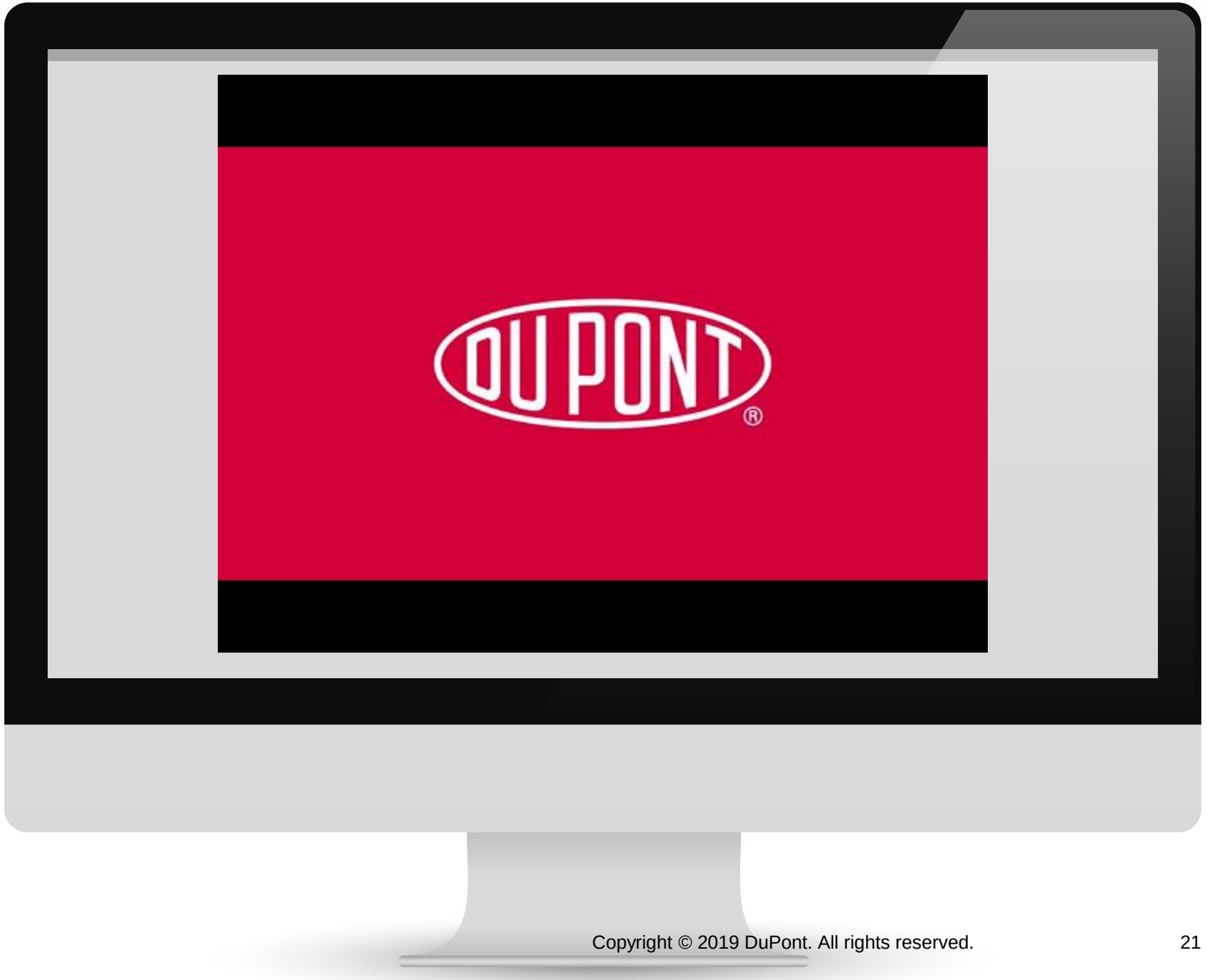
Protein in the Folded State
More stable

Role of naturally occurring osmolytes in protein folding and stability, Kumar 2009

Protection

- Osmolyte interaction in protein stabilization is additive
- Cells may contain different kinds of osmolytes
- Osmolytes do not occupy a significant fraction of the backbone surface
- Osmolytes have interchangeable complementary protective effects

Osmolytes video



GENENCARE® OSMS BA

Product overview

GENENCARE® OSMS BA - Identification

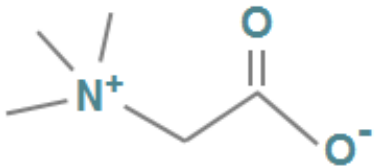
INCI name:
Betaine

Chemical name:
Trimethylglycine Glycine betaine

Formula:
$C_5H_{12}O_2$

CAS number:
107-43-7

White odorless crystalline powder



Typical Characteristics

Appearance	Free-flowing white crystals
Bulk density	0.6-0.8 g/ml
Melting point	241-242 ºC
Molecular weight	117.15 g/mol
pH (5% solution in DiH20)	5-7
Solubility in water **Solubility in Ethanol**	160 g/100 ml (25 ºC) readily soluble in water 8.7g/100 ml
Purity	min 99% d.s. betaine
Moisture	max 2% (when packed)

GENENCARE® OSMS BA

- Highly purified sugar beet extract in crystal form
- 100% naturally sourced
- Non GMO plant origin

Natural credentials

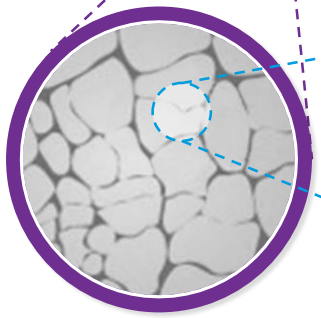
Approved by ECOCERT RAW MATERIAL COSMETICS

Approved by ECOCERT RAW MATERIAL COSMOS APPROVED

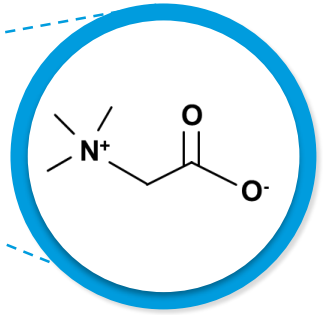
WWW.NATRUE.ORG

Source

Sugar Beet
(Beta vulgaris)

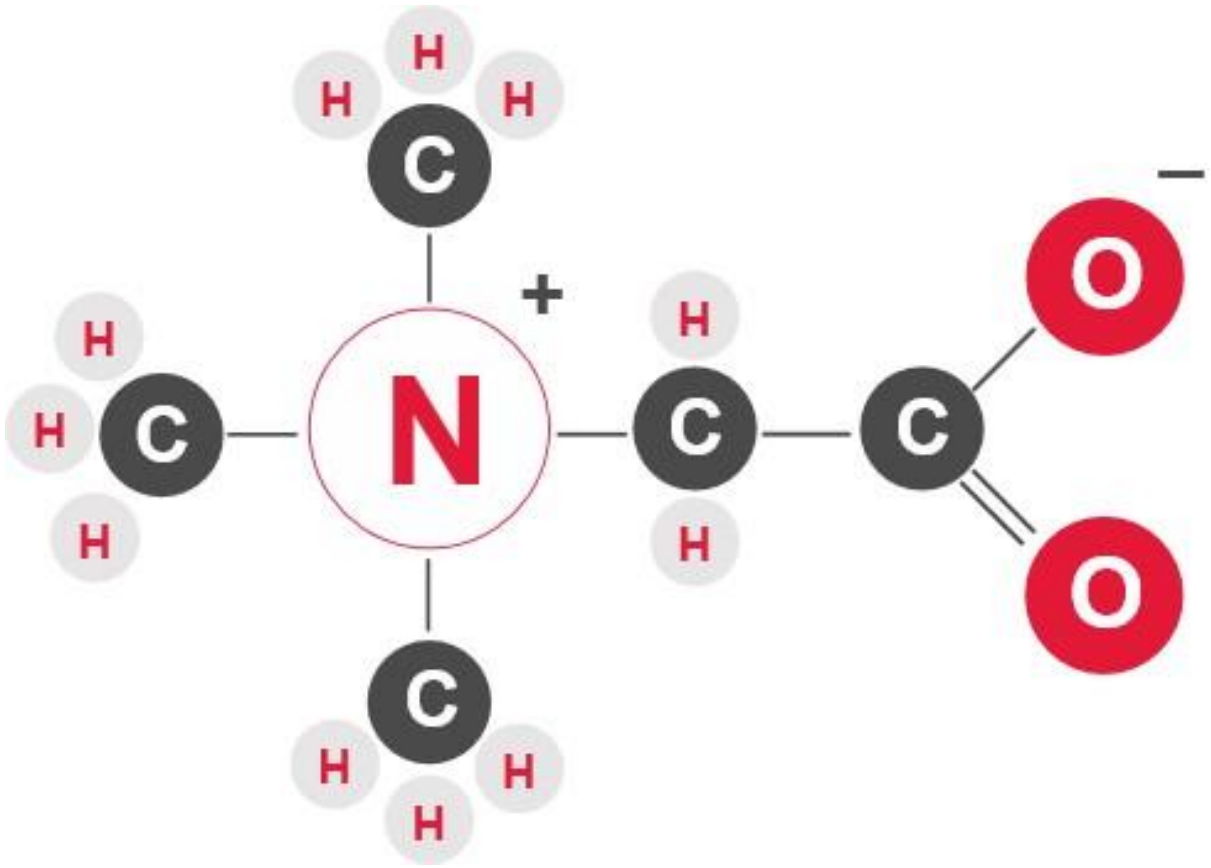


Beet root cells



Betaine

DUPONT



GENENCARE® OSMS BA – characteristics

Small amino acid derivative

Stable zwitterion (dipolar ion)

Neutral charge at isoelectric point (pH=5.5)

Very hydrophilic: Readily soluble in water

Naturally occurring osmolyte (animals, plants)

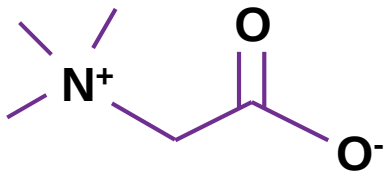
Hygroscopic: attracts water

Hydrotope: molecule performing water coordination

Naturally present in skin:
Component of the NMF (Natural Moisturizing factor)

As methyl-donor molecule, stimulates various biological processes

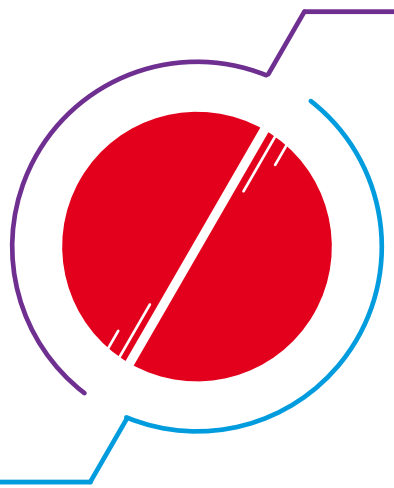
Different types of betaine



Betaine

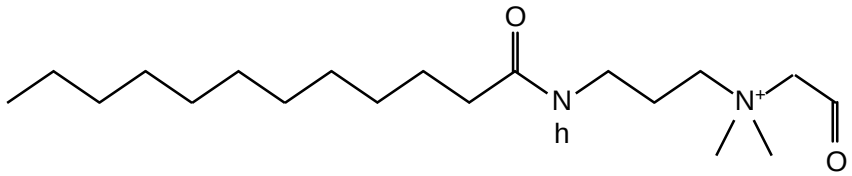
Trimethylglycine : GENENCARE® OSMS BA

- Natural origin from sugar beet roots
- Found in most plants and animals, also in our human body and especially in skin.
- White crystalline powder (99% pure)



Surfactant : Betaine derivatives

- Also a zwitterionic compound
- Synthetic origin and derived from trimethylglycine
- Used as surfactants for cleaning
- The most common is cocoamidopropylbetaine
- Clear viscous foaming liquid, diluted in water



cocamidopropylbetaine

GENENCARE® OSMS BA

Benefit drill-down

- Moisturization
- Sensory
- Protection

GENENCARE® OSMS BA helps to moisturize and protect the skin, providing perceivable sensorial benefits

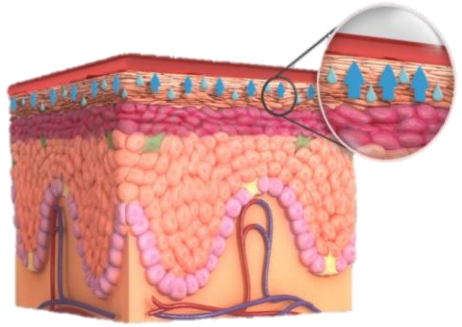
	MOISTURIZATION	PROTECTION	SENSORY
CLAIMS			
MECHANISM OF ACTION	**Humectancy** Osmoprotection / Osmosis : Control of water balance	**Osmoprotection** **Helps protect proteins against denaturation:** 1) Improvement of membrane protein integrity 2) Osmophobic effect: dehydration of first solvatation shell of proteins	**Water management** Betaine retains water but doesn't immobilize it.
IN-VITRO TESTS	Osmoprotection of keratinocytes and fibroblasts cultures (hyperosmotic and UV stress)	**TEER test :** strengthens keratinocytes' Tight Junctions **ZEIN test:** decreases solubility of zein protein	
CLINICAL TESTS	24 hours and 4 weeks moisturization studies	**TEWL test:** improves skin barrier integrity **Patch test :** Mitigates irritation of surfactants	**Sensory evaluation:** Helps reduce stickiness, improve spreadability, provide silky feeling, decrease oily feeling.

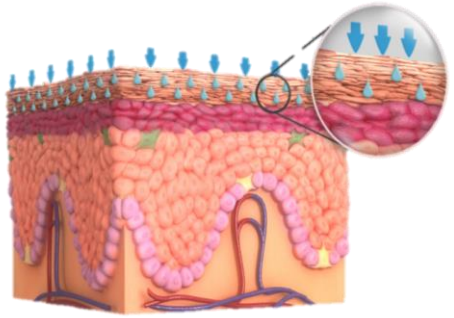
DUPONT

Skin moisturization strategies

SHORT TERM

Where?





What?

OCCLUSION

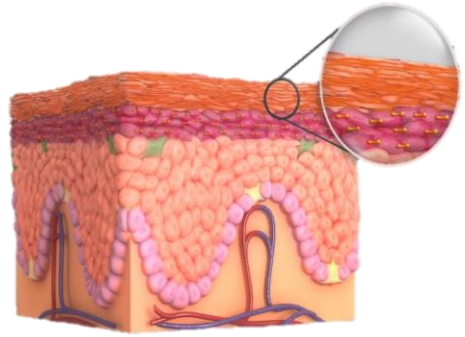
- Create a film at the surface
- Stop / Limit TEWL and dehydration

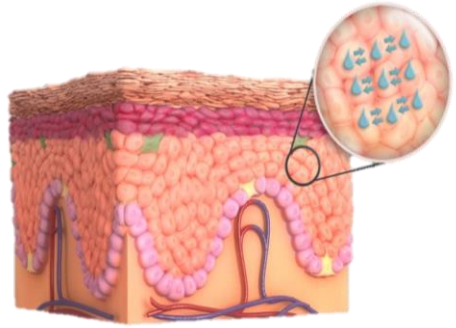
HUMECTANCY

- Attract and retain water in the Stratum Corneum

LONG TERM

Where?





What?

STRENGTHEN SKIN BARRIER

- Quality and cohesion of Stratum Corneum
- Strengthening Tight Junctions in Stratum Granulosum → Limit TEWL

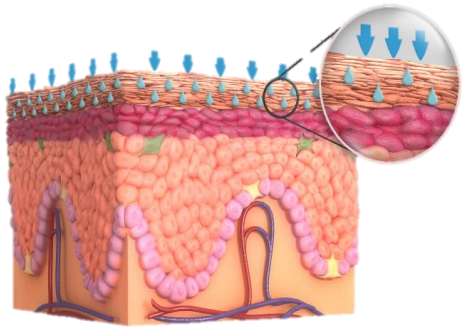
OSMOPROTECTION

- Improve water flow
- Restore cell water balance

GENENCARE® OSMS BA skin moisturization strategies

SHORT TERM

Where?



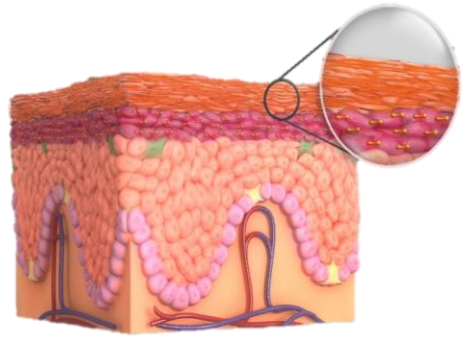
What?

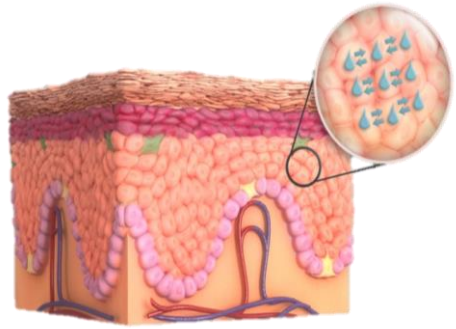
HUMECTANCY

- Water binding properties
- **Humectancy** at the skin surface
- Increase of the stratum corneum moisture content and elasticity

LONG TERM

Where?





What?

STRENGTHENS SKIN BARRIER

- Improves Tight Junctions integrity in Stratum Granulosum(1)
- **Strengthens** skin barrier and prevents water loss

OSMOPROTECTION

- Regulates cell water balance
- **Osmoprotection** (2) (3) (4) (5) (6)

1 Putaala & al 2010

2 K.Tiihonen unpublished

4 Janake et al 2003

5 Warskulat et al 2004, 2007

6 Warskulat et al. 2008

GENENCARE® OSMS BA moisturizes for short-term and long-term benefit.

1. Betaine is a humectant. Capture water molecules on the surface

   Betaine is a component of the NMF

2. Betaine strengthens keratinocytes' Tight Junctions integrity and reinforces the skin barrier cohesion.

3. Osmolyte strategy of keratinocytes: under oxidative (UV) or thermal stress, synthesis of specific membrane transporters for active osmolyte intake

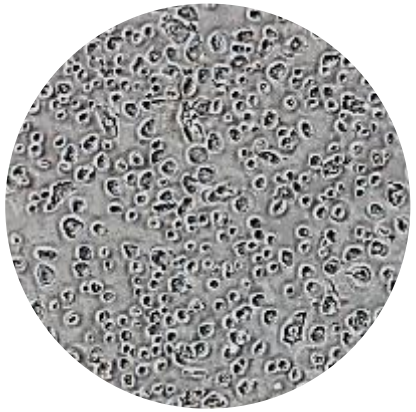
4. Fibroblasts use betaine to maintain cell homeostasis against osmotic stress

DUPONT

GENENCARE® OSMS BA manages water balance and protects keratinocytes from hyperosmotic stress

In vitro test

Cell survival an morphology assessment study



Without GENENCARE® OSMS BA

48 h pre-incubation
With 3% GENENCARE® OSMS BA (30mg/ml)

Normo-osmotic condition

Hyper-osmotic stress condition (150mM NaCl)

Source: Bio alternatives Jan 2017 Cell survival and morphology assessment of NHEK under hyperosmotic stress

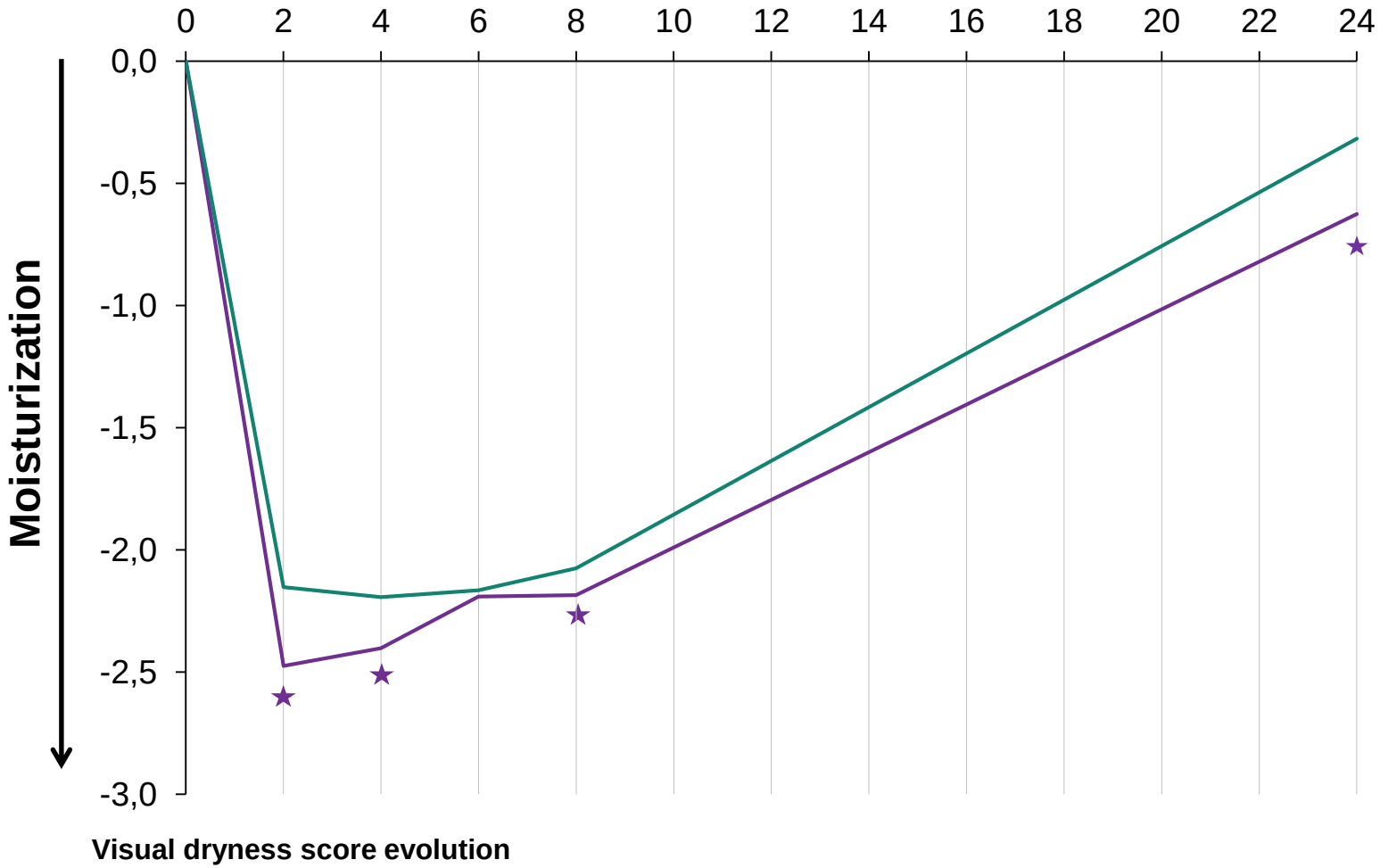
GENENCARE® OSMS BA contributes to control water homeostasis in cells and maintain keratinocyte size under hyperosmotic stress.

Flow cytometry results	CONTROL Normo-osmotic	CONTROL Hyperosmotic stress	3% GENENCARE® OSMS BA Hyperosmotic stress
Number of cells (% normo-osmotic control)	100	15*	80**
Cell size (% normo-osmotic control)	100	57*	73**
Cell viability (% viable cells)	68	32*	88.2**

Source: Bio alternatives Jan 2017 Cell survival and morphology assessment of NHEK under hyperosmotic stress
** Extremely significant versus normo-osmotic control p<0.0001*
*** Extremely significant versus hyperosmotic control p<0.0001*

DUPONT™

GENENCARE® OSMS BA significantly improves visual dryness



At 2h, 4h, 8h and 24h, the reduction of skin dryness with 4% GENENCARE® OSMS BA is higher than with the placebo

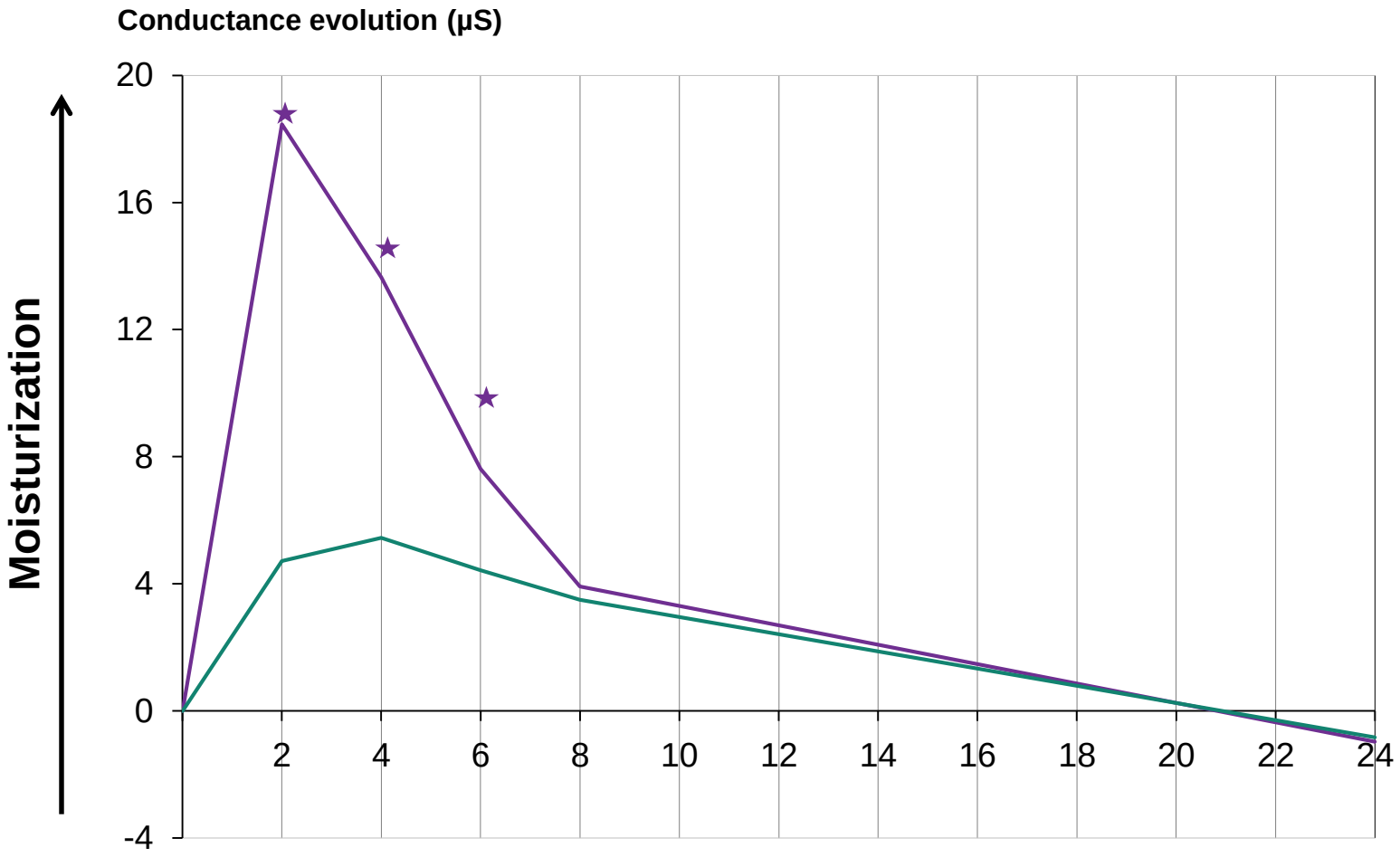
4%GENENCARE® OSMS BA

Placebo

★ Significant difference versus control placebo, based on fixed effect LS Means Tukey HSD

HillTop Research, 2013

GENENCARE® OSMS BA significantly improves skin moisturization



At 2h, 4h, 6h, 4% GENENCARE® OSMS BA significantly improves skin moisturization compared to the placebo

★ Significant difference versus control placebo, based on fixed effect LS Means Tukey HSD

HillTop Research, 2013

24h moisturization study (1) - methodology

Method

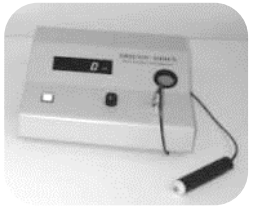
- single application
- 21 volunteers, 18-65 year old with dry skin (dryness grade 2 or greater)
- Winter period
- Lower outer legs (test sites 3 cm x 5 cm + untreated area)
- 2 ml/cm^2 = 30ml/test site
- O/W emulsions (polymeric structure)

Visual evaluation

- visual dryness assessment by expert
- Scale from 0 to 5 (extremely dry skin with deep cracking and evidence of bleeding)

Skicon

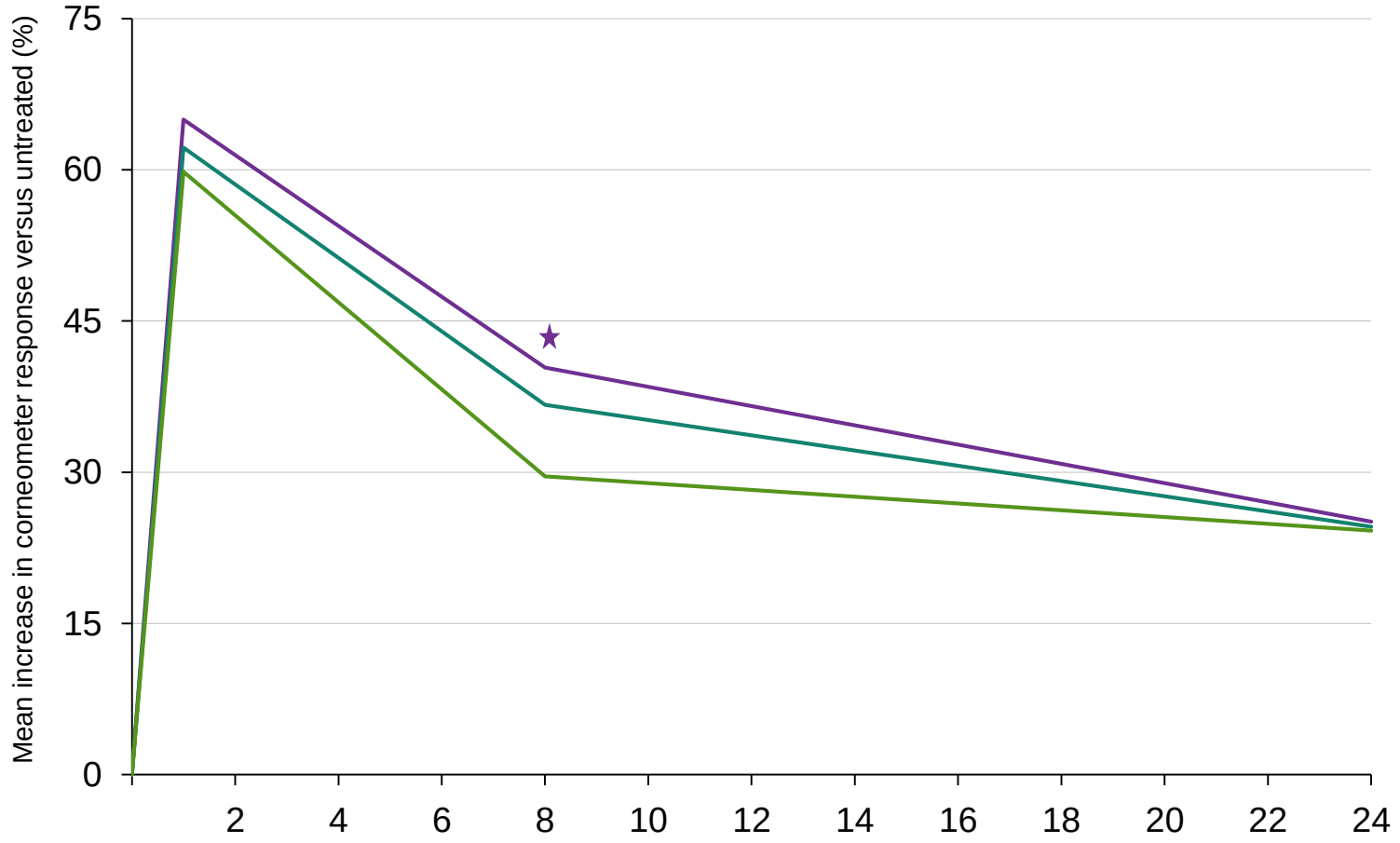
- Skicon 200EX, I.B.S. Company, Japan)
  with MT-8C probe
  (Measurement Technologies, USA)
- Measurement based on skin conductance (results in microsiemens, mS)
- More hydration leads to higher skicon values
- Measurement depth : up to 15 µm in the Stratum corneum



HillTop Research, 2013

DUPONT

GENENCARE® OSMS BA shows improvement of humectancy in combination with glycerin



At 8h, the blend of GENENCARE® OSMS BA and glycerin improves skin moisturization significantly better than the individual ingredients alone.

- 3.5% GENENCARE OSMS BA +3.5% Glycerin
- 7% Glycerin
- 7% GENENCARE OSMS BA

★ Significant difference, based on fixed effect LS Means Tukey HSD

Derma Consult Concept GmbH, 2008

24h moisturization study (2) - methodology

Method

- Single application
- 20 women, 26-63 year old
- Inclusion criteria:
  - female (age >18 y.o.), clinically healthy, with dry skin
  - maximum skin hydratation in the test area at start of the study :
    40 corneometer units (a.u.)
- Test period: May-June
- Test area: inner sides of forearms
- 2 mg/cm^2, 30 seconds of massaging the product into the skin
- Tested product : Lotion

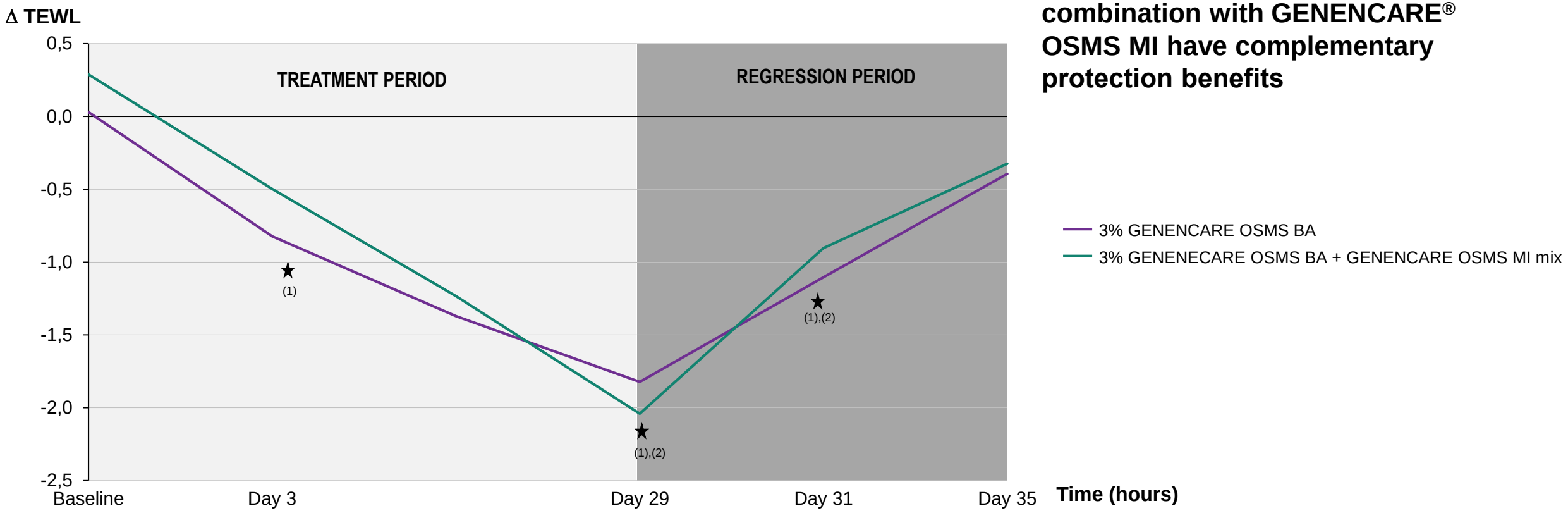
HillTop Research, 2013

Clinical evaluations

- Instrumental measurement : Corneometer CM 825 PC
- Descriptive statistics (average, median, minimum, maximum, variance, standard error, standard deviation), ANOVA, Tukey HSD

GENENCARE® OSMS BA improves skin barrier integrity

Evolution of the TEWL during a four weeks moisturization study



GENENCARE® OSMS BA in combination with GENENCARE® OSMS MI have complementary protection benefits

★ Significant differences
(1) Genencare® OSMS BA versus Control
(2) Genencare® OSMS BA+ MI versus Control
based on Fixed Effect LSMeans Tukey HSD

4-weeks moisturization study - methodology

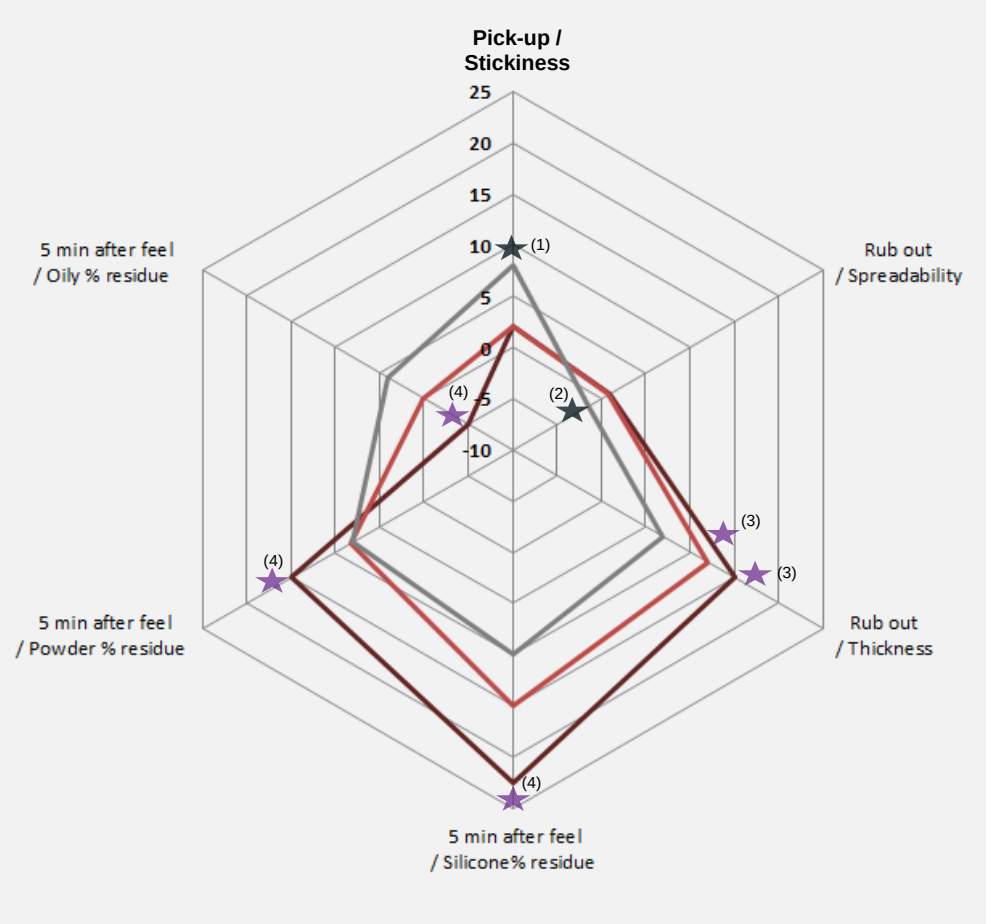
Method

- Randomized, evaluator blind, complete block design with 41 female volunteers
- Location: Lower outer legs (test sites 10 cm x 10 cm + untreated area)
- 2 ml/cm2
- Winter period
- Application twice a day during 29 days, then regression period up to Day 35.
- O/W cream-gel emulsion
- Product tested:
  - Control : O/W polymeric emulsion
  - O/W polymeric emulsion + 3% GENENCARE® OSMS BA
  - O/W polymeric emulsion + 3% Mix GENENCARE® OSMS BA + OSMS MI

Clinical evaluations

- Instrumental: TEWL with Tewameter
- Indicative of the barrier integrity

GENENCARE® OSMS provides sensory benefits such as improved silky after feel and reduction of stickiness



Sensory profiles of hand sanitizers

Sensory synergistic effect between GENENCARE® OSMS BA (2%) and glycerin (2%)

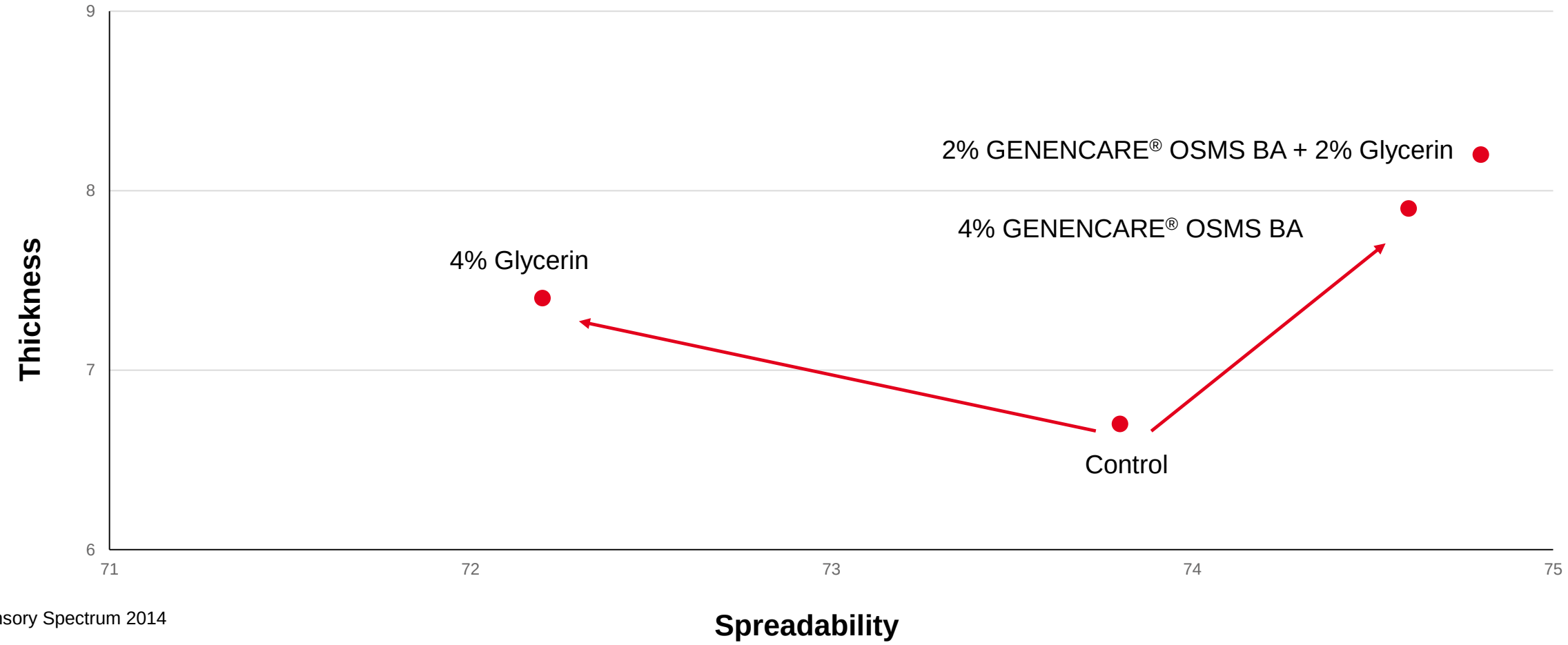
- 2% GENENCARE® OSMS BA + 2% Glycerin
- 4% GENENCARE® OSMS BA
- 4% Glycerin

Control as baseline «0».

★ Significant difference based on Fixed Effect LSMeans Tukey HSD :

1) Versus mix, vs betaine and vs control
2) Versus mix, versus betaine
3) Versus control
4) Versus glycerin

GENENCARE® OSMS BA enhances thickness and sensory profile without compromising on spreadability



Sensory Spectrum 2014

Sensory study - methodology

Panelists

- Sensory Spectrum, US
- 9-12 highly trained panelists
- Extensive training (minimum 100 hours)
- Strong selection

Products

- Hand sanitizers with 4% active (GENENCARE® OSMS BA, Glycerin or mixture of both) versus control
- Amount of product: 0.1 ml (pick-up) and 1 ml (application)

Method

- Quantitative Descriptive Analysis

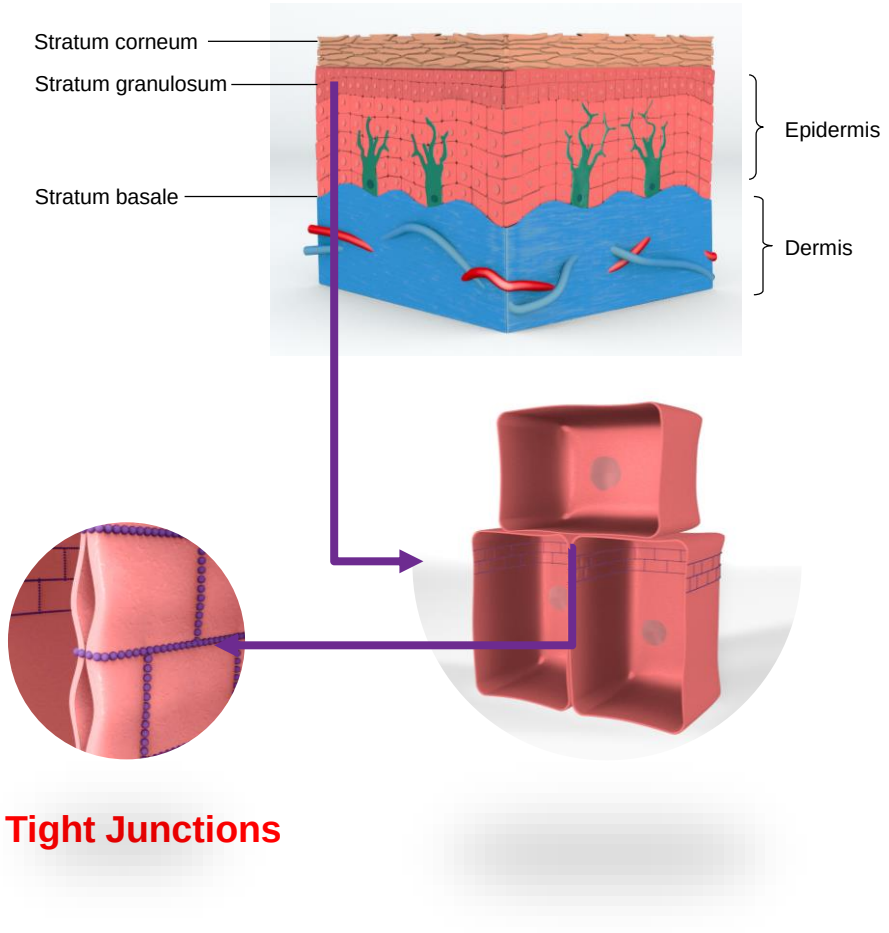
Standardized protocol

- Room conditions
- Skin preparation
- Evaluation

GENENCARE® OSMS BA – Protection mechanisms

	Mechanism of action	In vitro cells level	Clinical skin level
Protection against dehydration	Osmosis Control of water balance	Keratinocyte moisturization strategy (osmotic and oxidative stress)	SKIN MOISTURIZATION 24H and 4 week moisturization study
Protection against protein denaturation	Improvement of membrane proteins integrity	Increases keratinocytes' TEER Strengthens epidermis Tight Junctions	Strengthens skin barrier TEWL reduction
Protection against protein denaturation	Osmophobic effect: dehydration of the first solvatation shell of proteins	ZEIN test Decrease of the solubility of the Zein protein	Patch Test Mitigates irritation

What is a Tight Junction?



Tight Junctions[1] are expressed in **granular keratinocyte** layer

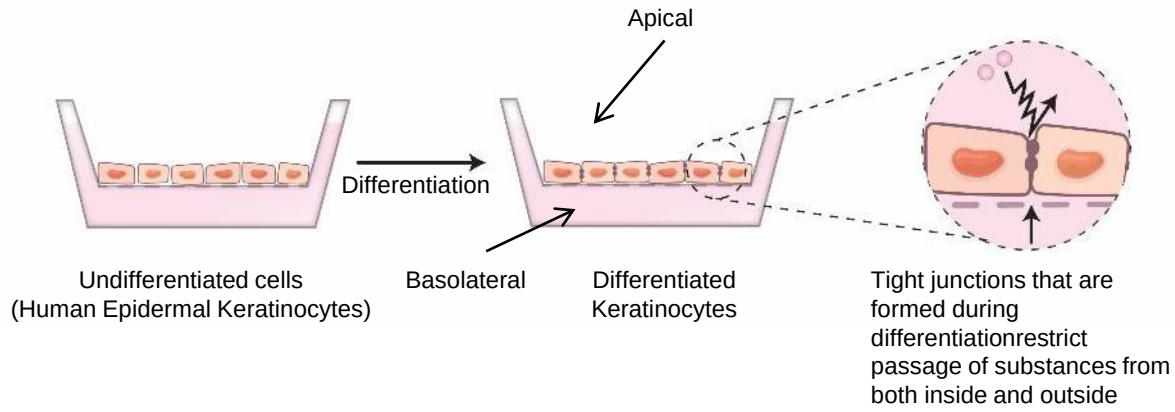
Tight junctions are **cell-cell junctions** that connect neighboring cells (keratinocytes)

They play a crucial role in the **epidermis cohesion** and **skin's barrier function**

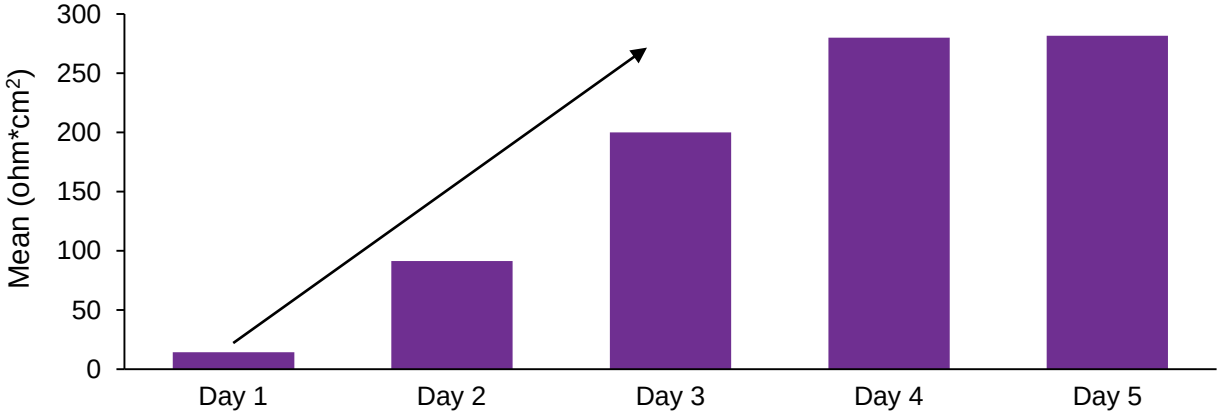
- **Control Inter-cellular pathway** of molecules
- **Prevents the penetration of harmful substances** such as allergens, pollutants into skin.
- **Prevents vaporization of water** (decreases Transepidermal Water Loss)

[1] Kirchner et al. 2010

Evidence of Tight Junctions formation in vitro



Resistance increases during the differentiation because the tight junctions are forming between cells



- Keratinocytes isolated from normal adult human skin are differentiated in cell culture inserts. During the differentiation the cells form Tight Junctions (=TJs) between the cells.
- The cell layer in itself is impermeable to water and water-soluble substances, but the flow of these solutes through TJs can be measured with chopstick electrodes (Trans Epithelial Electrical Resistance =TEER)
- The greater the resistance to ion flux across the TJs, the stronger the TJs between the cells and the higher the TEER values are.

GENENCARE® OSMS BA improves Tight Junction integrity and strengthens skin barrier

TEER:Trans Epithelial Electrical Resistance
% Change in TEER = Percentage change in TEER calculated from time point 0h; mean are shown

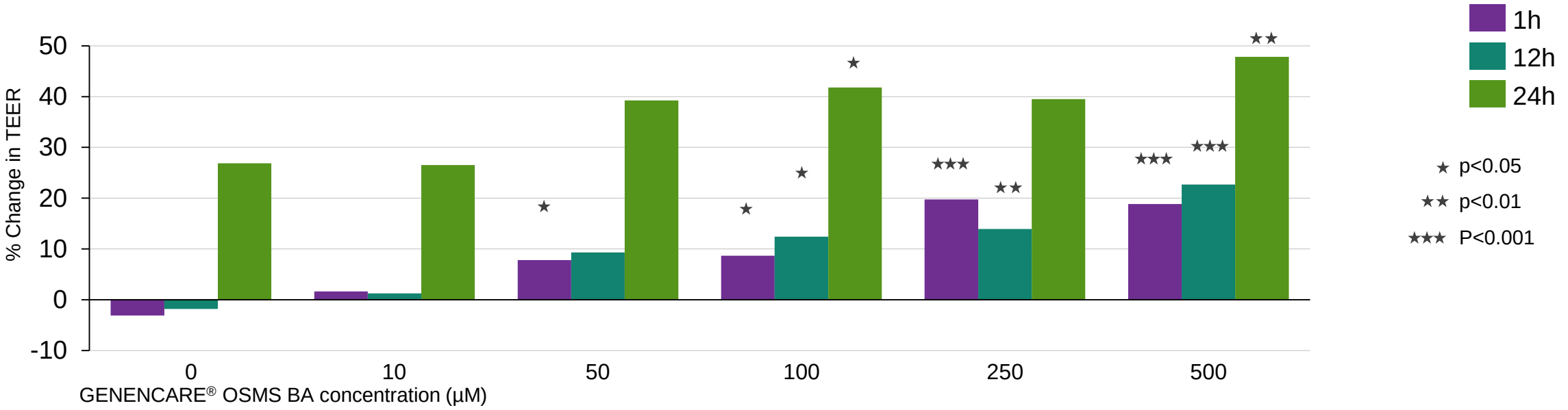
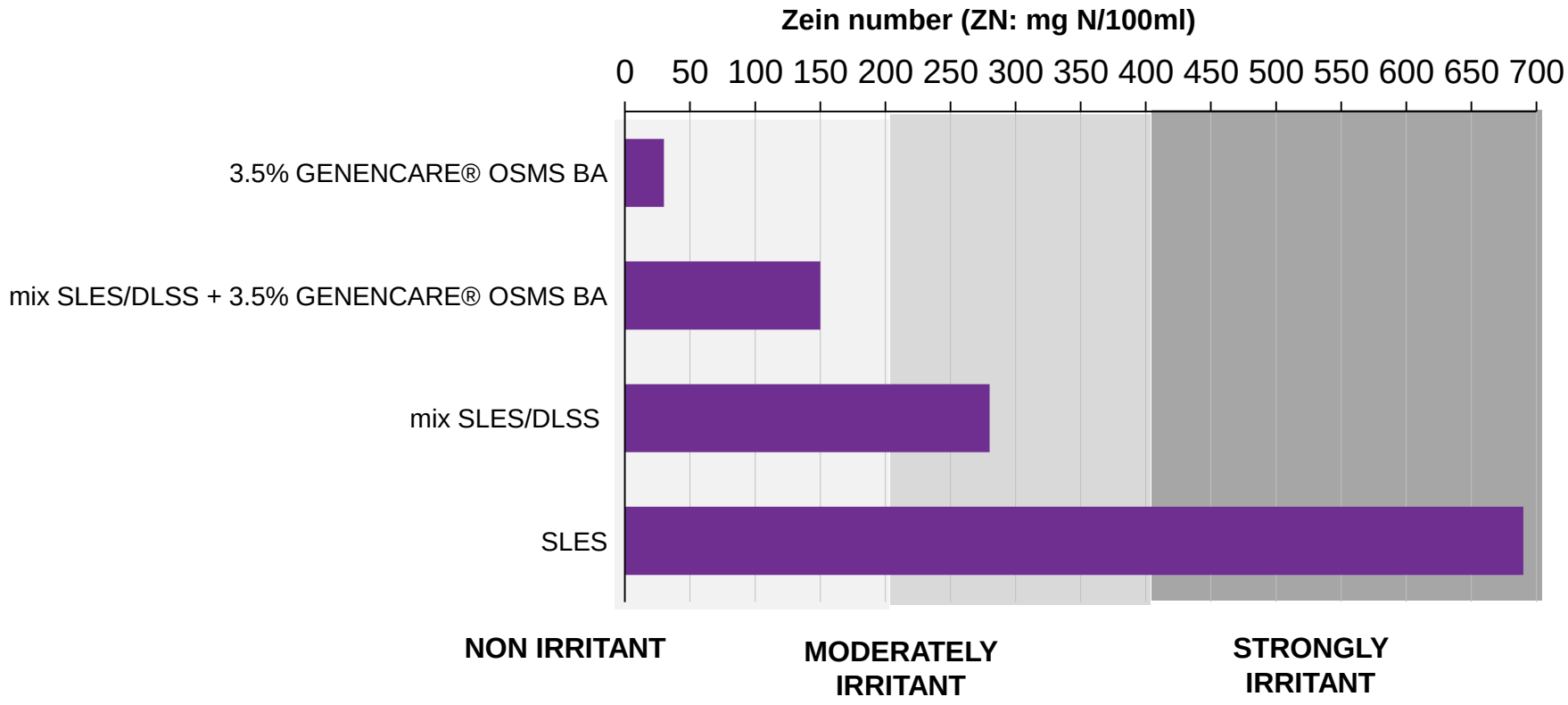


Fig.: The effect of GENENCARE® OSMS BA on the TEER of differentiated keratinocytes

Poster presentation:
Betaine increases tight junction integrity in epidermal keratinocytes; h. Putaala, K. Tiihonen, N. Rautonen: Danisco Finland Oy, Health & Nutrition, Sokeritehtaantie 20, 02460 Finland.
40th Annual Meeting of the European Society for Dermatological Research, Helsinki, Finland, September 8th to 10th 2010.

GENENCARE® OSMS BA decreases irritancy potential of surfactants

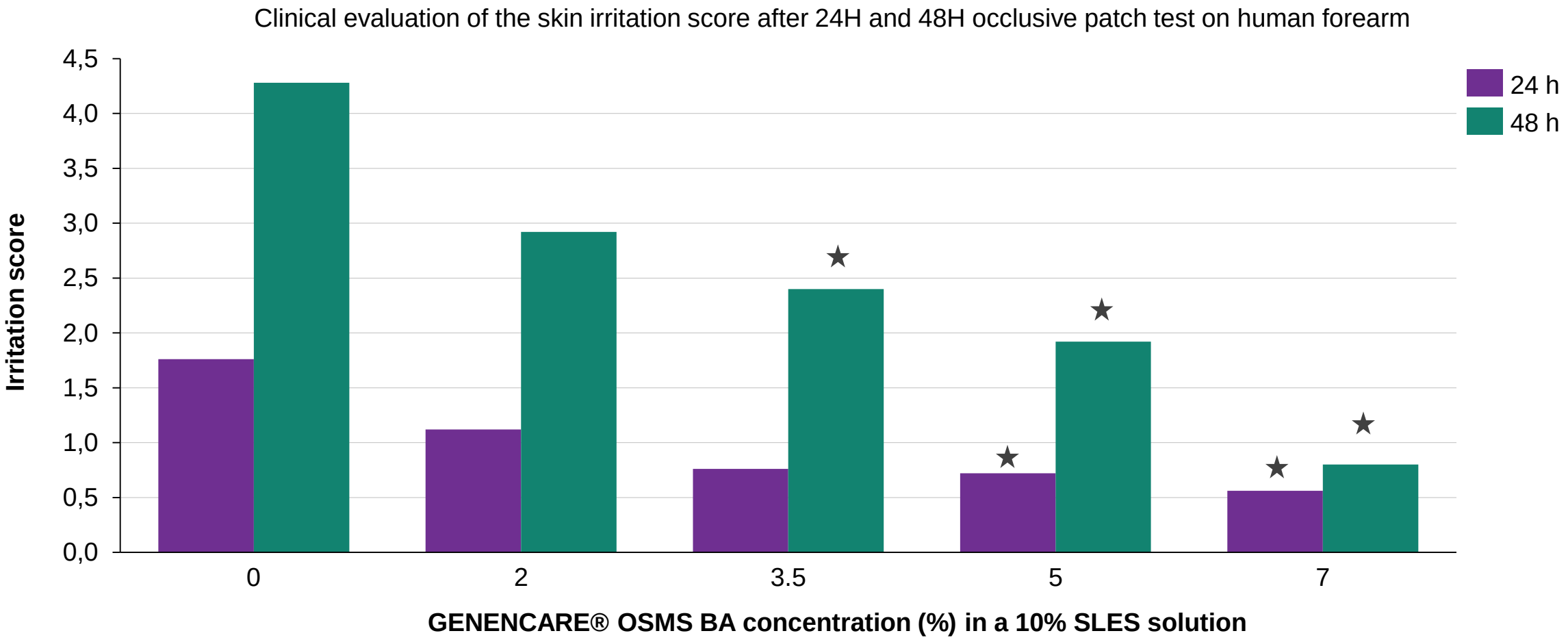
GENENCARE® OSMS BA reduces the solubilization of the Zein protein by surfactants, which correlates with *in vivo* reduction of skin irritation



Source : Cosmetics & Toiletries 115 (12), 47 - 54, 2000 L. Rigano et al.; EP0056595 B1, 1984 Konrad, h Mager and D Hoch (1984)

SLES: sodium lauryl ether sulfate; DLSS: disodium laurylsulfosuccinate
MIX SLES/DLSS: 50% SLES / 50% DLSS solution

GENENCARE® OSMS BA reduces the irritating effect of SLES



★ Significant differences compared to SLES 10% solution without betaine;based on Fixed Effect LS Means Turkey HSD p<0.05

Toxicol laboratories, Ltd, Test Ref V3-V10-8811 (1988)

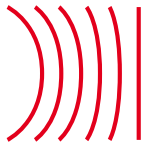
GENENCARE® OSMS MI

Product overview

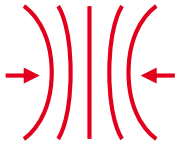
Improving Skin Elasticity

GENENCARE® OSMS MI increases or stabilizes 3 important skin biomechanical parameters:

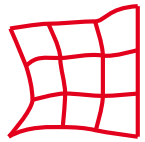
The effect of GENENCARE® OSMS MI is inverse as the effects of aging on these parameters*.



Rate of elastic recovery to total deformation



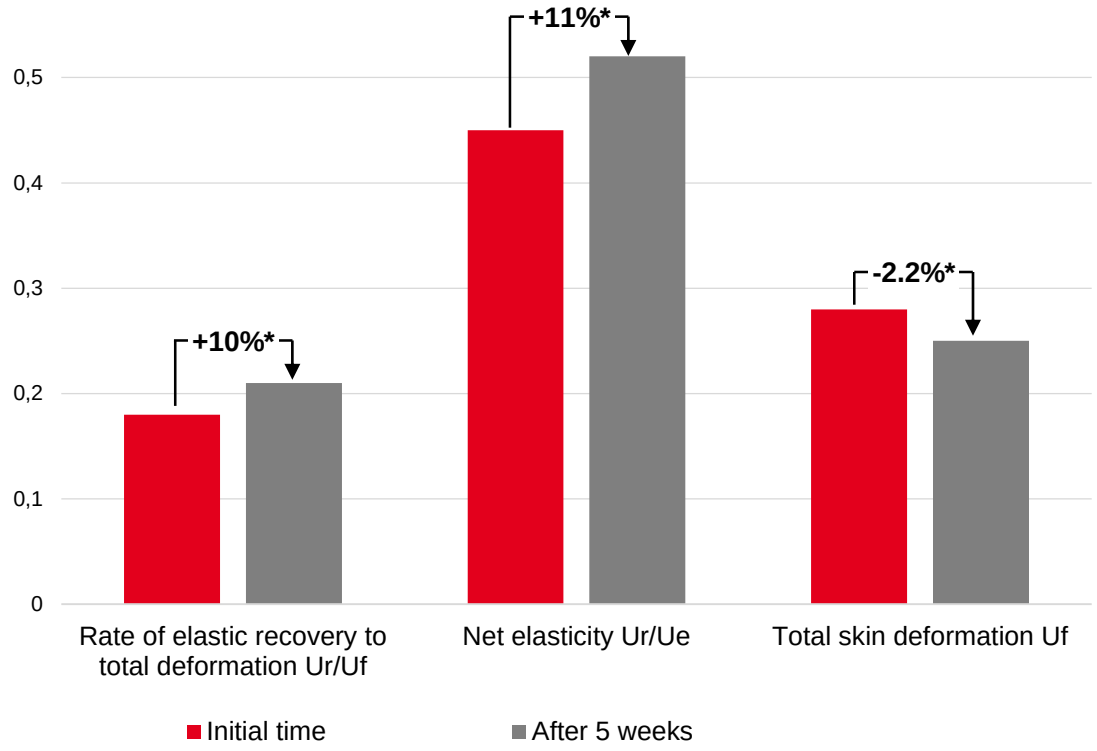
Net elasticity



Total skin deformation

Biomechanical properties of the skin measured by cutometer on face

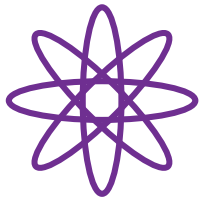
5-week clinical study, 40 volunteers, twice daily application of a cream with 3% GENENCARE® OSMS MI versus placebo.



Significant effect versus To and versus control cream (Student T test)

* p<0.01 ; ** p<0.05

GENENCARE® OSMS MI: the natural Invigorating Osmolyte

	Energy	Oxygen	Water
MECHANISM OF ACTION	 • Stimulates skin cells metabolism • Stimulates keratinocytes differentiation	 • Improves cells and skin oxygenation	 • Protection of keratinocytes against hyperosmotic stress • Maintains cell size/volume and water homeostasis of keratinocytes under hyperosmotic stress
IN-VITRO TESTS	• Filaggrin expression • Energy and protein content in fibroblasts	• Mitochondrial respiration of fibroblasts	• Hyperosmotic stress on keratinocytes
CLINICAL TESTS	• 5-week study, measurement of the skin biomechanical properties (cutometer)	• 1-week study, measurement of the skin partial pressure of oxygen (electrodes)	• 4-week study, TEWL (Tewameter)

GENENCARE® OSMS MI

- Highly purified sugar beet extract in crystal form
- 100% naturally sourced
- Non GMO plant origin

Natural credentials

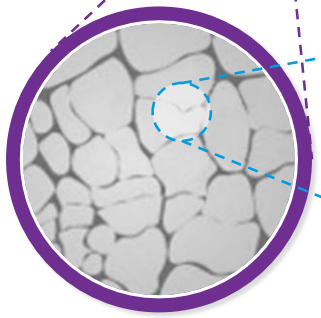
Approved by ECOCERT RAW MATERIAL COSMETICS

Approved by ECOCERT RAW MATERIAL COSMOS APPROVED

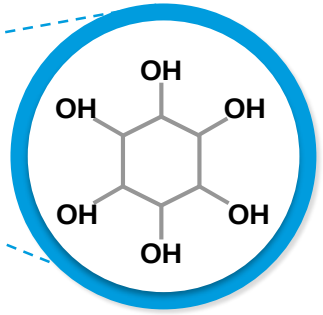
WWW.NATRUE.ORG

Source

Sugar Beet
(Beta vulgaris)



Beet root cells



Myo Inositol

DUPONT

Myo-Inositol as a dietary supplement is known to have health benefits ranging from Immune support to stress reduction

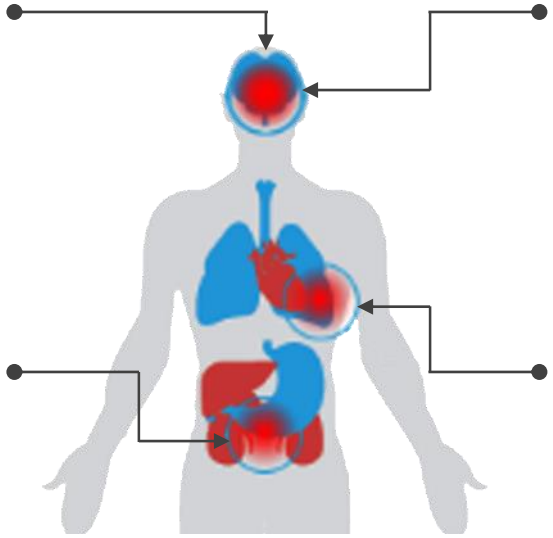
- Naturally occurs in plants and animals (skin, muscles)
- Inositol and its phosphates are mostly found in fruits like oranges and cantaloupe.
- Carbohydrate, isomer of glucose, source of energy
- It plays a **major role in the metabolism of fats and cholesterols**

Panic disorder relief [1]

Small-scale studies of inositol show early promise in controlling panic attacks, especially those caused by a fear of open spaces (agoraphobia). One such study showed thath this use of inositol was as effective as prescription medication.

Reproductive health support [3]

Oral supplements of inositol may help manage the symptom (PCOS), which primarily include unhealthy circulation and an unhealthy cholesterol profile.



Mood support [1]

Inositol is needed to support the proper function of several brain neurotransmitters, including serotonin, which is needed to avoid mood disorders.

Immune support [2]

Inositol may also help support the immune system and the body's natural ability to maintain thermoregulation.

Source:
Medfiles report based on literature search

1. *Einat & Belmaker 2001; Levine 1997; Taylor and al. – Inositol for depressive disorders- Cochrane database of systematic reviews 2004*
2. *Jiang et al., 2016*
3. *Unfer et al. 2016; Nordio and Proietti, 2012*

GENENCARE® OSMS MI

Benefit drill-down

- Skin elasticity

Mode of action drill-down

- Energy
- Oxygen
- Water

GENENCARE® OSMS MI contributes to improve skin elasticity

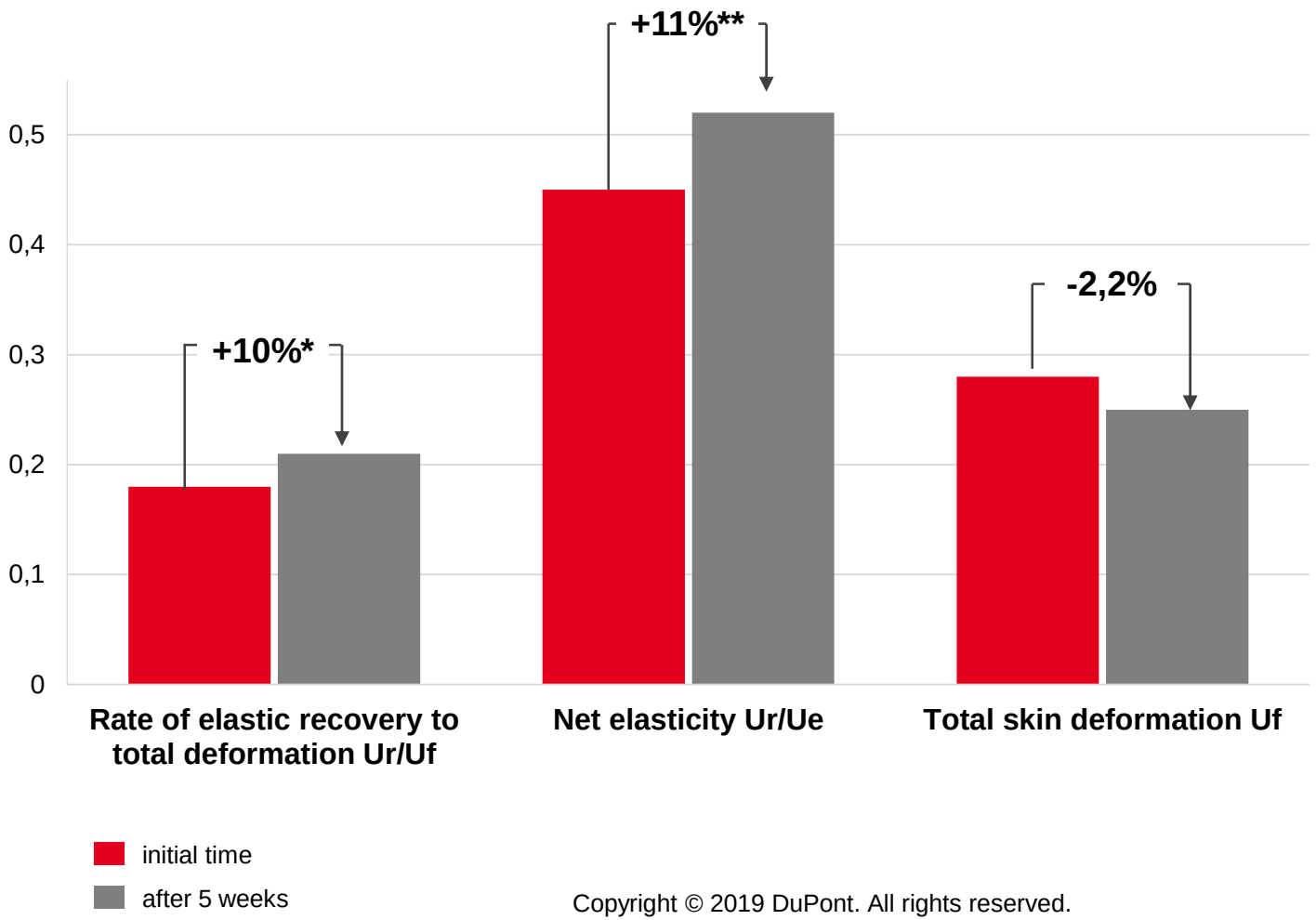
Clinical test, 5-week study

- 5-week in-use test
- 40 volunteers (40-65 y.o.)
- Twice daily application
- Cream with 3% GENENCARE® OSMS MI versus placebo

GENENCARE® OSMS MI increases or stabilizes 3 important biomechanical parameters related to skin elasticity.

The effect of GENENCARE® OSMS MI is inverse as the effects of aging on these parameters.

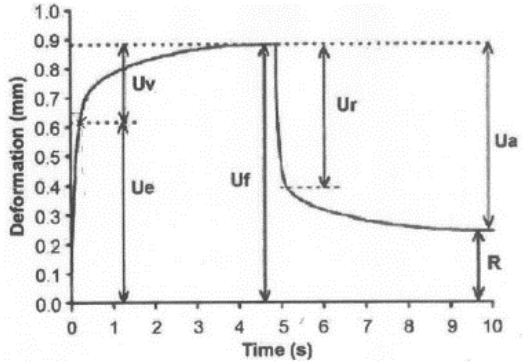
Biomechanical properties of the skin measured by cutometer on face



Significant effect versus To and versus control cream (Student T test) * p<0.01; ** p<0.05

Skin elasticity decreases with age

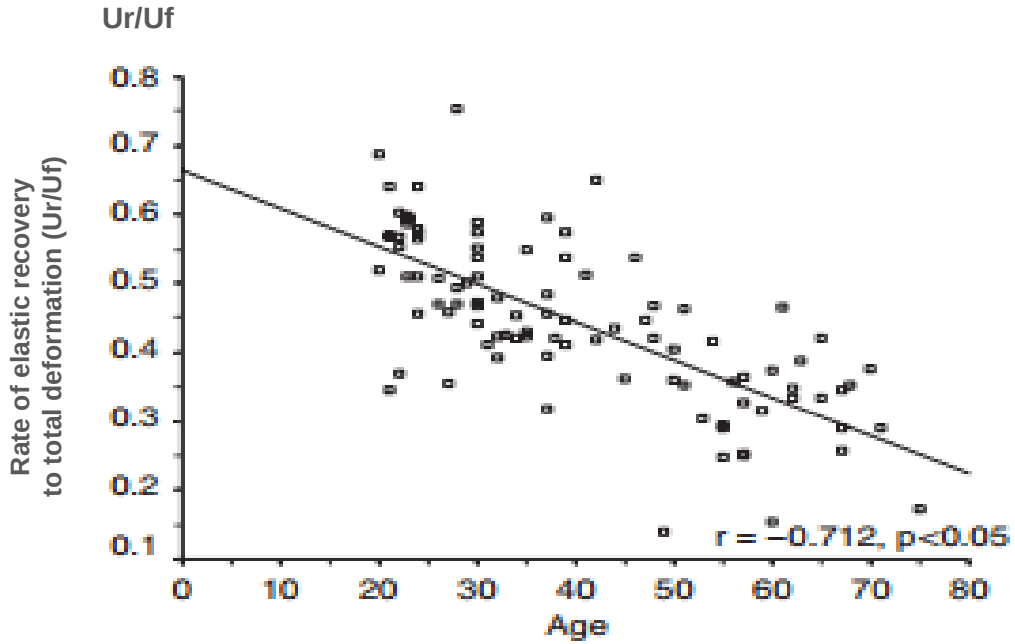
Theory of skin biomechanical properties



Uf	**Passive behavior of skin to force**
Uf - Ua	Return to original skin state
Ua/Uf	Gross elasticity =>1
Ur / Ue	**Net elasticity =>1**

Uv / Ue	Viscoelasticity =>0
Ua/Uf	Gross elasticity =>1
Ur / Uf	**Ratio of elastic recovery to the total deformation =>1**
Ua	Return to original skin state =>0

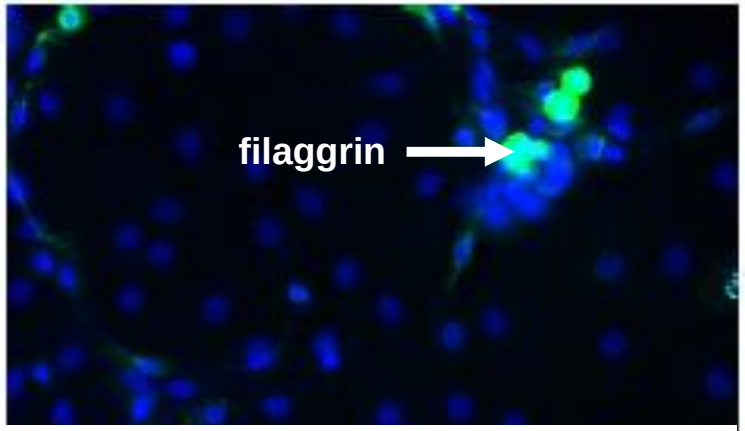
Evolution of skin elasticity in relation to age(1)



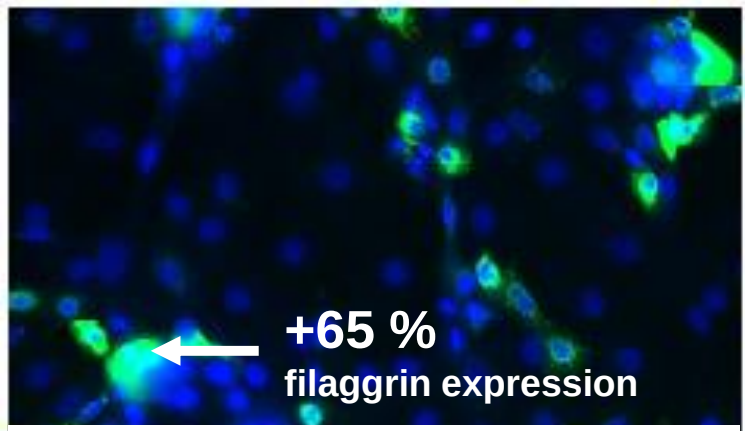
- The rate of elastic recovery to total deformation (Ur/Uf) correlates with the age of healthy adult and decreases with age(1)
- The total deformation parameter (Uf) tends to increase with age.

GENENCARE® OSMS MI helps stimulate keratinocytes metabolism

3% GENENCARE® OSMS MI contributes to increase filaggrin expression of keratinocytes by **65%.**



CONTROL
keratinocytes culture



+ 3 %
GENENCARE® OSMS MI

Filaggrin
is a key element in the epidermis:

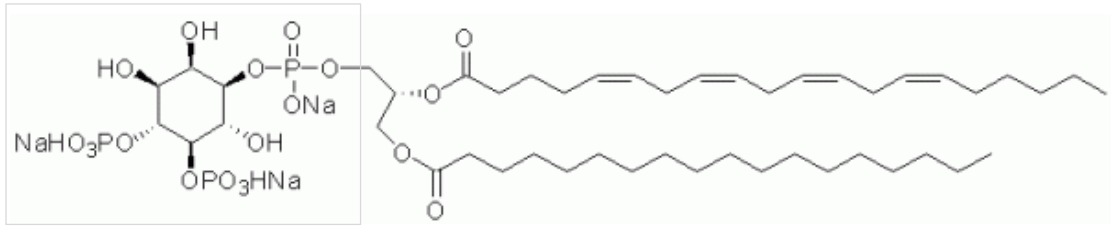
- **Differentiation marker of keratinocytes**
- Precursor protein of the **Natural Moisturizing Factor** (NMF.) for **water retention** in the stratum corneum.
- regulation of **epidermal homeostasis** and **skin barrier** function.

Source:
Bioalternatives- BM160408- Effect of GENENCARE® OSMS MI on the expression of filaggrin by NHEK- Jan 2017

Myo-Inositol is a building block of key skin elements

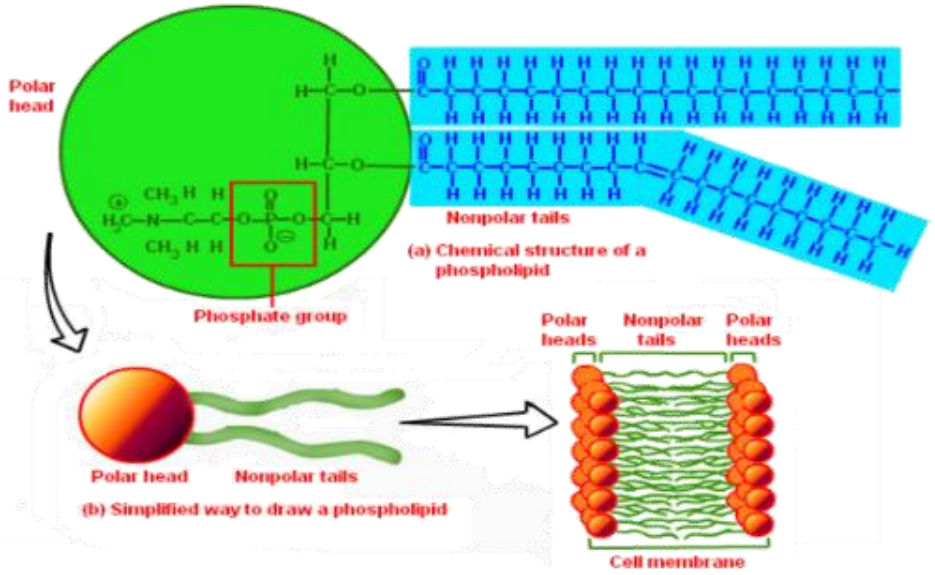
Inositol is a building block of **membrane phospholipids**

- PI: Phosphatidyl-inositol
- PIP: Phosphatidylinositol phosphate
- PIP2: phosphatidyl Inositol 2 phosphate



Inositol plays an important role as the structural basis for a number of **secondary messengers** in eukaryotic cells, the various **inositol phosphates**

- IP3: Inositol triphosphate
- IP6: Inositol hexaphosphate



In vitro test

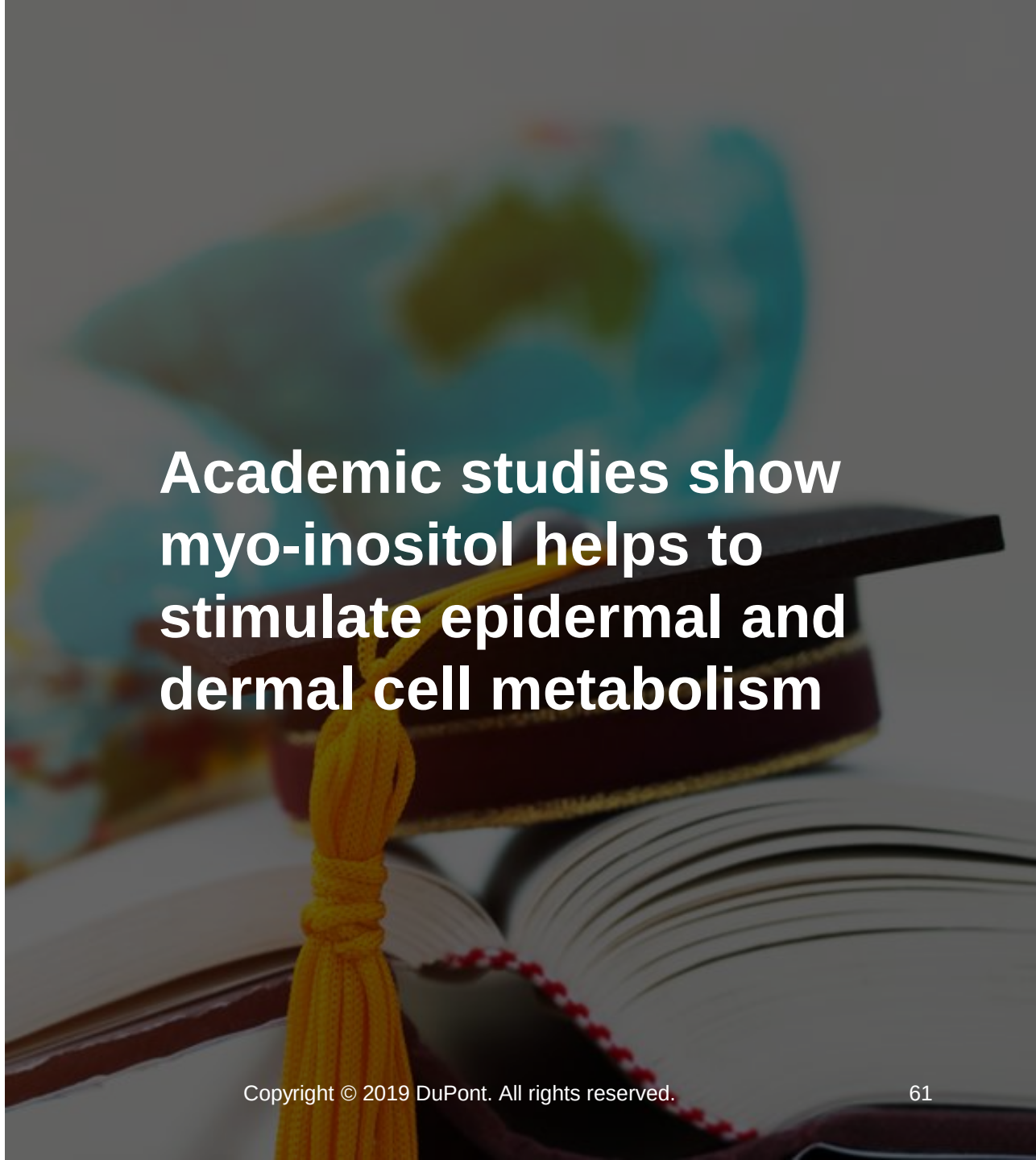
- Increases the production of PDGF-BB[1], a growth factor for dermal stem cells
- Is a required nutrient for keratinocyte growth[2]
  At an optimal concentration of 55 pM, myo-inositol approximately tripled keratinocyte yield compared to paired cultures in basal medium containing 0.3 pM.
- Improves dermal fibroblast and human endothelial cell metabolism and growth[3]

Source:

[1] Shiseido, "Shiseido Develops New Anti-Aging Skincare Technology that Enhances Skin' Self-Restoring Capabilities using Dermal Stem Cells", News release (April 2012)

[2] Gordon, P.R. et al. «Inositol is a required nutrient for keratinocyte growth», J. Cellular Physiolog. 135, 416-424 (1988)

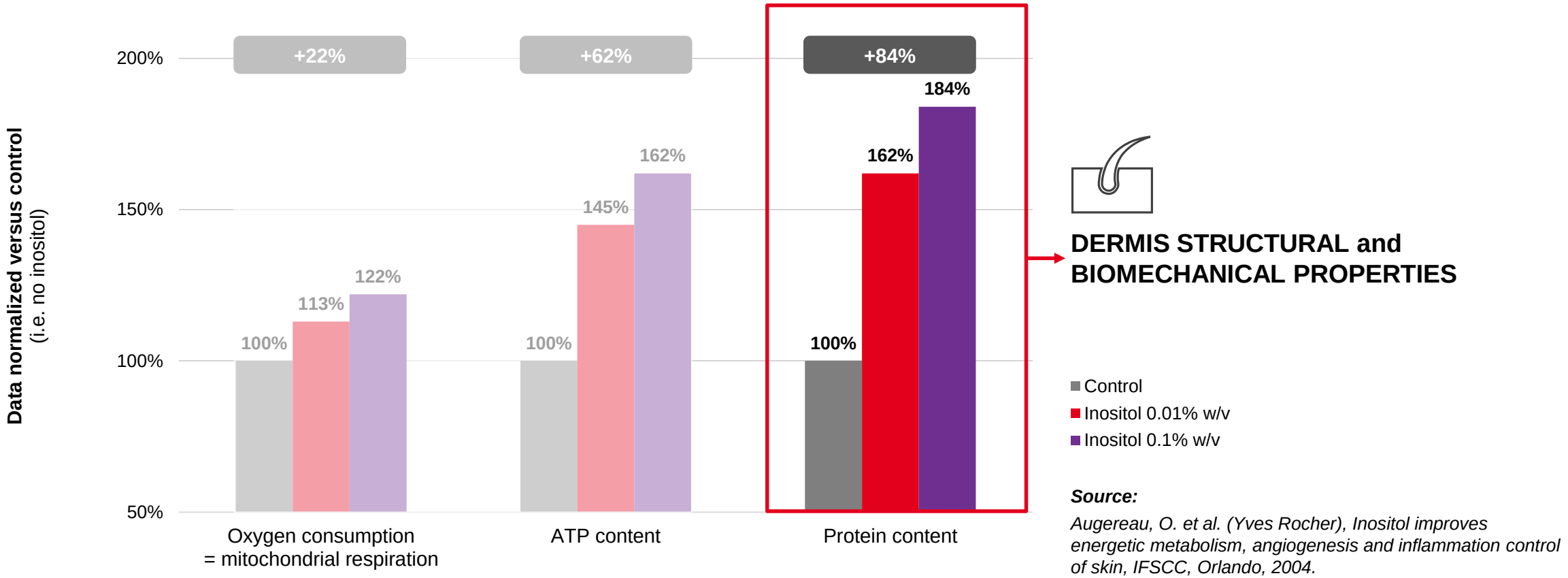
[3] Augereau, O. et al. (Yves Rocher), Inositol improves energetic metabolism, angiogenesis and inflammation control of skin, IFSCC, Orlando, 2004.



Academic studies show myo-inositol helps to stimulate epidermal and dermal cell metabolism

Academic studies show myo-inositol contributes to enhancing fibroblast metabolism

In vitro test on dermal fibroblast culture



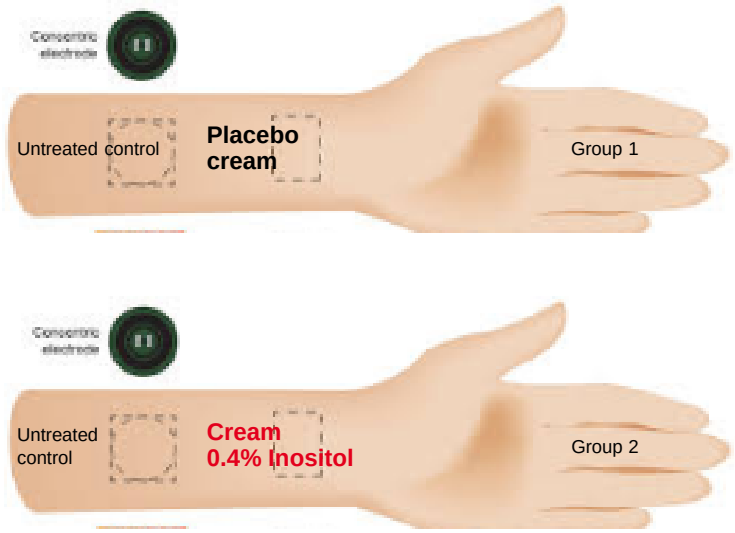
Source:

Augereau, O. et al. (Yves Rocher), Inositol improves energetic metabolism, angiogenesis and inflammation control of skin, IFSCC, Orlando, 2004.

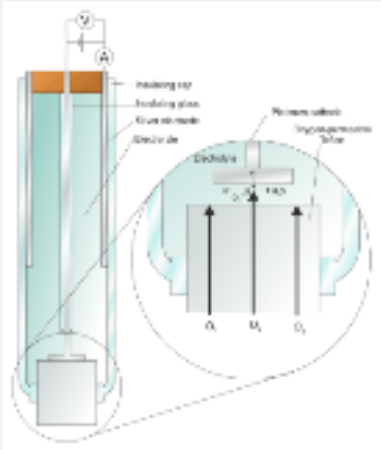
Myo-Inositol contributes to increase skin oxygen consumption in-vivo

Myo-inositol contributes to increasing skin oxygen consumption.

After 1 week application of a cream containing 0.4% inositol, skin oxygenation increases by 10% versus control.



1 week application



Measurement of the **partial pressure of oxygen** with a polarographic electrode

→ **NO CHANGE** versus control zone

→ **+10% skin oxygenation versus control**

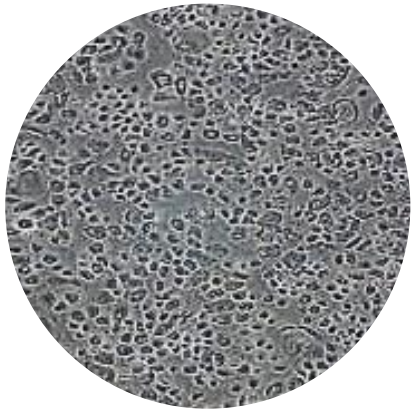
Oxygen partial pressure decreases at the skin surface which corresponds, in the absence of any change in the blood flow, to a higher skin oxygenation (for the same surface) and a higher metabolic activity of cells.

(1) Source: Rolland, Yves Rocher, WO2004075821A2, Cosmetic use of inositol, 2004

GENENCARE® OSMS MI helps to manage water balance and protect keratinocytes from hyperosmotic stress

In vitro test

Cell survival an morphology assessment study



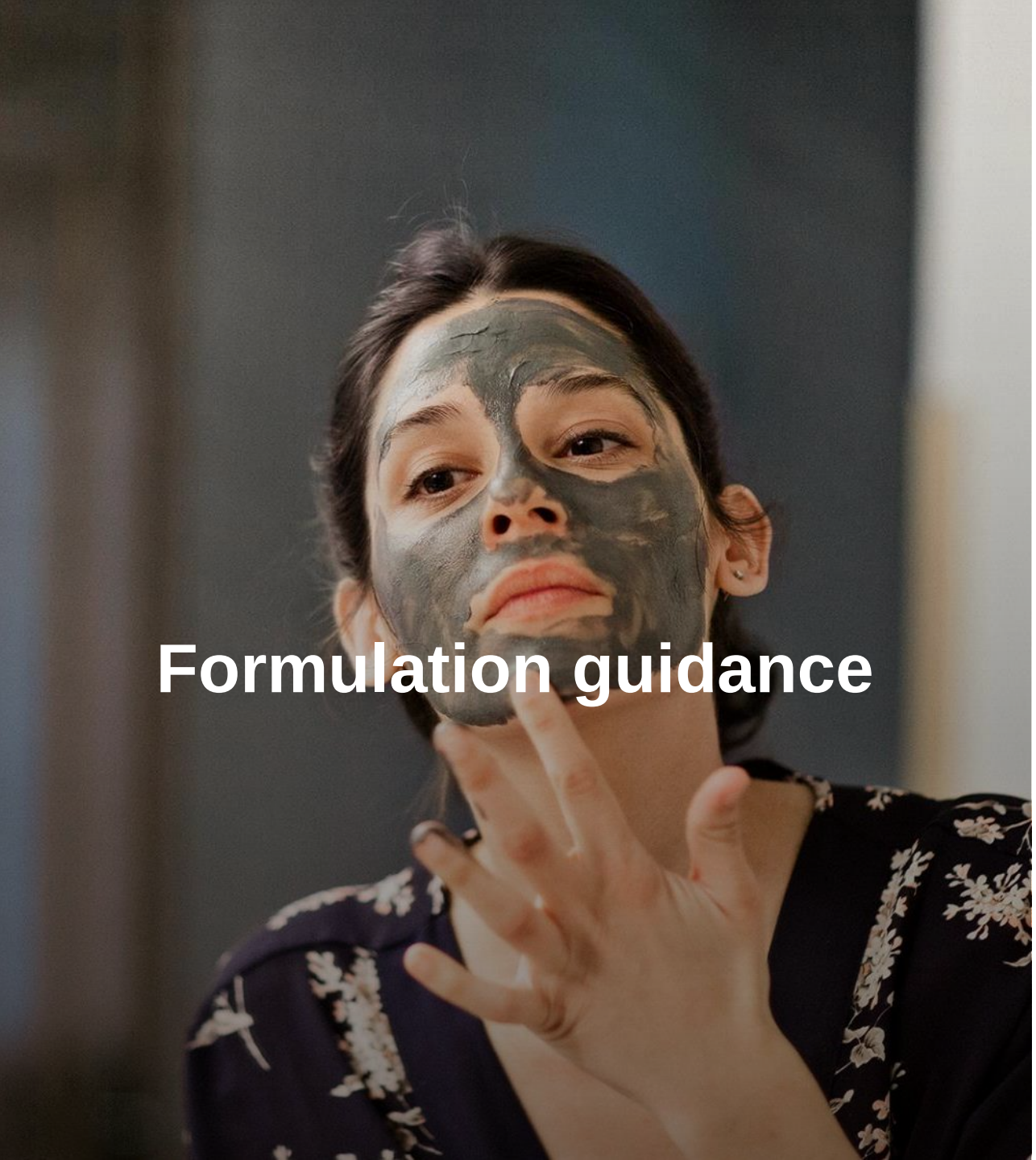
Without GENENCARE® OSMS MI

48 h pre-incubation
+ 3% GENENCARE® OSMS MI

Normo-osmotic condition

Hyper-osmotic stress condition (150mM NaCl)

Source:
Bio alternatives Jan 2017 Cell survival and morphology assessment of NHEK under hyperosmotic stress



Formulation guidance

Properties

- INCI: myo-inositol
- Free flowing white crystals
- Solubility in water: 14g/100 ml (25°C)

Recommendations

- Pay attention to solubility. May need to be dissolved in the main water phase.
- Gives colorless solutions in water.
- Known compatibility challenges:
  - Niacinamide (Vitamin B3) may create formulation instability. Dose driven and formulation dependent.
- Recommended use level: 0.5% - 3%

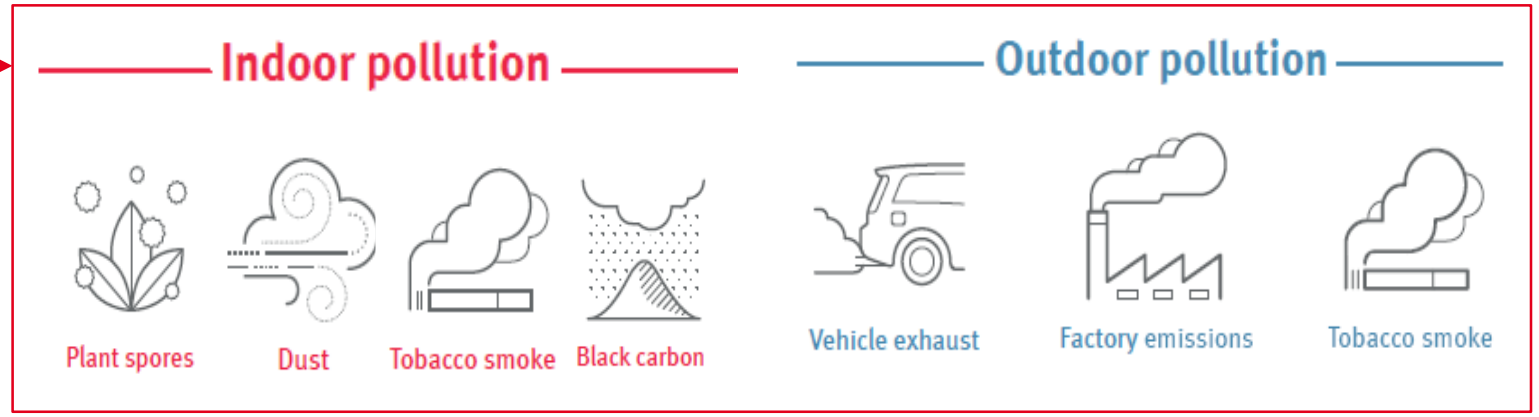
GENENCARE® OSMS PRO

Product overview

Pollution is one of the causes of skin aging

Skin aging

- Pollution
- Nutrition
- Stress
- Lack of sleep
- Temperature
- Tobacco
- Sun radiation



Effects of Pollution on Skin

- Premature skin aging (wrinkling)
- Pigmentation (dark spots)
- Uneven skin tone
- Redness
- Dull skin
- Increase of atopic and urticarial skin (Sensitive skin)
- Skin inflammation and worsening of inflammatory skin diseases such as eczema, psoriasis and acne

"UV was really the topic in skin protection for the last 20-30 years. Now, I think air pollution has the potential to keep us busy for the next few decades"

- Professor Jean Krutmann, Director Leibniz Research Institute for Environmental Medicine

J. Krutmann et al. / Journal of Dermatological Science 85 (2017) 152–161

DUPONT

L'Oréal studies in Mexico and Shanghai show the negative impact of pollution on skin.

L'Oréal study April 2015

- **Evaluation of the impact of urban pollution on the quality of skin: a multicenter study in Mexico**; comparing two populations: one living in polluted area, one living in healthier conditions
- Direct consequences of pollution on skin: significant quantitative and qualitative modification of skin surface biochemical parameters:
  - Increased level of sebum production rate
  - Lower level of vitamin E and squalene in sebum
  - Increase of lactic acid
  - Higher level of carbonylated proteins (surface oxidation by free-RL)
  - Lower level of IL 1a
- At the clinical level:
  - Higher frequency of atopic and urticarial skins (sensitive skins)
  - Higher erythematous index on the face (redness)
- Results confirmed after a similar controlled study in China.

Download full articles:

Mexico study : hiip://onlinelibrary.wiley.com/doi/10.1111/ics.12203

Shanghai study : hiips://onlinelibrary.wiley.com/doi/abs/10.1111/ics.12270

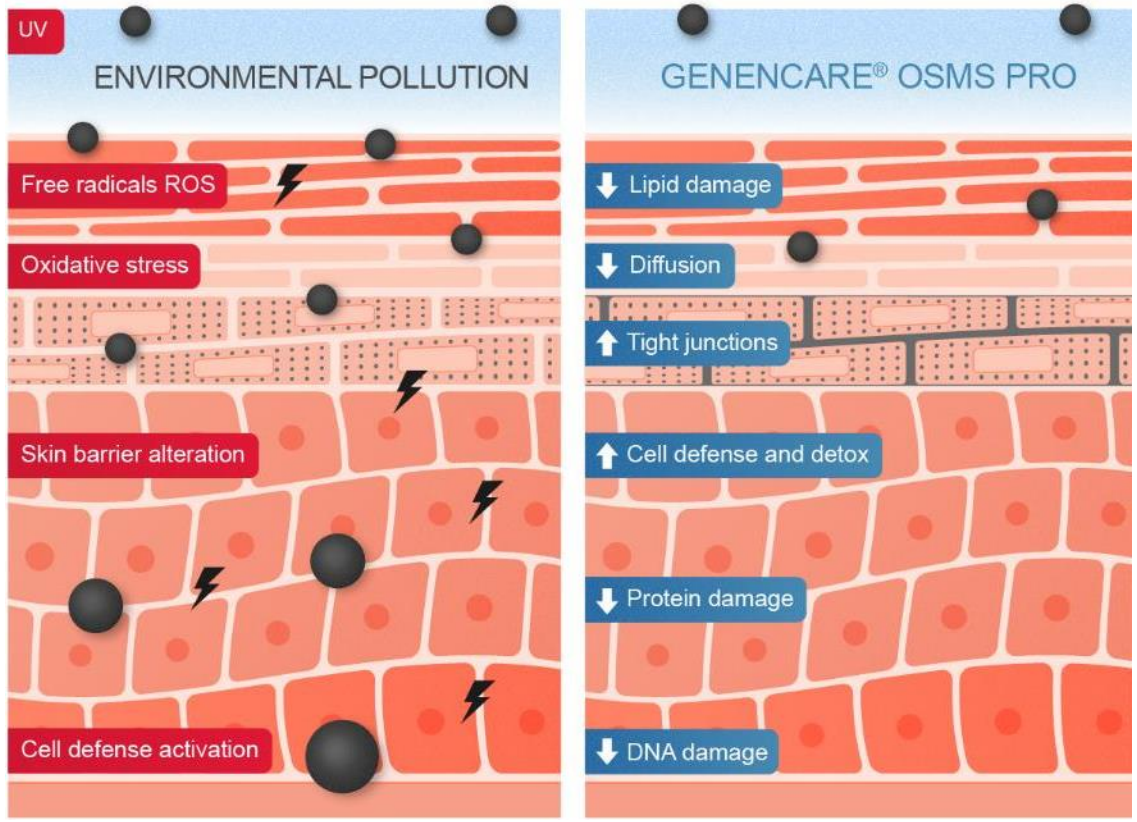
GENENCARE® OSMS PRO is a natural osmolyte pollution protection complex.

Contributes to protect the skin via three main defence strategies:

1. **ANTI-OXIDATION***
   - Helps protect skin against oxidative stress
   - Reduces pollution induced lipids peroxidation
   - Reduces UV-induced carbonylated proteins
   - Reduces UV-induced DNA damage

2. **DETOXIFICATION***
   - Induces oxidative stress transcription factors
   - AhR (Aryl Hydrocarbon Receptor),
   - Nrf2 (Nuclear factor erythroid 2-related factor)
   - Anti-oxidant enzyme HO-1 (Heme–Oxygenase 1)
   - Decreases metal regulation protein MT-1H (Metallothionein1) induced by pollution

3. **SKIN BARRIER FUNCTION improvement***
   - Strengthens keratinocytes tight junctions
   - Decreases substance penetration



Source:
BIOEC, Bioalternatives, DuPont Kantvik.

GENENCARE® OSMS PRO is easy to formulate

1

PRODUCT CHARACTERISTICS

- **Aspect:** clear liquid
- **pH:** 4, 5 – 5,5
- **Solubility:** water soluble
- **Thermo-stability:** recommended temperature of addition is below 40°C
- **Storage:** store at room temperature, avoid cold/freeze conditions
- **Naturality:** 99,6% natural according to ISO 16128

2

APPLICATION

- **Aspect:** clear liquid
- **Recommended use:** 1 - 4 %
- **pH range tested:** stable at pH = 3.5 – 7 (sensory bar formulations), not limited.
- **Starting point formulations:** Sensory Bar 2018 «ARCTIC» line

3

REGULATORY INFORMATION

- **INCI/CTFA:** Water, Betaine, Proline, Serine, Inositol
- **Contains:** sodium benzoate and 0.8% citric acid
- **Countries regulation:** authorized for use in EU, USA, China (IECIC 2015), South Korea, Japan approved for ordinary cosmetics.

GENENCARE® OSMS PRO NATURAL OSMOLYTE pollution protection complex

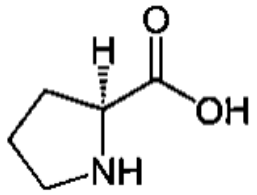
- Unique combination of pure AMINO-ACIDS and OSMOLYTES
- Naturally sourced: fermentation and sugar beet extract (NI and NOI 0.8 according to ISO 16128)
- Combined pollution protection and osmoprotection benefits

GENENCARE® OSMS PRO – what is inside the natural osmolyte pollution protection complex

- Unique combination of pure AMINO-ACIDS and OSMOLYTES
- Combined pollution protection and osmoprotection benefits
- All naturally occurring in skin, part of the NMF (Natural Moisturizing Factor) and involved in metabolic pathways

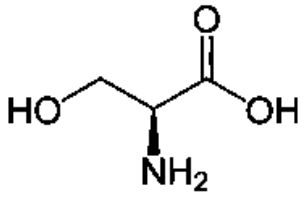
INCI: Proline

L-Proline Amino-acid with anti-oxidant and heavy metal scavenging properties. Precursor for collagen synthesis.



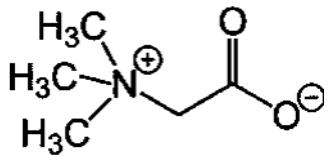
INCI: Serine

L-Serine Amino-acid precursor of key elements of the skin barrier (ceramides, phospholipids). Stabilizes proteins/enzymes.



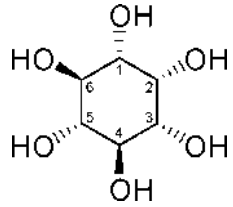
INCI: Betaine

Tri-methyl glycine natural moisturizing osmolyte



INCI: Inositol

Myo-inositol natural energizing osmolyte



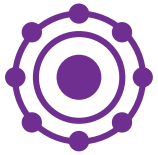
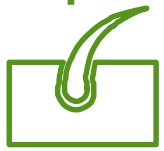
OSMOPROTECTANTS, CELL WATER BALANCE

GENENCARE® OSMS PRO

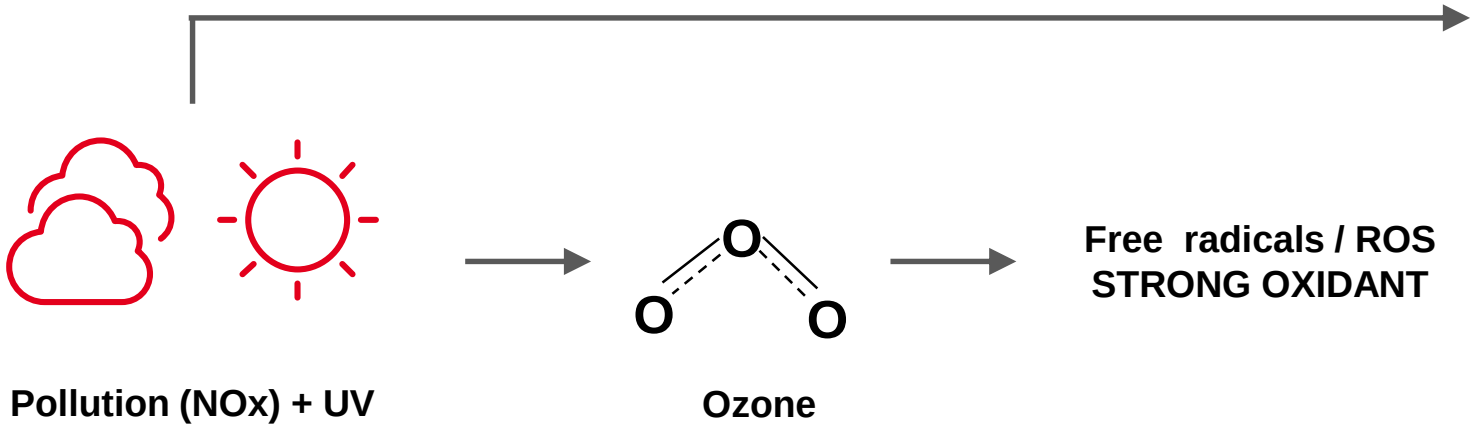
Benefit drill-down

- Anti-oxidation
- Detoxification
- Skin barrier function improvement
- Osmoprotection

GENENCARE® OSMS PRO benefit matrix

	Anti Oxidation	Detoxification	Skin barrier function improvement
MECHANISM OF ACTION	 • Reduction of lipids peroxidization • Reduction of UV-induced DNA damage • Reduction of UV-induced protein carbonylation	 • Induction the detoxification mechanisms to prepare the cells to fight pollutants • Inhibition of heavy metals effect on anti-oxidant enzymes	 • Contribution to strengthen the keratinocytes tight junctions in the stratum granulosum • Helps to limit the penetration of harmful substances through epidermis
IN-VITRO / EX-VIVO TESTS	Ex vivo test on skin explant detecting • *MDA induced by pollution* • *TUNEL marker* • *FTZ marker of surface protein oxidation*	Ex vivo test on skin explant detecting • Oxidative stress transcription factors: Nrf2, AhR • Anti-oxidant enzyme: HO-1 MT-1H b	• TEER test • Caffeine diffusion test on reconstructed skin

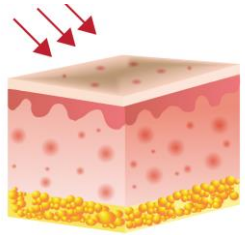
Oxygen free radicals impact key cell structural skin elements



Pollution (NOx) + UV

Ozone

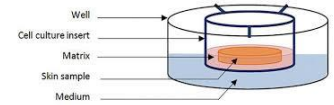
Free radicals / ROS
STRONG OXIDANT



Oxidation of key cell structural skin elements

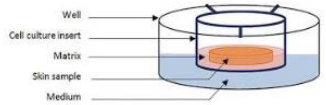
- Lipid peroxidation
- DNA damage
- Protein carbonylation

POLLUBOX® SYSTEM TEST methodology

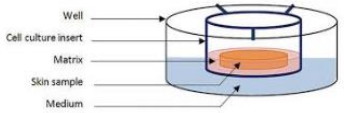


Living human skin explant

Test products

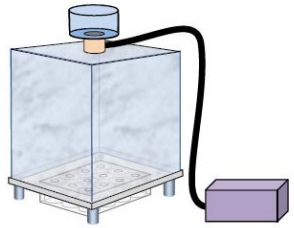


GENENCARE® OSMS PRO



Control

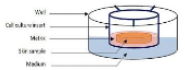
Smoke nebulisation over the cells: Benzene, toluene, xylene, Anthracène, Naphtol, Heavy Metals



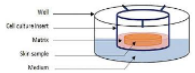
PolluBox® system

AIR PARIF

POLLUTED SAMPLES + HM

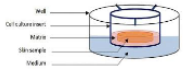


GENENCARE® OSMS PRO

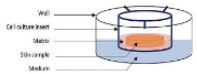


Control

NON POLLUTED SAMPLES - HM



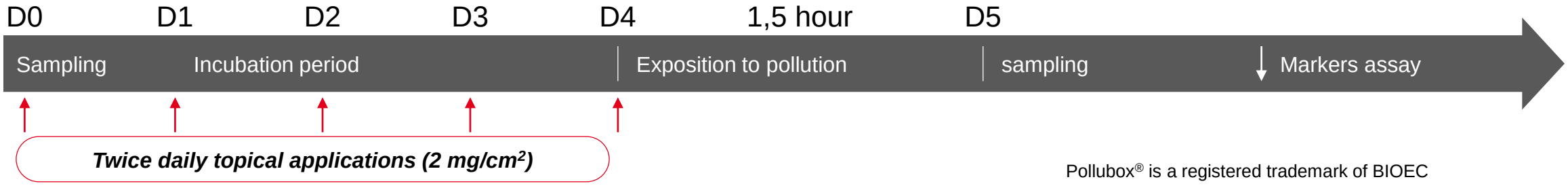
GENENCARE® OSMS PRO



Control

Δ HM = (+HM vs –HM)

- MDA assay (TBARS)
- Immunostaining of oxidative stress and detoxification markers
  - Nrf2
  - AHR
  - HO-1
  - MT-1H



Pollubox® is a registered trademark of BIOEC

DUPONT

GENENCARE® OSMS PRO helps to reduce lipid peroxidation induced by pollution

MDA, malondialdehyde is a product arising from the oxidative degradation of cell membranes lipids by free radicals, resulting in cell damage.

- **The exposure of skin explants to the mix of pollutants has a significant effect on lipid peroxidation : + 48%*****
- **4 % GENENCARE® OSMS PRO reduces the lipid peroxidation induced by the mix of pollutants by 44 %*.**
- **GENENCARE® OSMS PRO contributes to protect against pollution-induced lipid peroxidation.**

+ HM : Heavy Metal exposure

- HM : no heavy metal exposure

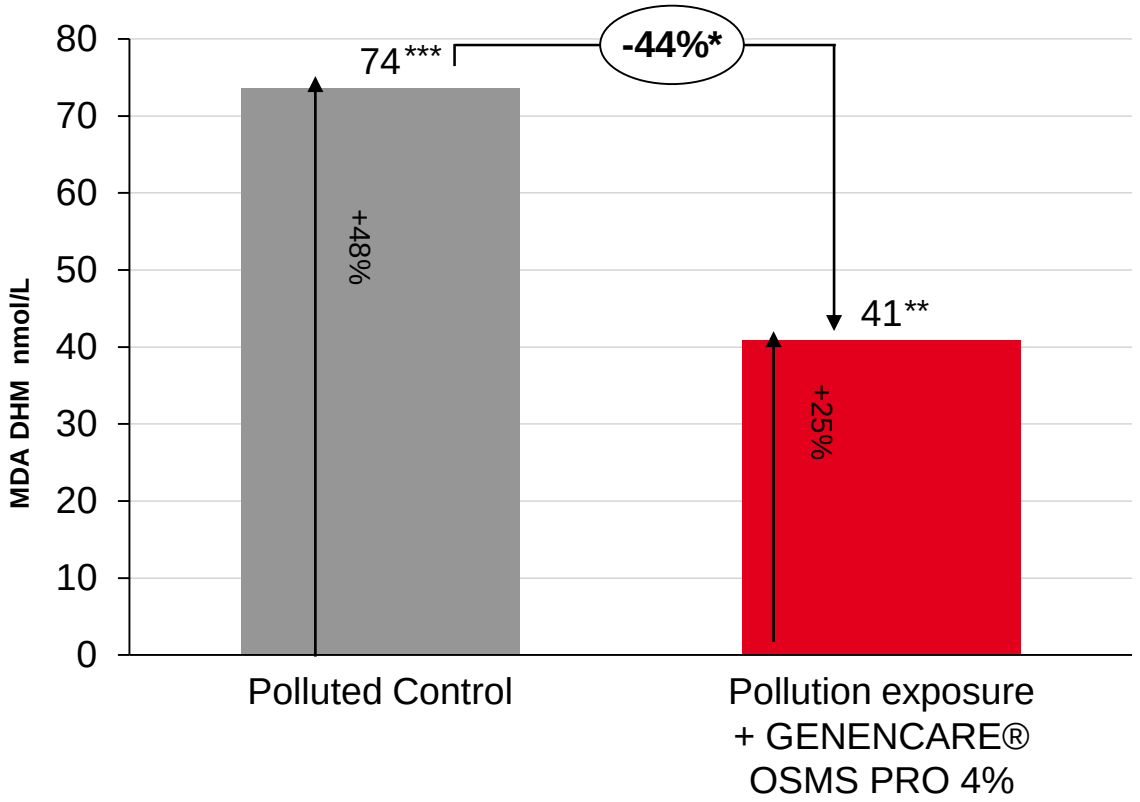
Δ HM: delta (increase) of MDA induced by HM for each explant compared to the average of the batch without HM.

* Significant for p<0.1 (90%)

** Significant for p<0.05 (95%)

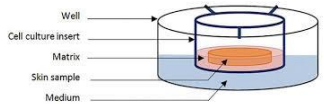
*** Significant for p<0.01 (99%)

MDA induction by pollutant exposure (Day 5)



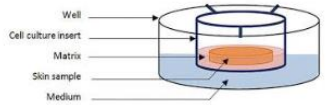
UV exposure test methodology

BIOLOGICAL MODEL

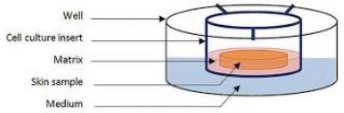


Living human skin explant

Test products



GENENCARE® OSMS PRO

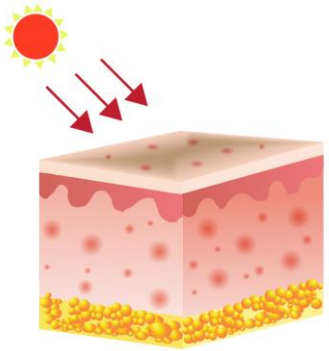


Control

OXIDATIVE STRESS

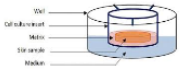
UV exposure

UVA 18 J/cm2 (4 MED*)
+ UVB 0,3 J/cm2 (2 MED).

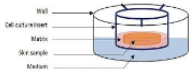


IRRADIATED SAMPLES + UV

BATCHES

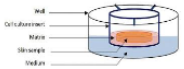


GENENCARE® OSMS PRO

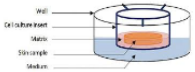


Control

NON IRRADIATED SAMPLES - UV (dark)



GENENCARE® OSMS PRO



Control

END POINTS

TUNEL marker assay

→ DNA damage

Immunostaining of FTZ marker

→ Carbonylated protein

D0 D1 D2 D3 1,5 hour D4

Sampling | Incubation period | UV irradiation | sampling | Markers assay

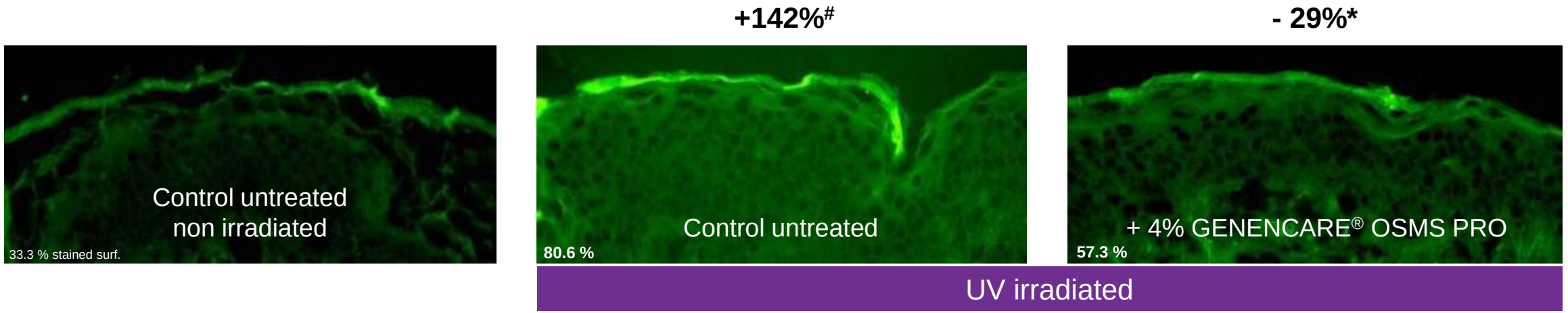
Twice daily topical applications (2 mg/cm²)

*MED = Minimal Erythemal Dose

GENENCARE® OSMS PRO helps to reduce UV-induced protein carbonylation

Carbonylated proteins induction

Representative images of FTZ immunostaining of skin explant on day 4



Protein carbonylation is a type of protein oxidation that can be promoted by reactive oxygen species (ROS), generated by UV exposure.

- **The exposure of skin explants to UVA and UVB has a significant effect on protein carbonylation + 142%#**
- **4% GENENCARE® OSMS PRO reduces the UV-induced protein carbonylation by 29%*.**

Significant for p<0.01 (99%) versus control non irradiated
* Significant for p<0.05 (95%) versus irradiated control

DUPONT™

GENENCARE® OSMS PRO helps to reduce UV-induced DNA damage

TUNEL marker induction

Representative images of TUNEL marker staining (DNA damage) of skin explant on day 4

+58%# | **- 18%***

UV irradiated

DNA oxidization is a type of damage that can be promoted by reactive oxygen species (ROS), generated by UV exposure.

- **The exposure of skin explants to UVA and UVB has a significant effect on DNA damage + 58%**
- **4% GENENCARE® OSMS PRO reduces the UV-induced DNA damage by 18%**

Significant for p<0.01 (90%) versus non irradiated control
* Significant for p<0.05 (95%) versus irradiated control

DUPONT™

Effect of pollution on the activation of cell detoxification pathways

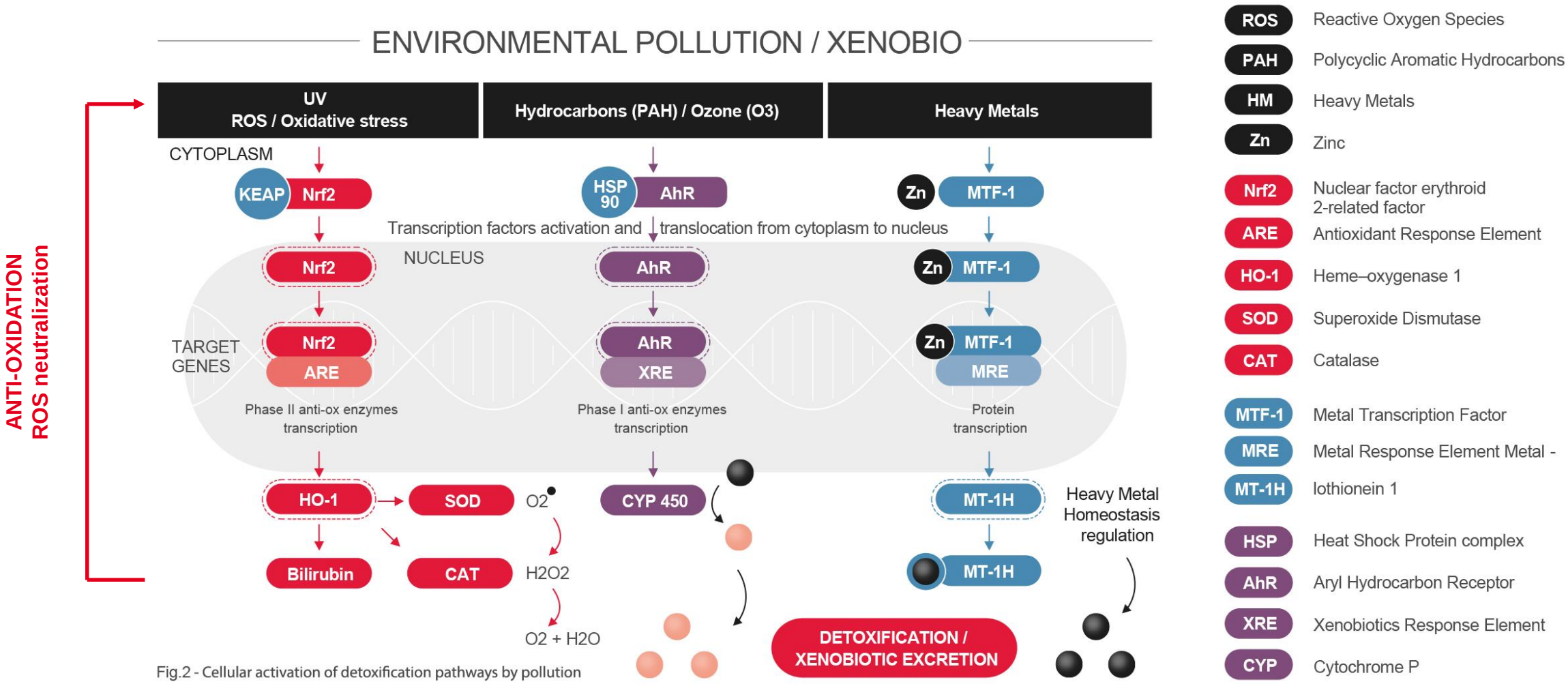


Fig.2 - Cellular activation of detoxification pathways by pollution

ROS Reactive Oxygen Species
PAH Polycyclic Aromatic Hydrocarbons
HM Heavy Metals
Zn Zinc
Nrf2 Nuclear factor erythroid 2-related factor
ARE Antioxidant Response Element
HO-1 Heme–oxygenase 1
SOD Superoxide Dismutase
CAT Catalase
MTF-1 Metal Transcription Factor
MRE Metal Response Element Metal -
MT-1H Iothionein 1
HSP Heat Shock Protein complex
AhR Aryl Hydrocarbon Receptor
XRE Xenobiotics Response Element
CYP Cytochrome P

GENENCARE® OSMS PRO helps to increase Nrf2 induction for skin detoxification

Nrf2 induction

Representative images of Nrf2 immunostaining of skin explant on day 5

+41%*

Nrf2 is a transcription factor and is the first cell answer to an oxidative stress, involved in skin detoxification mechanisms.

* Significant for p<0.05 (95%) versus control

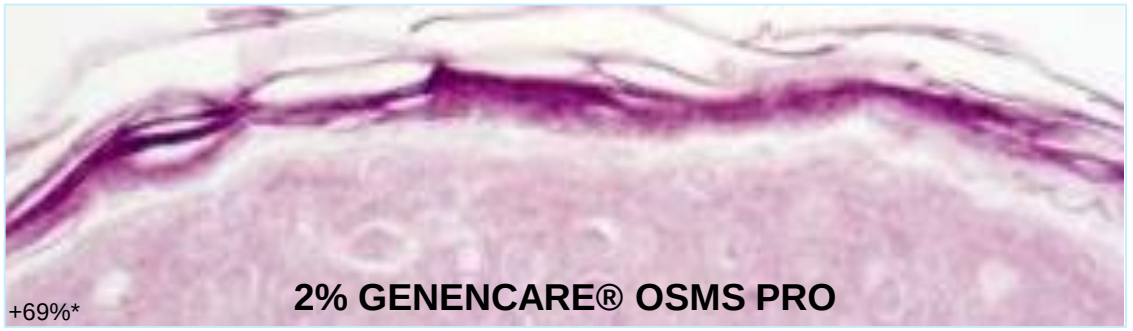
- **2% GENENCARE® OSMS PRO help increase Nrf2 induction by 41 %***
- **Nrf2 induction contributes to increase the amount of defense enzymes like HO-1, helping the skin to be ready to neutralize pollutants.**

GENENCARE® OSMS PRO helps to increase AhR induction for skin detoxification

AhR induction

Representative images of AhR immunostaining of skin explant on day 5

70%*



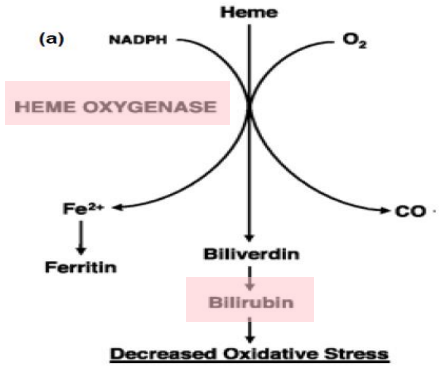
AhR is a transcription factor involved in activation of cytochrome family genes and detoxification enzymes

*Significant for p<0.05 (95%) versus control

- **2% GENENCARE® OSMS PRO help increase AhR expression by 69 %*.**
- **AhR induction contributes to increase the amount of phase I detoxification enzymes like CYP450 in cells, helping the skin to be ready to get rid of pollutants.**

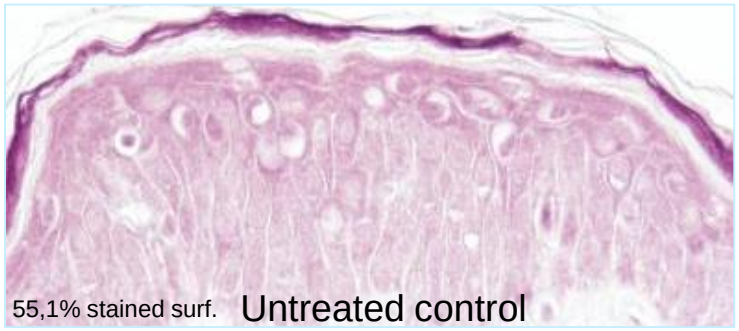
GENENCARE® OSMS PRO helps to increase HO-1 induction for skin detoxification

Anti-oxidant

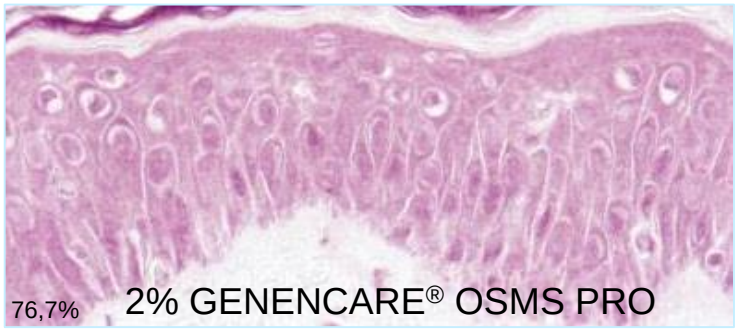


HO-1 induction

Representative images of HO-1 immunostaining of skin explant on day 5



+39%**



Heme-Oxygenase is a phase II skin anti-oxidant enzyme which generates cell anti-oxidant molecules. HO-1 expression is considered as a specific molecular indicator of cellular oxidative stress

** Significant for p<0.01 (99%) versus untreated control

- **2% GENENCARE® OSMS PRO contributes to increase HO-1 induction by 39%**.**
- **HO-1 induction contributes to increase the amount of anti-oxidant in the cells, helping the skin to be ready to neutralize pollutants.**

GENENCARE® OSMS PRO helps to reduce MT-1H induction by pollution

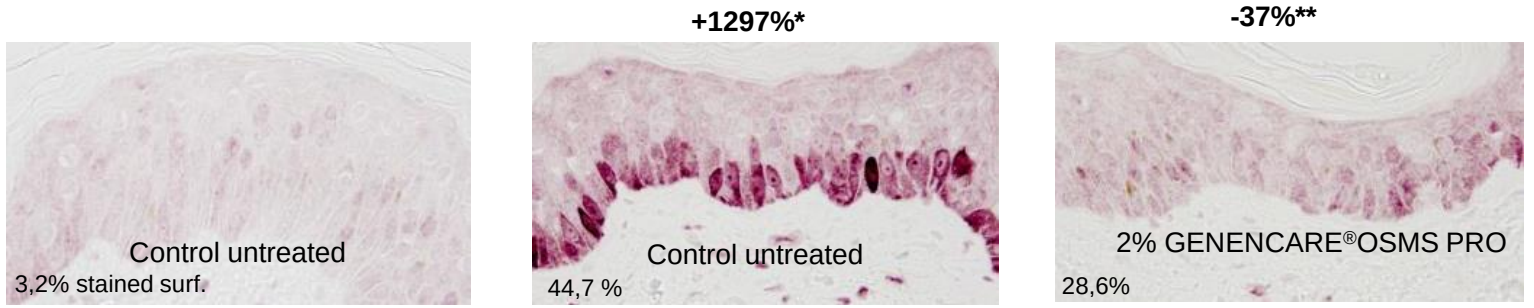
Metallothionein, MT-1H, is a regulator of metal homeostasis in the cell. MT-1H is involved in stress resistance and cellular detoxification via binding heavy metals.

- **The exposure of skin explants to heavy metals (pollution mixture) has a significant effect on MT-1H induction + 1297%***
- **2% GENENCARE® OSMS PRO significantly limits the pollution-induced MT-1H induction by 37%**.**
- **GENENCARE® OSMS PRO contributes to inhibit the effect of the pollutants on MT-1H induction. It helps to capture the heavy metals before they reach the cell and induce a defense reaction.**

**Significant for p<0.01 (99%) versus unpolluted*
***Significant for p<0.01 (99%) versus control Δ HM*

MT-1H induction by pollutant exposure

Representative images of MT-1H immunostaining of skin explant on day 5



Test condition	CONTROL - HM	GENENCARE® OSMS PRO 2% - HM	CONTROL + HM	GENENCARE® OSMS PRO 2% + HM
% Stained surface	3,2%	2,4%	44,7%**	28,6%**
Δ HM (surface %) = (+ HM) – (-HM)	-	-	41,5%	26,2%**

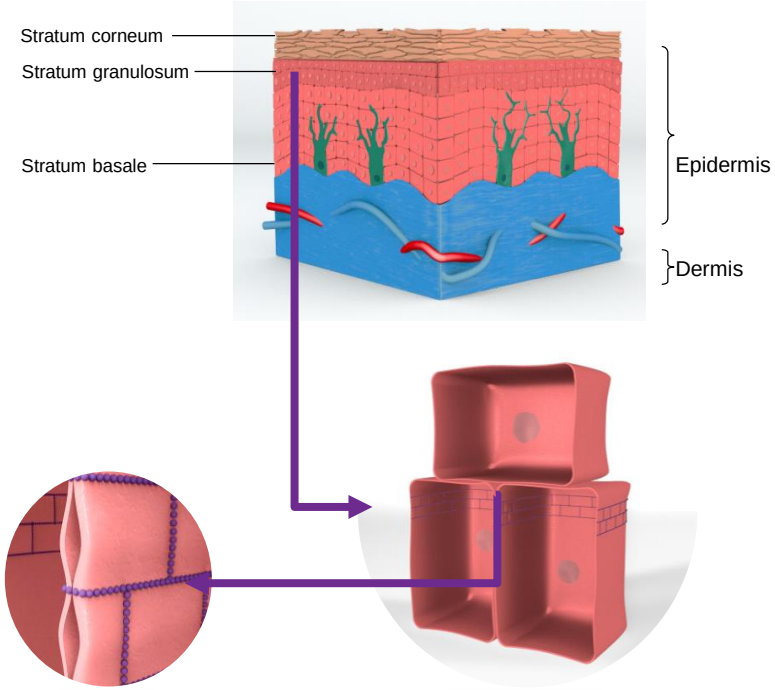
+ HM : Heavy Metal exposure

- HM : no Heavy Metal exposure

Δ HM: delta (increase) of MT-1H induced by HM for each explant compared to the average of the batch without HM.

Tight Junctions and TEER test

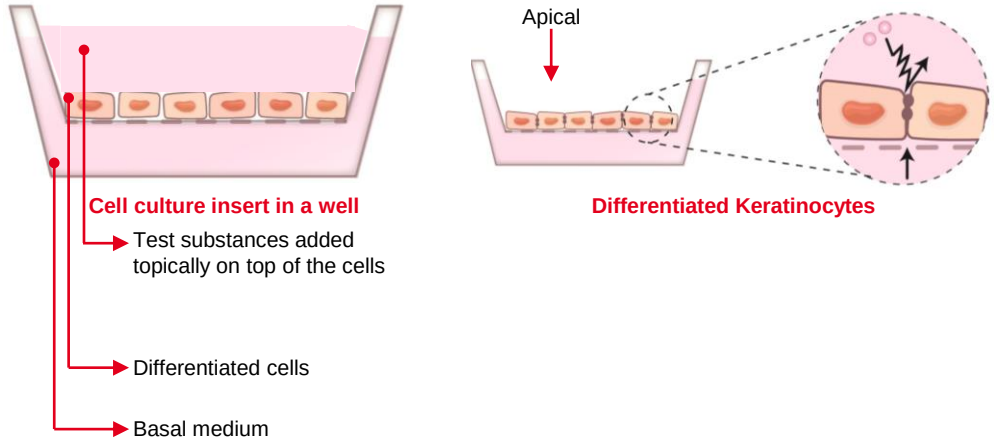
Tight Junctions



Tight Junctions are epidermis cell-cell junctions that restrict the passage of substances from both inside and outside

[1] Kirchner et al. 2010

Test Method



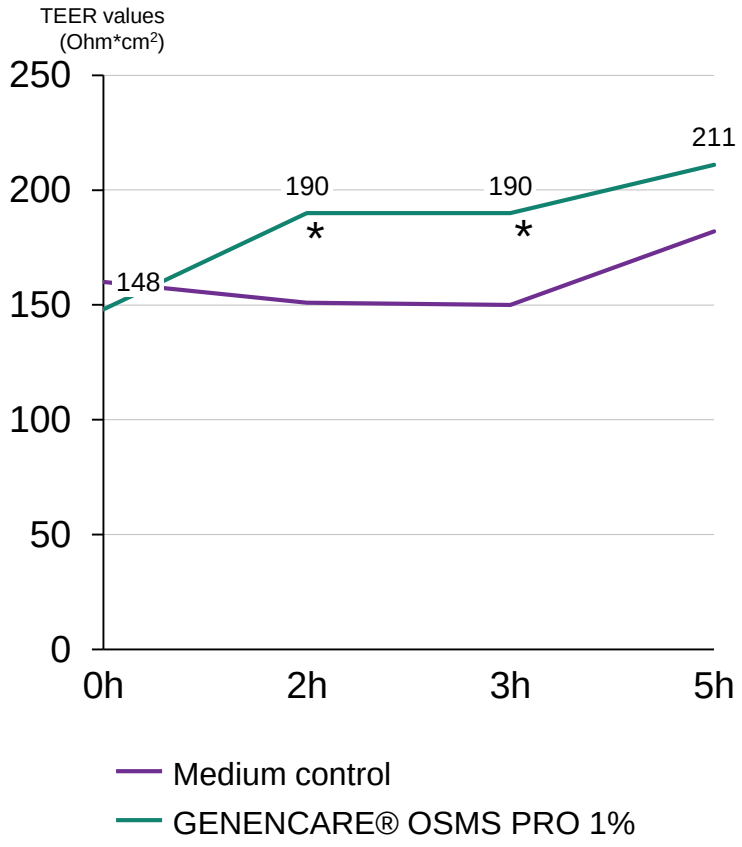
- Keratinocytes cultures treated with GENENCARE® OSMS BA
- Measurement of the **TEER = Trans Epithelial Electrical Resistance** with chopstick electrodes.
- Electrical resistance increases during keratinocytes differentiation as the tight junctions are forming between cells.
- **Higher electrical resistance = stronger Tight Junctions**

GENENCARE® OSMS PRO contributes to increase Tight Junctions' strength related to skin barrier function

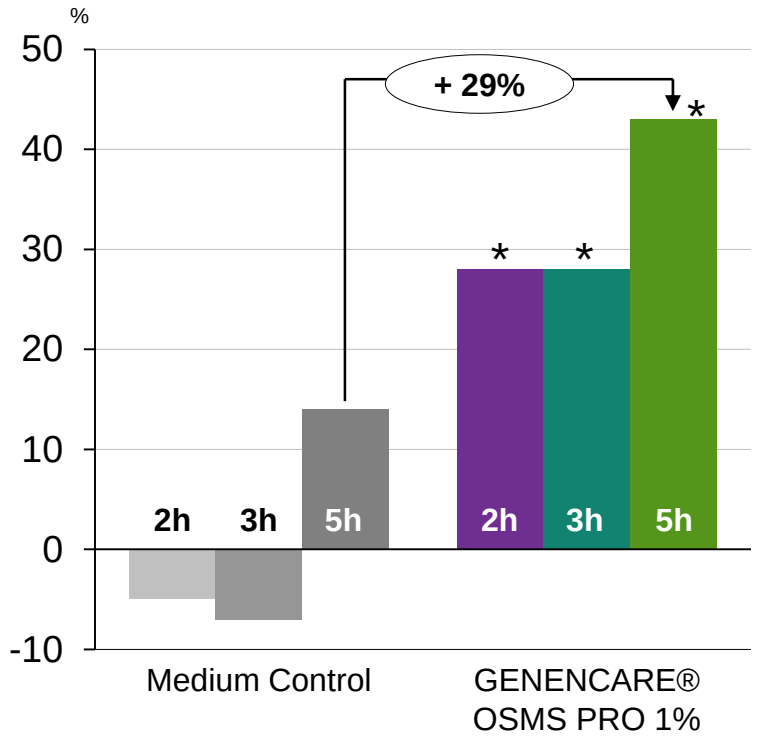
Tight Junctions are keratinocyte's cell-cell junction in the stratum granulosum. The strength of Tight Junctions is related to the skin barrier function.

- **1% GENENCARE® OSMS PRO helps to increase the TEER by 29%* after 5 hours.**
- **The higher is the TEER, the stronger are the Tight Junctions between the cells.**

Evolution of TEER over time

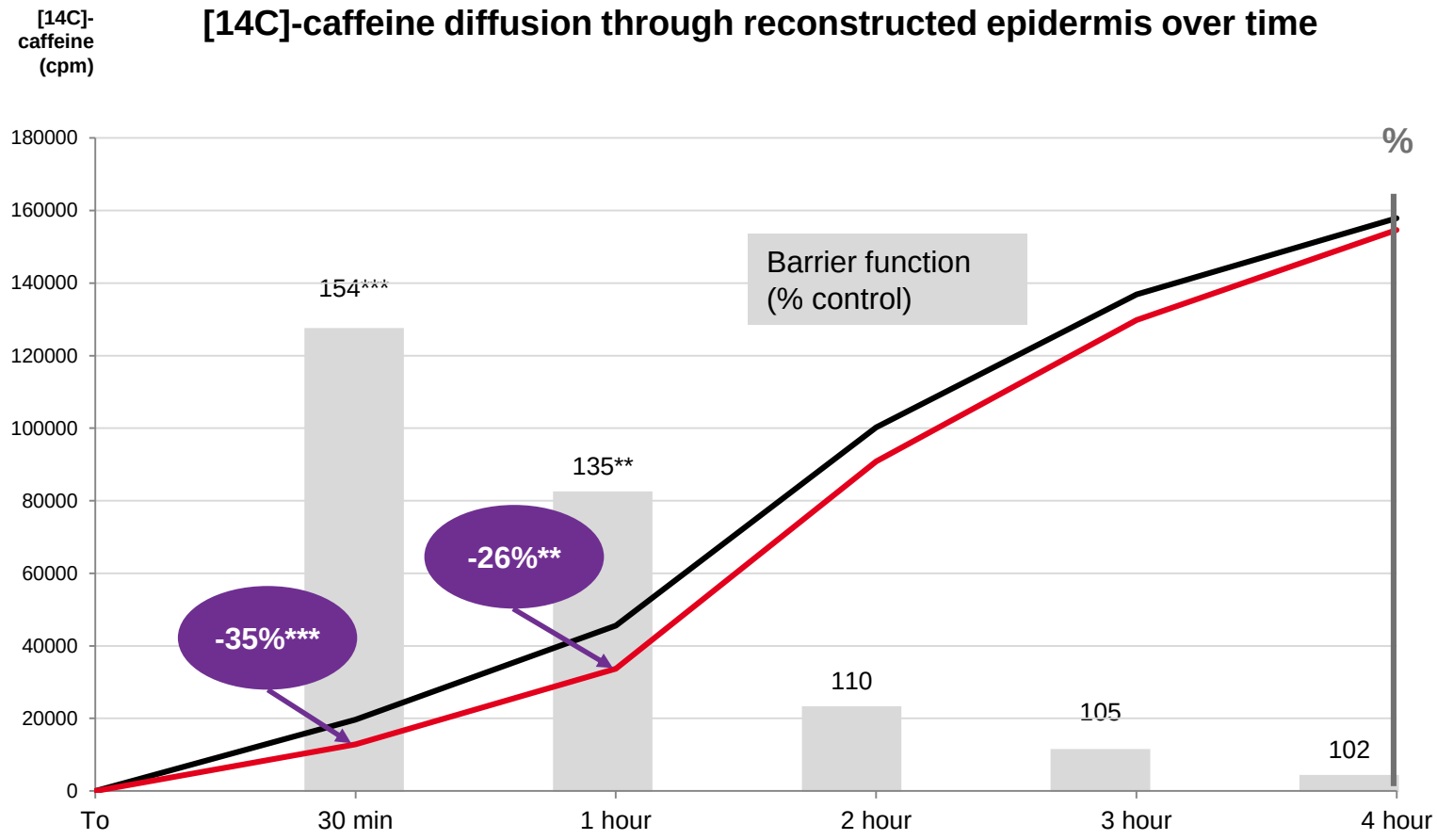


% change in TEER compared to 0 h



* Significant results according to Dunnett's multiple comparison test p<0.05 versus medium control

GENENCARE® OSMS PRO helps to limit the penetration of xenobiotics through epidermis



- **After 30 min, 2% GENENCARE® OSMS PRO helps to decrease caffeine diffusion by 35%*****
- **It contributes to decrease caffeine diffusion after the 4 first hours**
- **GENENCARE® OSMS PRO helps improving skin barrier function.**

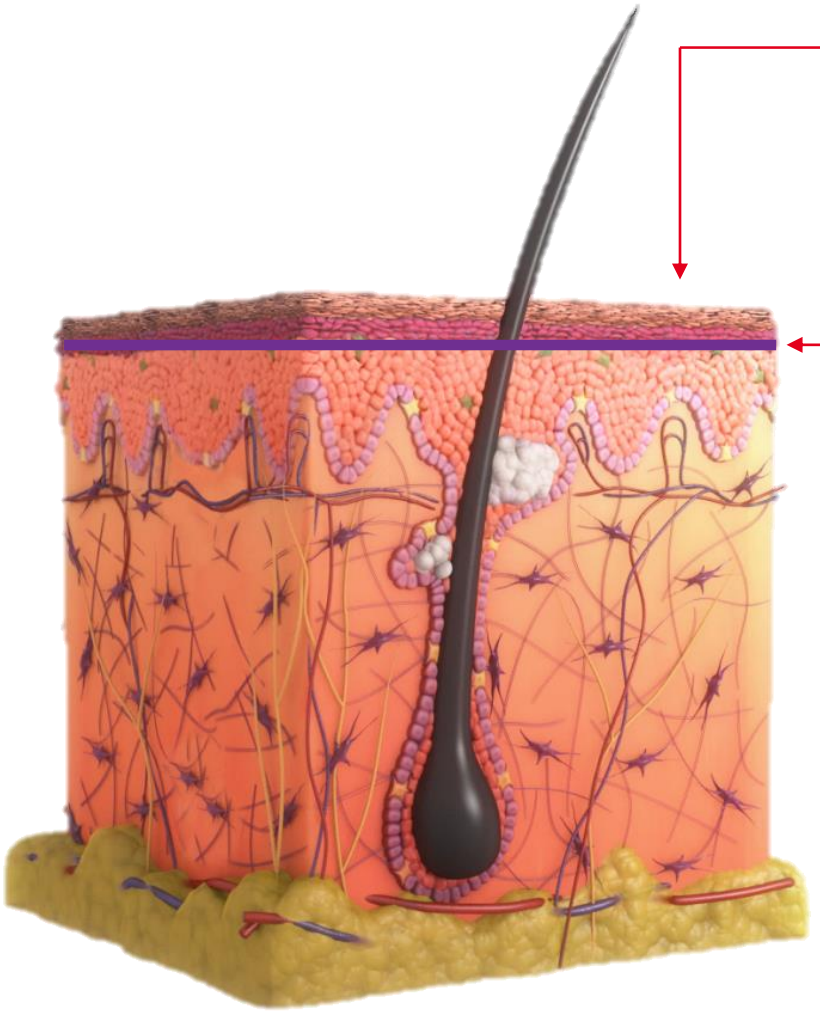
*** Significant for 0.01<p<0.001 (99%-99.9%) versus control*

**** Significant for p<0.001 (99.9%) versus control*

CONTROL

GENENCARE® OSMS PRO 2%

GENENCARE® OSMS PRO helps to improve skin barrier function



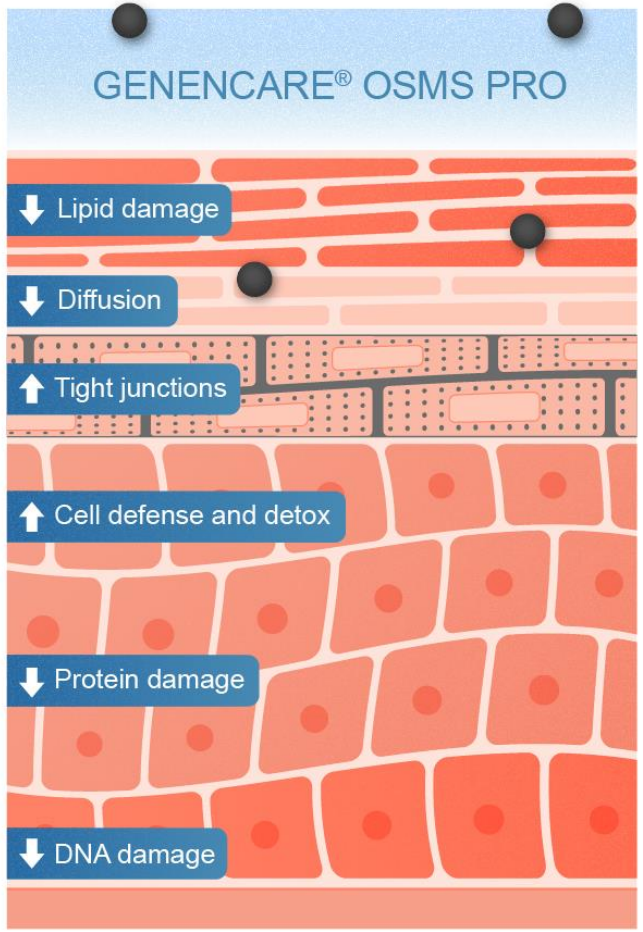
Caffeine diffusion test

GENENCARE® OSMS PRO contributes to limit the penetration of caffeine

TEER test

GENENCARE® OSMS PRO strengthen Tight Junctions in the Stratum Granulosum

GENENCARE® OSMS PRO contributes to protect the skin via three main defense strategies



GENENCARE® OSMS PRO

Anti-Oxidation

At 4 % helps reduce

-44%

lipid peroxidation

-29%

protein carbonylation

-18%

DNA Damage

Detoxification

At 2% contributes to induce

+69%

Transcription factor AhR

+41%

Transcription factor Nrf2

+39%

Heme Oxygenase

and to decrease the pollution-induced

-37%

Metal regulation protein MT1-H (Metallothioneine)

Skin barrier function Improvement

At 2%

+29%

Tight junction strenghtening

-35%

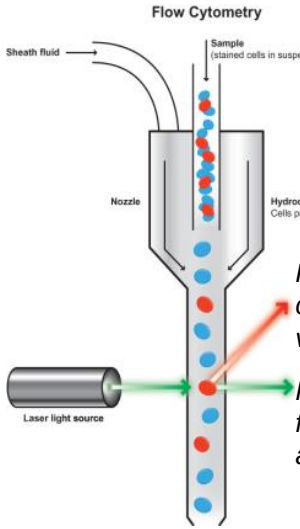
Substance penetration reduction

Source: BIOEC, Bioalternatives, DuPont Kantvik..

Osmoprotection cell survival and morphology assessment

METHOD

- NHEK, preincubated with/without osmolytes for 24 h + 48H incubation in hyper-osmotic stress condition
- Microscope observations: cell morphology *(Fig.1)*
- Flow cytometry *(Fig.2)*
  - Cell number
  - Cell size (FSC-A) - *Fig.3*
  - Cell viability (% viable cells) – *Fig.4*

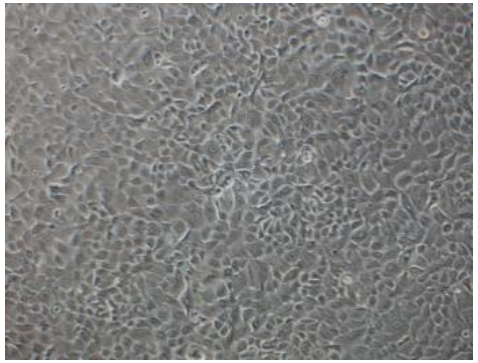


Fluorescence emitted from stained cells (PI+) ➔ % dead cells ➔ cell viability

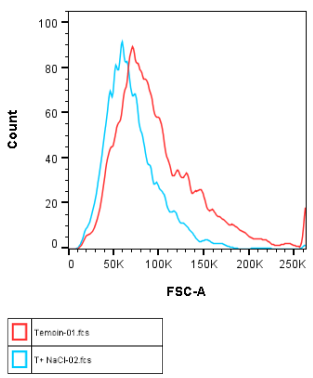
Forward and side scattered light from all cells detected ➔ cell size and granulometry

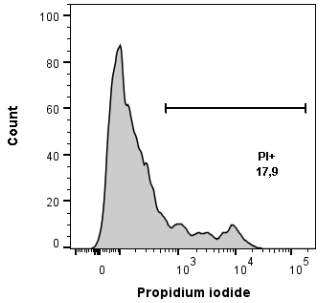
NORMO-OSMOTIC
Control
292 mOsm/L

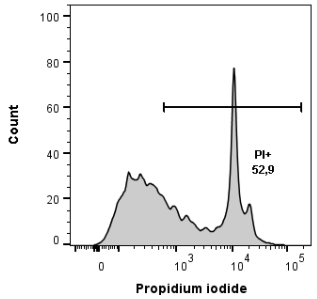
HYPEROSMOTIC STRESS
NaCl - 150 mM
592 mOsm/L



+ 48h







Morphological changes

↘ **84 %**
Cells number

↘ **25 %**
Cell Size

↗ % PI+ cell
% dead cells

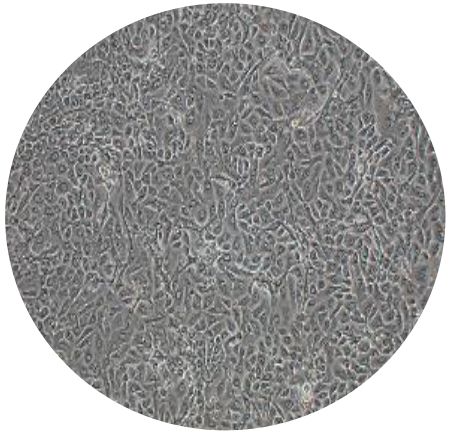
▼

↘ **38 %**
Viability

GENENCARE® OSMS PRO contributes to protect keratinocytes against hyperosmotic stress

Normo-osmotic CONTROL

Without GENENCARE® OSMS PRO



48 H pre-incubation + 2% GENENCARE® OSMS PRO

NO STRESS

HYPEROSMOTIC STRESS (150 mM NaCl)

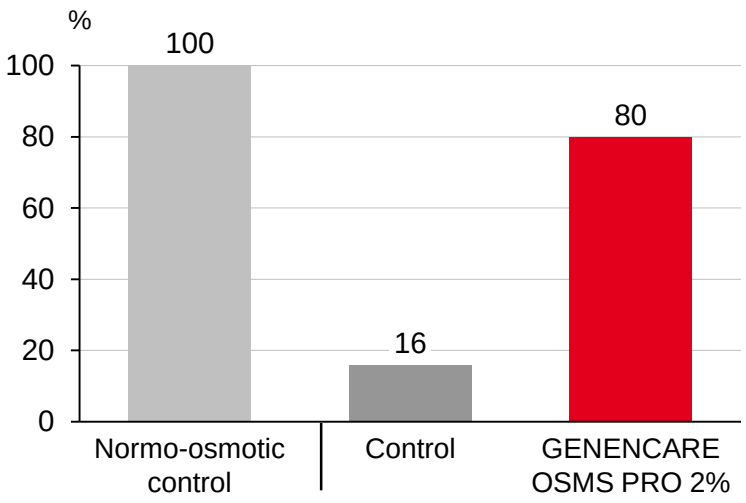
2% GENENCARE® OSMS PRO helps to limit keratinocytes' decrease in number, viability and size under hyperosmotic stress conditions.

- 84 %	Cell number(1)	- 20 %
- 25 %	Cell size(1)	- 13 %
- 30 %	% viable cells(1)	+ 14 %

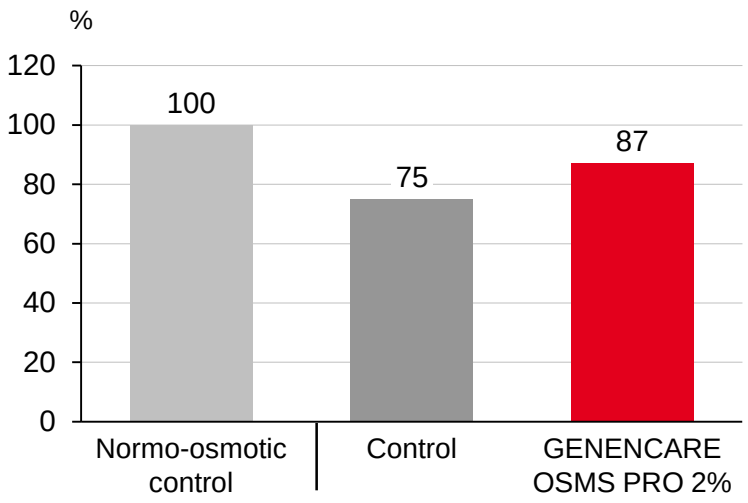
(1) versus normo-osmotic control (no stress condition)

GENENCARE® OSMS PRO contributes to protect keratinocytes against hyperosmotic stress

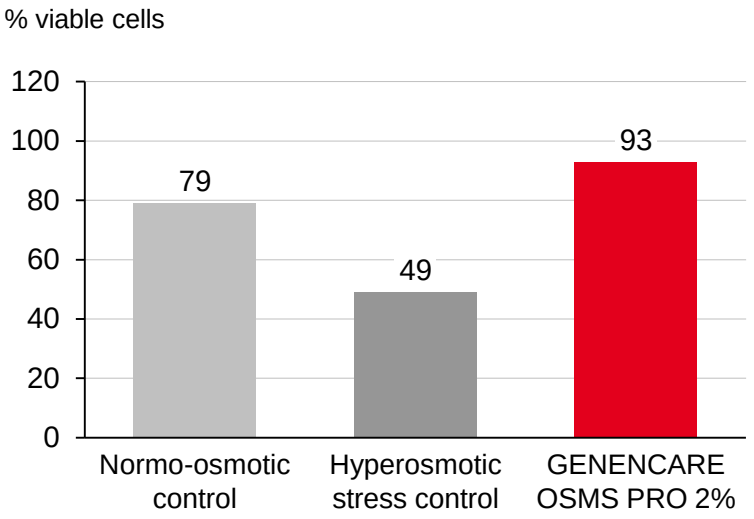
Number of cells (% normo-osmotic control)



Cell size (% normo-osmotic control)



Cell viability

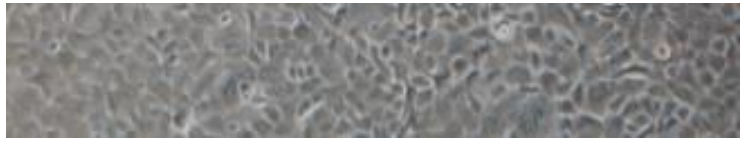


2% GENENCARE® OSMS PRO helps to limit keratinocytes' decrease in number, viability and size under hyperosmotic stress conditions.

GENENCARE® OSMS PRO, as an osmoprotectant complex, contributes to protect keratinocytes against hyperosmotic stress.

GENENCARE® OSMS PRO contributes to protect keratinocytes against hyperosmotic stress

NO STRESS

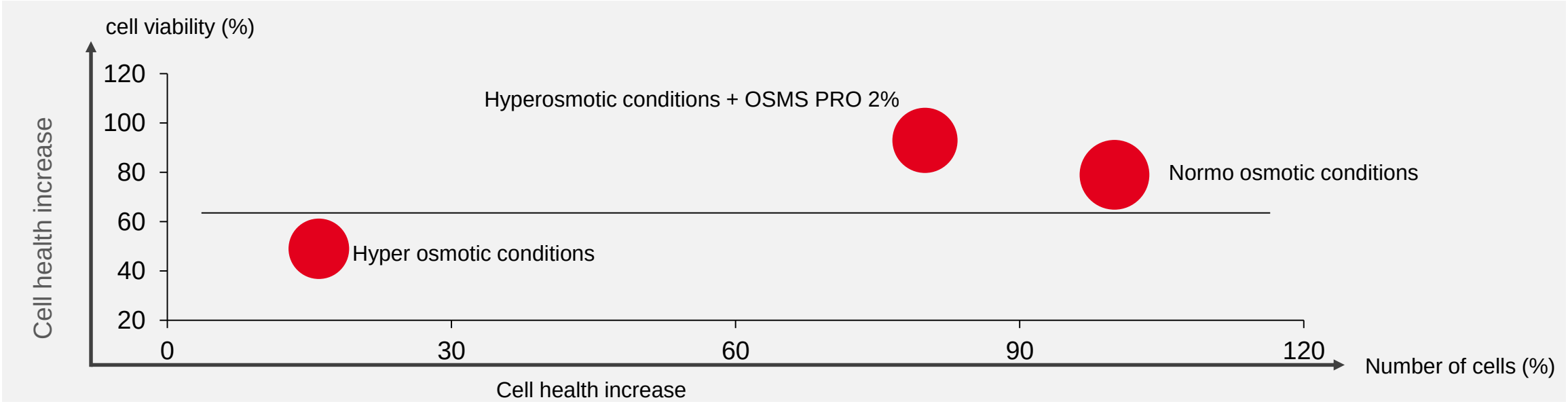


Normo-osmotic conditions
CONTROL

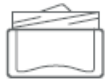
HYPEROSMOTIC STRESS

Hyperosmotic stress
(150 mM NaCl)
without GENENCARE®

Hyperosmotic stress
(150 mM NaCl)
+ 2% GENENCARE® OSMS PRO



Four main categories for Anti-pollution products

	1. Clean	2. Protect	3. Repair	4. Strengthen
Benefits	Remove dust, dirt, impurities.	Establish barrier against pollution, external aggression, shield from polluted air, UV, dust, etc.	To repair the harm or hunt to the skin brought by pollution, like to recover the energy.	Reinforce skin's defense system, strengthening skin barrier, or improve self-recovery/self-moisturizing of skin.
Related claims	Cleansing, oil-control, anti-acne, anti-dullness/yellowish.	Anti-UV, whitening.	Moisturizing, whitening, detoxifying, anti-dullness/ yellowish, even skin tone.	Moisturizing, anti-aging,
Related categories	 Facial cleanser  Make-up remover  Cleaning device	 UV-block  BB/CC cream  Primer, foundation	 Sleeping mask  Treatment lotion  Mist/spray	 Serum  Booster  Ampoules

Source: Mintel research for DuPont

Anti-pollution products and ingredients solutions

PRODUCT categories	**1. Clean**	**2. Protect**	**3. Repair**	**4. Strengthen**
	Remove dust, dirt, impurities.	Estabish barrier against pollution, external aggresstion, shield from polluted air, UV, dust, etc.	To repair the harm or hunt to the skin brought by pollution, like to recover the energy.	Reinforce skin's defense system, strengthening skin barrier, or improve self-recovery/self-moisturizing of skin.
INGREDIENTS categories Anti-pollution actives categories are used in synergies	**Film forming** • shielding action to limit the deposition of particulate matters		• Repair damages from free-RL • **Reduce the effects of pollution:** moisturization, brighter/even skin tone, lipid balance restoration, decrease redness / skin sensitivity, revert the signs of aging	• **Reinforcement of skin barrier** to limit the penetration of harmful substances
	• help removal of particulate matters	**UV filters** • limit synergy of UV with pollutants and the generation of free-radical and UV-induced skin damages		
		• **Anti-oxidant**: neutralize free-radicals and limit their deleterious effects on skin • **Detoxification :** improvement of skin natural detoxification processes • **Anti-inflammatory**		

GENENCARE® OSMS PRO

Mode of action

Definition of POLLUTION

Degradation of the environment by substances (natural, chemical or radioactive), waste (household or industrial) or various nuisances (sound, light, thermal, biological, etc.).

In cosmetics, we want to protect the skin from the atmospheric pollution in cities or from indoor pollution.

UV light is an aggravating factor of the impact of the pollution on skin.
UV + pollutants → free radicals toxic for skin.

Atmospheric pollution ? Grey cloud, dust, colorless gas, ozone layer ?

Primary pollutants:

directly issued from «fixed» sources (heating, industry) and «mobile» sources (cars, planes)

- SO2 → H2So4 (acid rains)
- NOX – nitrogen oxides (NO2) → generate ozone (O3) under the action of UV
- VOC - volatile organic compounds : hydrocarbons (benzene, toluene, xylene), methane (CH4)
- PAH (polycyclic aromatic hydrocarbons) → cancerogenic
- CO (carbon monoxide)
- HM: Heavy metals

Secondary pollutants:

- Ozone, coming from the degradation of primary pollutants by the action of UV + heat,
- H2SO4, HNO3 acids

Solid pollutants:

fine particles (PM = particulate matter). Vehicles for other substances like cancerogenic PAH. Below 1 microns, they can penetrate the lungs alveoli and blood circulation.

PUBLICATIONS / LITERATURE

How to make anti-pollution skincare, tips and recommendations for formulators

hiips://formulabotanica.com/how-to-make-anti-pollution-skincare/

L'Oréal Pollution Study

hiip://www.loreal.com/media/news/2016/apr/loreal-scientific-publication-on-pollution-impact-on-the-skin-awarded-by-ifscc

The full articles are enclosed or downloadable from there:

Mexico study :
hiip://onlinelibrary.wiley.com/doi/10.1111/ics.12203

Shanghai study:
hiips://onlinelibrary.wiley.com/doi/abs/10.1111/ics.12270

News Articles

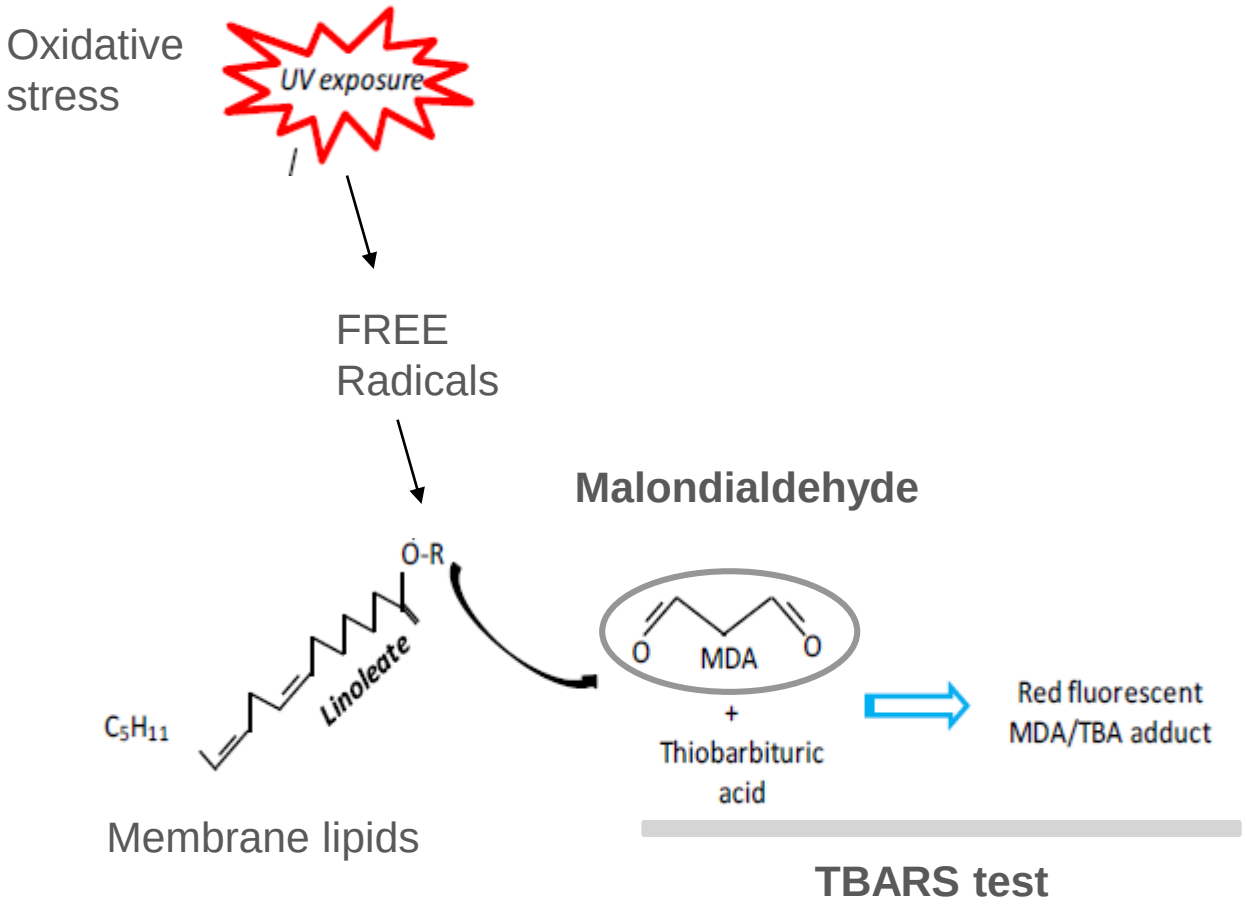
10 best anti-pollution skincare products | The Independent | August 2017

Anti-pollution skincare more popular than ever, but are products worth the investment | the Telegraph | October 2017

Is 'Anti-Pollution Skincare' The New Beauty Buzzword In Asia | Forbes | November 2017

Not only the environment and health, pollution affects your skin too | Outlook India | November 2017

MDA assay – marker of cell membrane lipid peroxidation

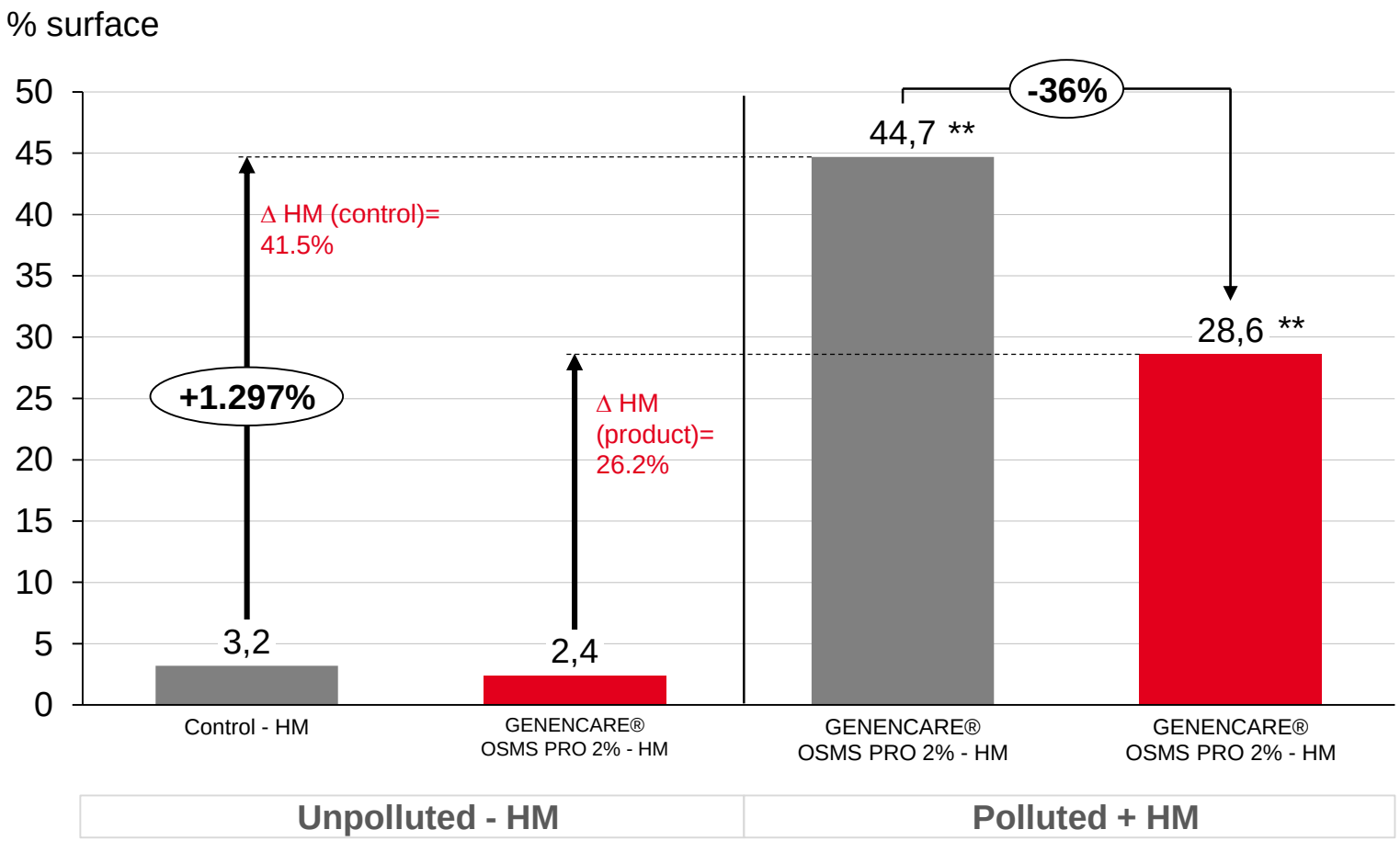


- Malondialdehyde (MDA) is a product arising from **lipid peroxidation of the cell membranes.**
- The **free radicals induced by oxidative stress** (UVA, pollutants, heavy metals, pesticides…) **degrade the polyunsaturated lipids and generate hydroperoxides**
- ➔ formation of radical intermediates and aldehydes, particularly **MDA.**
- MDA is one of the several end products formed via the decomposition of certain primary and secondary lipid peroxidation products.

TBARS : Thiobarbituric Acid Reactive Species

GENENCARE® OSMS PRO helps to reduce MT-1H induction by pollution

Surface % of the epidermis covered by the MT-1H staining on day 5

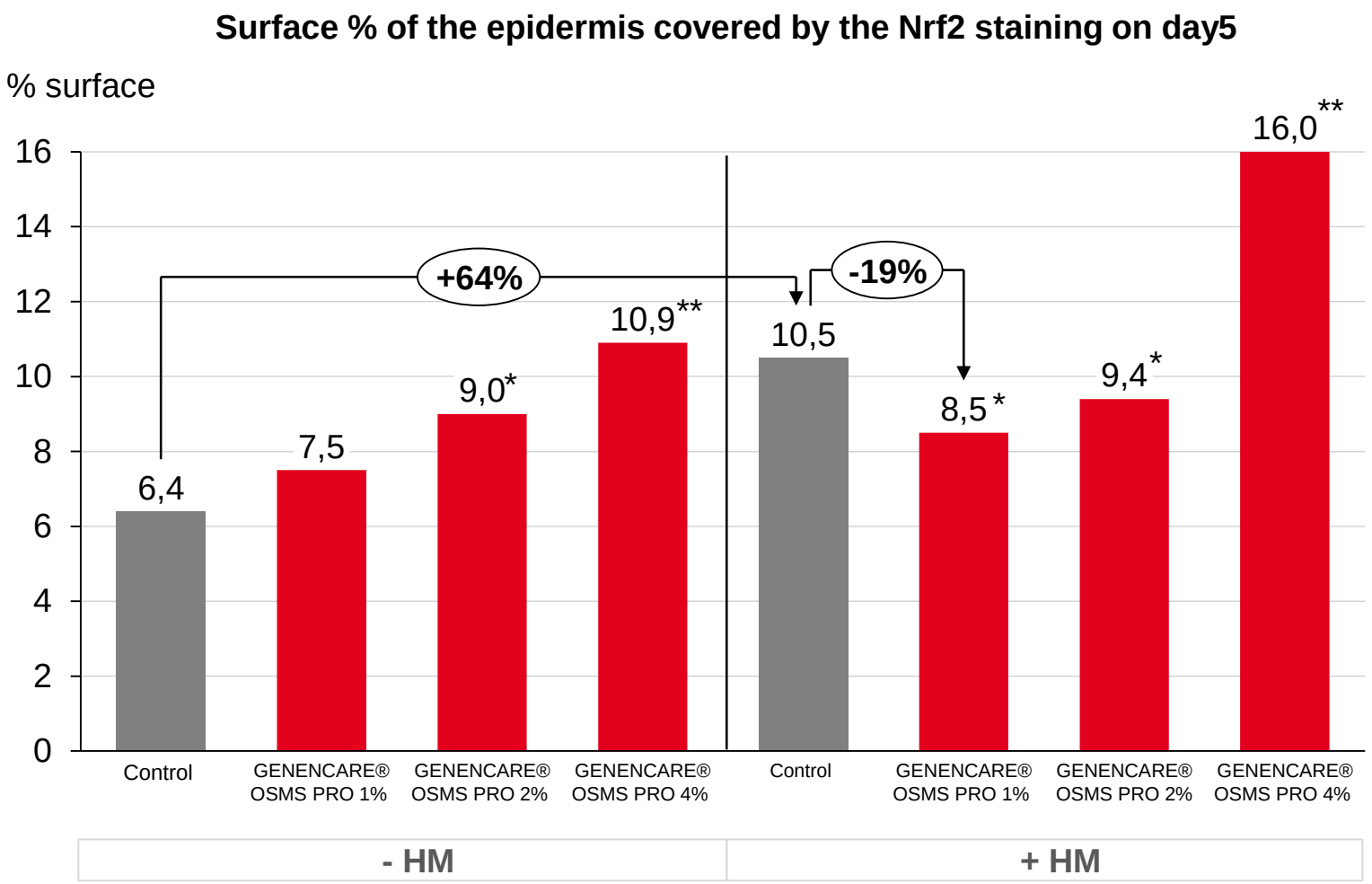


+ HM : Heavy Metal exposure
- HM : No Heavy Metal exposure

Δ HM: delta (increase) of MT-1H induced by pollution (Heavy Metals) for each explant compared to the average of the batch without pollution.

*** Significant for p<0.01 (99%) versus control*

NRf2 induction with/without pollution exposure



+ HM :Heavy Metal exposure

- HM : no heavy metal exposure

*Significant for p<0.05 (95%) versus control

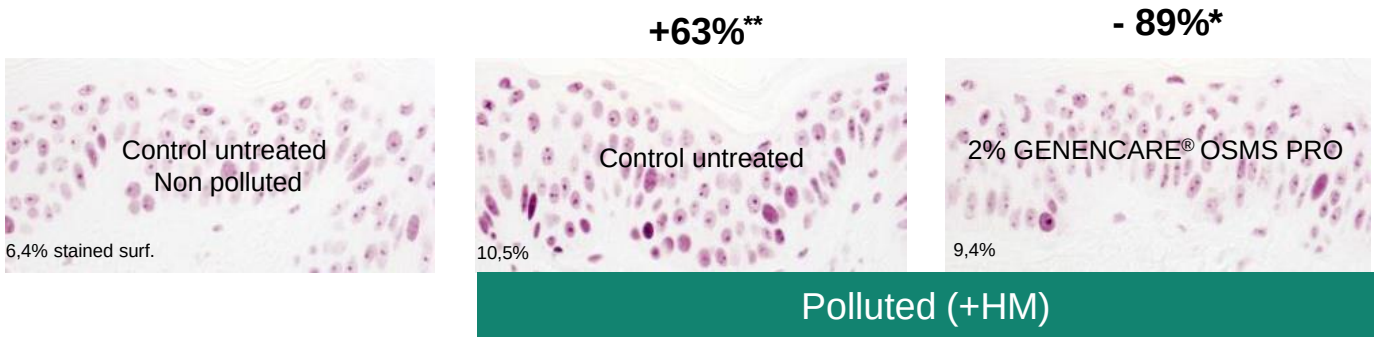
** Significant for p<0.01 (99%) versus control

GENENCARE® OSMS PRO HELPS TO REDUCES NRF2 INDUCTION BY POLLUTION

Nrf2 is a transcription factor, the first cell answer to an oxidative stress, involved in skin detoxification mechanisms.

Nrf2 induction by pollutant exposure

Representative images of Nrf2 immunostaining of skin explant on day 5



Test condition	CONTROL Untreated Non polluted -HM	CONTROL Untreated Polluted +HM	GENENCARE® OSMS PRO 1% +HM	GENENCARE® OSMS PRO 2% +HM
% stained surface	6,4%	10,5%	8,5%*	9,4%*
Δ HM (% stain surf.)	-	4,1%**	1%*	0,4%*

- The exposure of skin explants to heavy metals (pollution mixture) has a significant effect on Nrf2 induction → + 63%**
- 2 % GENENCARE® OSMS PRO significantly limits the pollution-induced Nrf2 increase by 89%*.
- After pollutant exposure, GENENCARE® OSMS PRO almost totally inhibits the effect of the pollutants on Nrf2 induction.

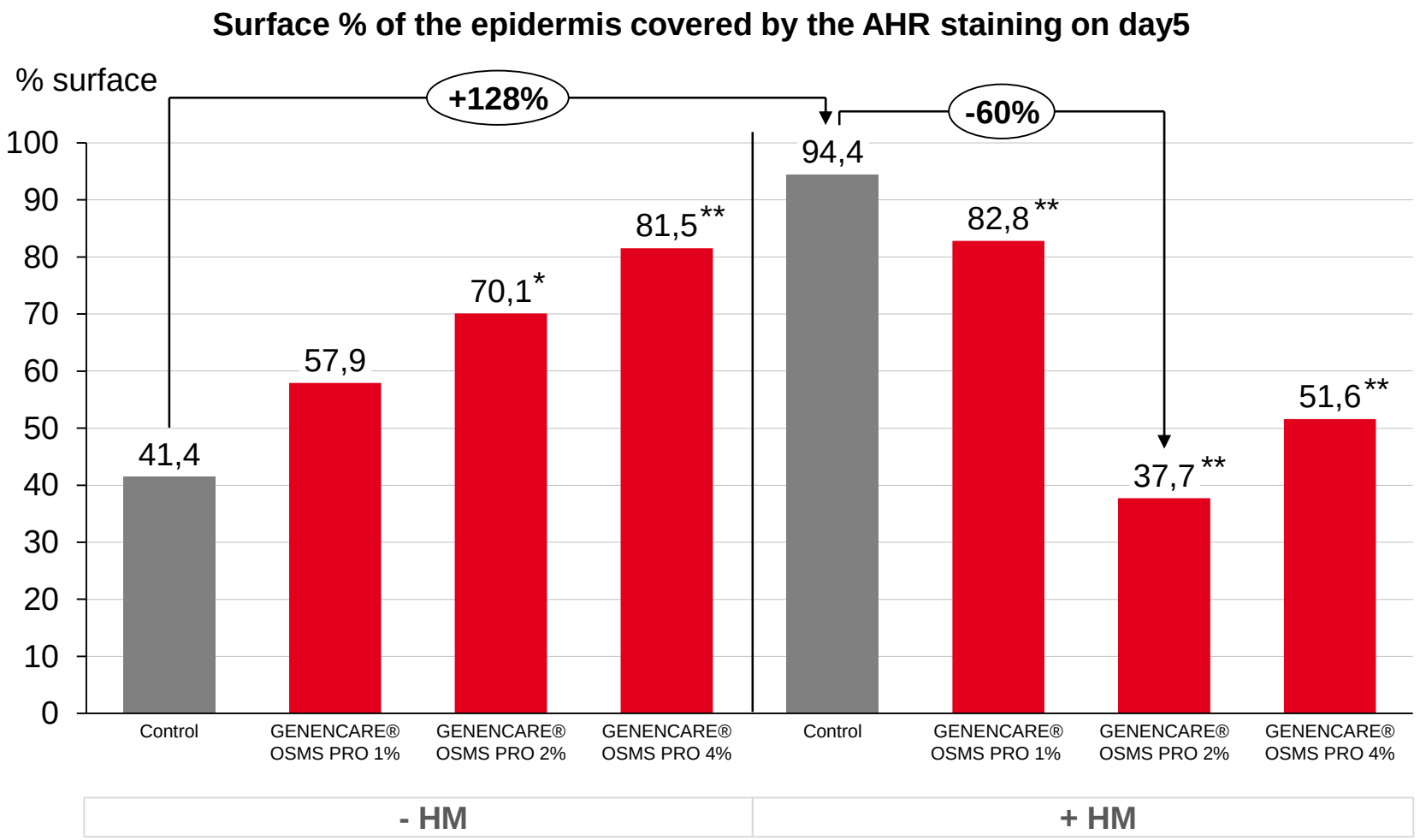
+ HM :Heavy Metal exposure
HM : no heavy metal exposure
HM: delta (increase) of Nrf2 induced by HM for each explant compared to the average of the batch without HM.(+HM vs –HM)

Pollubox® is a registered trademark of BIOEC

* Significant for p<0.05 (95%) versus control DHM

** Significant for p<0.01 (99%) versus non polluted control

AHR induction with/without pollution exposure



+ HM :Heavy Metal exposure

- HM : no Heavy Metal exposure

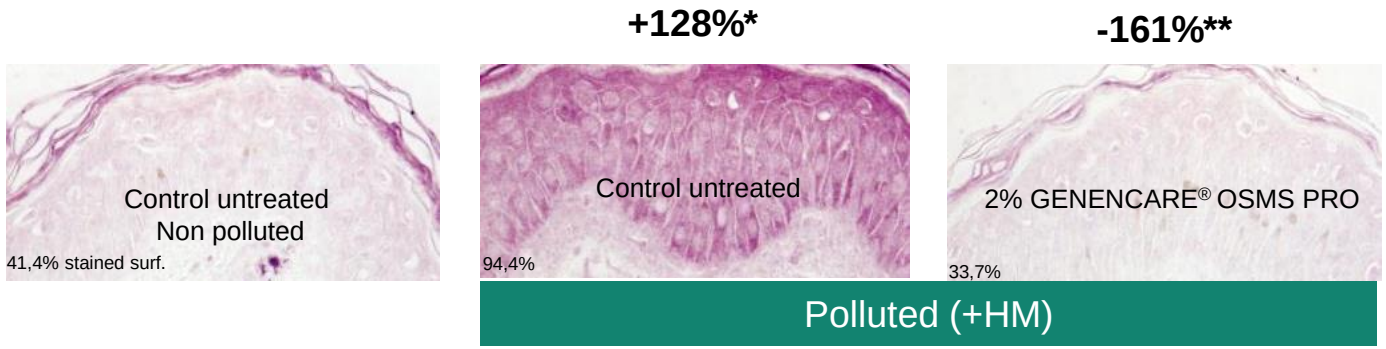
* Significant for p<0.05 (95%) versus control

** Significant for p<0.01 (99%) versus control

GENENCARE® OSMS PRO helps to reduce AhR induction by pollution

AhR is a transcription factor involved in activation of cytochrome family genes and detoxification enzymes

AhR induction by pollutant exposure
Representative images of AhR immunostaining of skin explant on day 5



Test condition	CONTROL Untreated Non polluted -HM	CONTROL Untreated Polluted +HM	GENENCARE® OSMS PRO 1% +HM	GENENCARE® OSMS PRO 2% +HM
% stained surface	41,4%	94,4%	82,8%*	33,7%*
Δ HM (% stain surf.)	-	53%	-24,9%**	-32,4%**

- The exposure of skin explants to heavy metals (pollution mixture) has a significant effect on AhR induction + 128%**
- 2 % GENENCARE® OSMS PRO significantly limits the pollution-induced AhR induction by 161%**.
- After pollutant exposure, GENENCARE® OSMS PRO almost totally inhibits the effect of the pollutants on ArR induction with a dose dependent effect .

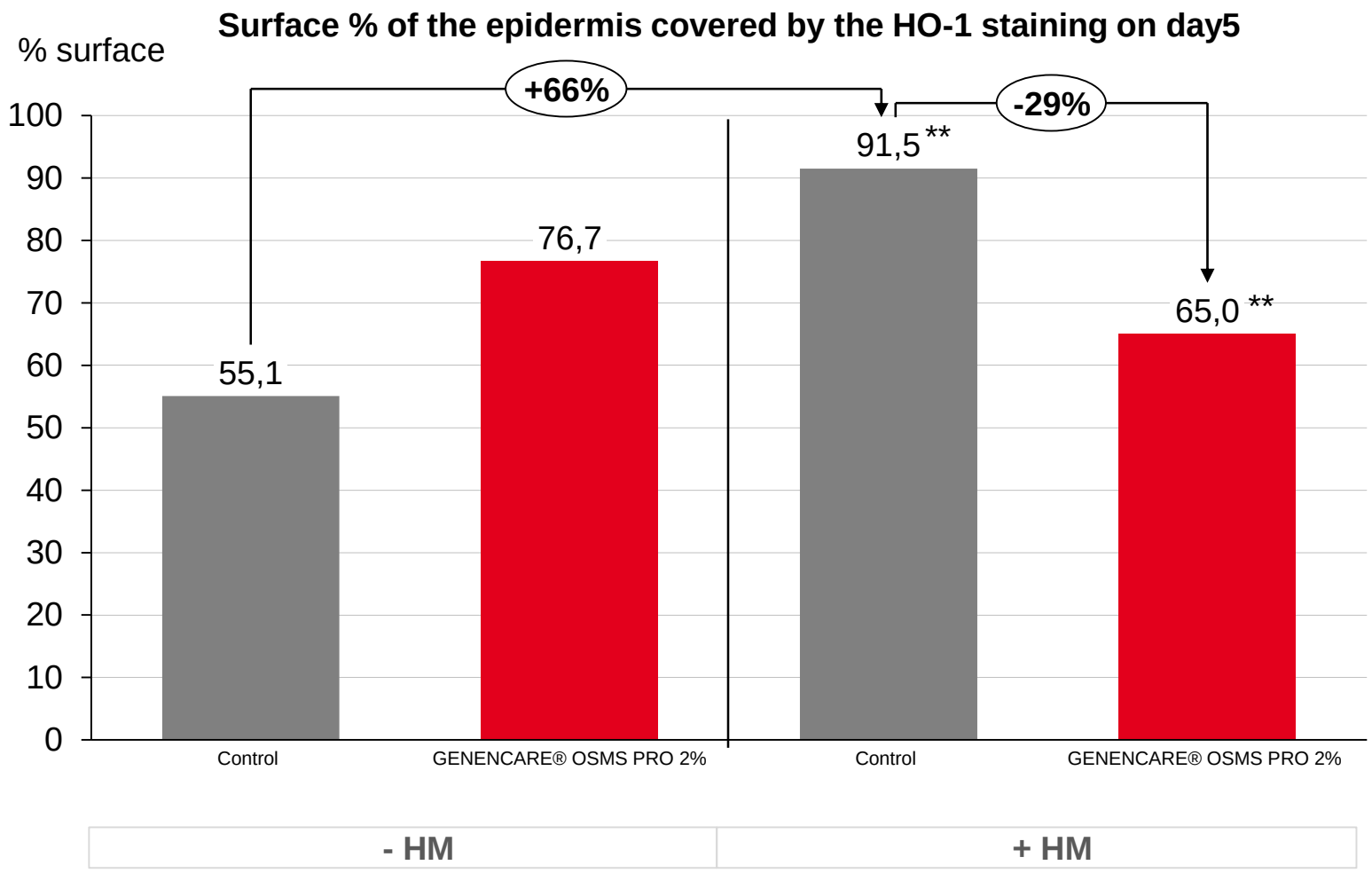
+ HM :Heavy Metal exposure
- HM : no heavy metal exposure
Δ HM: delta (increase) of AhR induced by HM for each explant compared to the average of the batch without HM.

Pollubox® is a registered trademark of BIOEC

* Significant for p<0.01 (99%) versus unpolluted control

** Significant for p<0.01 (99%) versus control Δ HM

HO-1 induction with/without pollution exposure



+ HM :Heavy Metal exposure
- HM : no Heavy Metal exposure

** Significant for p<0.01 (99%) versus control

GENENCARE® OSMS PRO helps to reduce HO-1 induction by pollution

HO-1 expression is considered as a specific molecular indicator of cellular oxidative stress

Representative images of HO-1 immunostaining of skin explant on day 5

Test condition	CONTROL Untreated Non polluted -HM	CONTROL Untreated Polluted +HM	GENENCARE® OSMS PRO 2% Polluted +HM
% stained surface	55,1%	91,5%*	65,0%*
Δ HM (% stain surf.)	-	66%	-11,7%**

- The exposure of skin explants to heavy metals (pollution mixture) has a significant effect on HO-1 induction + 66%*.
- 2 % GENENCARE® OSMS PRO significantly limits the pollution-induced HO-1 induction by 132%**.
- After pollutant exposure, GENENCARE® OSMS PRO contributes to totally inhibit the effect of the pollutants on HO-1 induction.

+ HM :Heavy Metal exposure
- HM : no heavy metal exposure
Δ HM: delta (increase) of HO-1 induced by HM for each explant compared to the average of the batch without HM.

Pollubox® is a registered trademark of BIOEC

*Significant for p<0.01 (99%) versus unpolluted control

* *Significant for p<0.01 (99%) versus control D HM

#makersofnew

DUPONT™