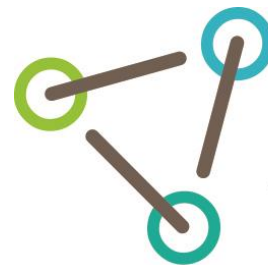




# Aqua-Osmoline™

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targets water control for improved hydration and skin glow.



**Ashland™**  
always solving

# outline

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designed to improve **skin glow** and **hydration** by targeting **water channeling**, and to help preserve **morphology** & **cohesion** during stress



# aqua-osmoline™ biofunctional

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- origin, positioning, benefits
- summary of test results
- mode of action

# overview

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## origin

- purified *Ceratonia siliqua* (carob) seed extract rich in amino acids and peptides

## positioning

- designed to improve skin glow and hydration by targeting water channeling and by helping preserve morphology & cohesion during stress

## benefits

- boosts hyaluronic acid expression linked with water filling and regeneration effects
- targets water channeling and water sealing
- may help to contribute to preserve cell shape and cohesion during stress
- may help boost expression of proteins linked with epidermal differentiation and *stratum corneum (SC)* plasticity
- enhances skin hydration and perception of skin glow

in addition, aqua-osmoline™ biofunctional boosts skin's natural adaptability to water challenging stress conditions (cold temperatures, low humidity, air conditioning, peelings, dermabrasions, etc.)

# summary of benefits and test results

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## water filling and regeneration

- increases hyaluronic acid expression in human skin (*ex vivo*)
- increases hyaluronic acid CD44 receptor expression in human skin (*ex vivo*)

## water channeling and water sealing

- increases aquaporin-3 to facilitate water exchanges between the dermis and the epidermis (*in vitro*, *ex vivo*)
- increases claudin-1 in tight junctions to reduce paracellular permeability (*ex vivo*)

## morphology and cohesion

- may help preserve cell shape and cohesion (*in vitro*, *in vivo*: *vivascope\** evaluation)
- helps improve skin regeneration (*in vitro* experimental skin repair model)

## epidermal differentiation and *stratum corneum* (SC) plasticity

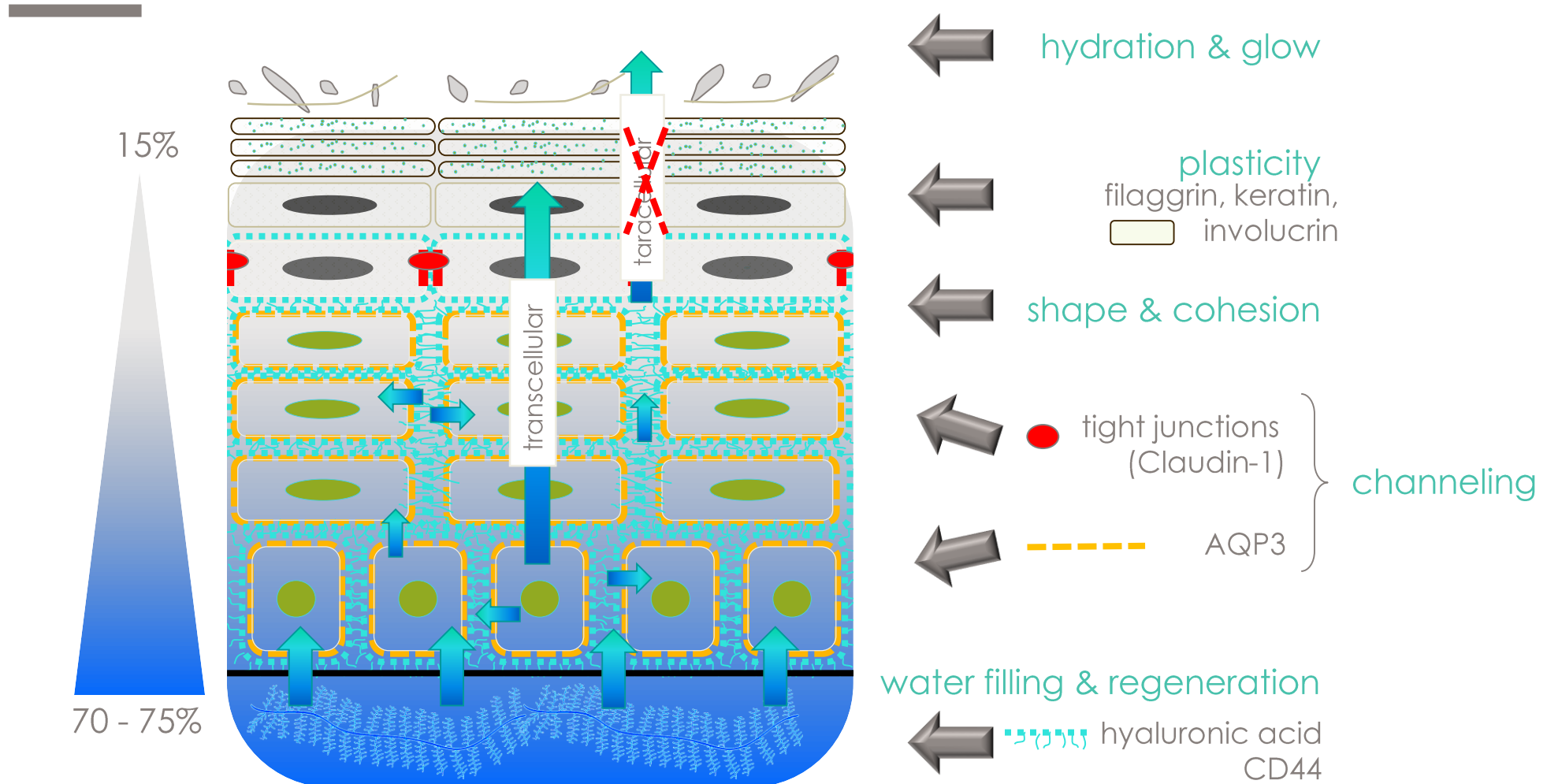
- helps increase natural moisturizing factor precursor (filaggrin) and PAD1 (*ex vivo*)
- helps increase epidermal differentiation markers cytokeratin 10 and involucrin (*ex vivo*)

## skin hydration and skin glow

- boosts skin hydration (clinical study, corneometer\*)
- increases the perception of skin glow (clinical study, expert scoring)

\*trademark owned by a third party

# targets all major water-related proteins



# aqua-osmoline™ biofunctional

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- going one step further in skin hydration
- hyaluronic acid: water filling and regeneration
- AQP3 water channels
- claudin-1, a component of tight junctions
- osmotic stress

# going one step further in skin hydration

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the skin is a living tissue that depends on **water** for optimum function and beauty

in a water-challenging environment, the skin is less able to naturally and effectively prevent and correct the premature **signs of aging**

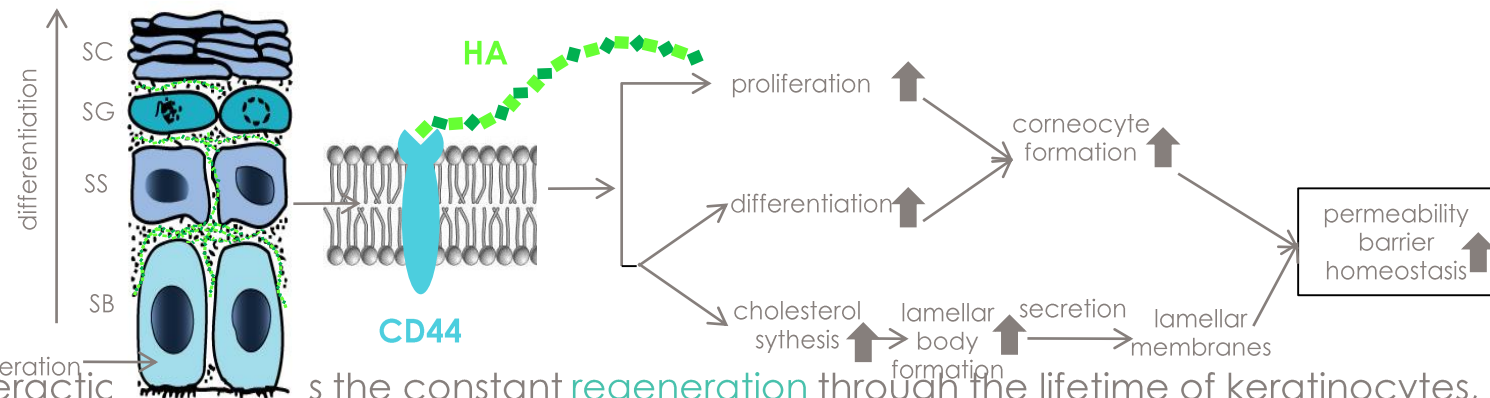
the hydration status of the skin is dependent upon the rates of **water movements into and out** of this tissue, as well as the ability of skin to **retain** and **channel** water through water-related proteins

aqua-osmoline™ biofunctional is going one step further in skin hydration by targeting **several key water-related molecules** (hyaluronic acid, aquaporin-3, claudin-1, filaggrin), to help sustain skin's moisture and glow over time, and to resist the appearance of premature aging



# hyaluronic acid: water filling and regeneration effects

- hyaluronic acid (HA) or hyaluronan is a glycosaminoglycan found as a part of the skin extracellular matrix of the dermis, where it has **water binding** and **electrolyte control** properties.
- HA is also actively metabolized by **keratinocytes** (KC) and secreted in the extracellular space where it facilitates the **diffusion of water** and nutrients.
- HA is not only a space filler: anchored to a **receptor CD44**, HA is involved in KC **proliferation** and **differentiation**: in the suprabasal layers, HA activates **corneocyte** and **lamellar body** formation<sup>[1]</sup>.

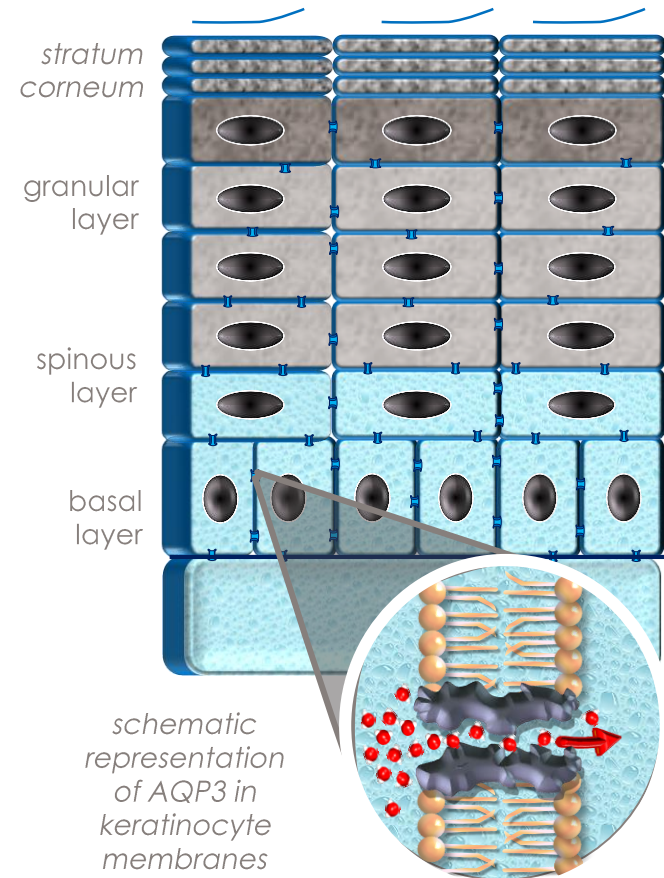


- HA-CD44 interaction is the constant **regeneration** through the lifetime of keratinocytes, emerging from division of a columnar basal cell and ending as a flattened corneocyte.
- HA is a critical **water-related molecule** involved in the formation of a waterproof skin barrier.

[1] Hyaluronan-CD44 interaction stimulates keratinocyte differentiation, lamellar body formation/secretion, and permeability barrier homeostasis. Bourguignon et al., *J Invest Dermatol.* 2006 126(6):1356-65

# aquaporin-3 water channels

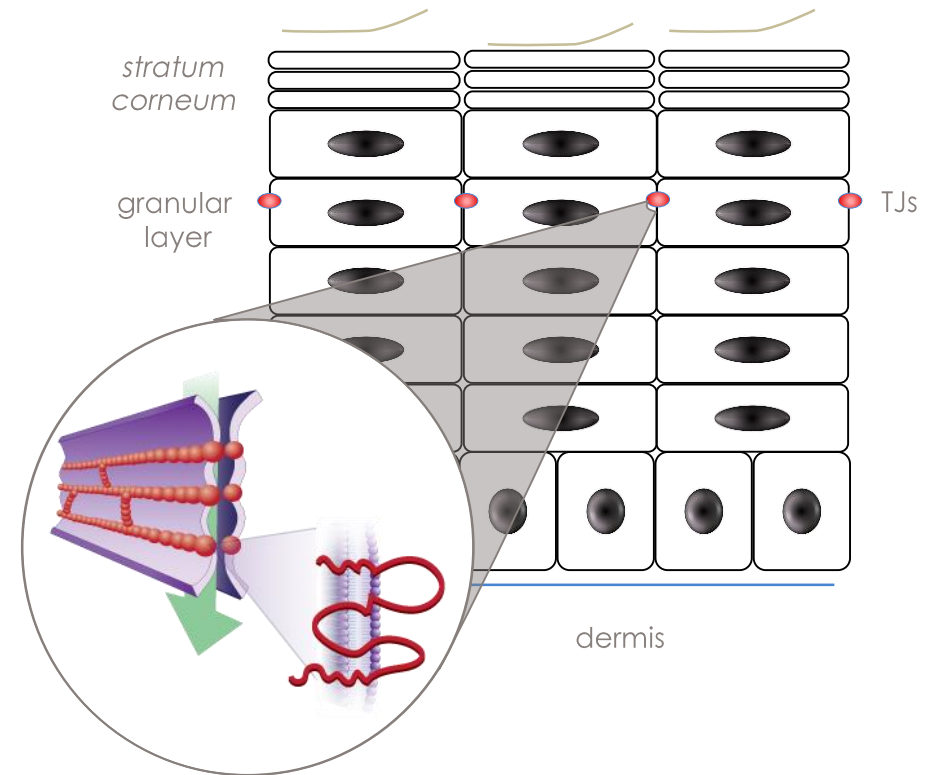
- discovered in 1988 by Peter Agre, Nobel prize in chemistry in 2003, aquaporins are **water channels** that play a crucial role in transporting water.
- aquaporin-3 (AQP3) is the most abundant skin aquaglyceroporin. both **water** and **glycerol** transport by AQP3 appear to play an important role in **hydration** of skin epidermis<sup>[2]</sup>.
- AQP3 is also believed to be important in **skin repair**, as a water channel by facilitating cell migration, and as a glycerol transporter by enhancing keratinocyte proliferation and differentiation<sup>[2]</sup>.
- AQP3 deficiency is related to a loss of epidermal **firmness**, a decreased skin **barrier function** and **moisture content** in the *stratum corneum* leading to a **very dry skin**<sup>[2]</sup>.



[2] Skin aquaporins: function in hydration, wound healing, and skin epidermis homeostasis. Boury-Jamot et al., *Handbuch Exp Pharmacol.* 2009;(190):205-17.

# claudin-1: a component of tight junctions

- claudin-1 is an integral membrane protein and a component of **tight junctions** (TJs)
- TJs are located in the granular layer
- TJs constitute continuous, **circumferential seals** around cells
- they serve as a **primary barrier** preventing solutes and water from passing freely through the **paracellular** pathway
- claudin-1 deficiency is reported to cause **wrinkling** and **dry skin** suggesting a role in age-related skin dryness<sup>[3]</sup>



[3] Claudin-based tight junctions are crucial for the mammalian epidermal barrier: a lesson from claudin-1-deficient mice. Furuse et al, *J Cell Biol.* 2002 18;156(6):1099-111.

# osmotic stress

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- intracellular environments are **osmotically controlled** to preserve cell shape and function. this control is particularly important in the epidermis exposed to the dryness of the environment.
- retention of water by the *stratum corneum* is attributed to the generation of **hygroscopically** active products of **filaggrin** proteolysis<sup>[4]</sup>
- the keratinocytes possess an **osmolyte strategy**, which is important to preserve cell **volume homeostasis** and optimal functioning during osmotic stress<sup>[5]</sup>
- the **osmolyte strategy** of keratinocytes seems to be part of skin response to other stress, such as **UV**, and could be implicated in fighting **oxidative stress** in the skin<sup>[5]</sup>
- aqua-osmoline™ biofunctional boosts skin's natural response to osmotic stress conditions, to help preserve **morphology**, **cohesion**, and **moisture** content

[4] Is endogenous glycerol a determinant of *stratum corneum* hydration in humans? Choi *et al.*, *J Invest Dermatol.* 2005 125(2):288-93.

[5] The osmolyte strategy of normal human keratinocytes in maintaining cell homeostasis. Warskulat *et al.*, *J Invest Dermatol.* 2004 123(3):516-21 *J Cell Biol.* 2002 Mar 18;156(6):1099-111.

# aqua-osmoline™ biofunctional

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- water filling & regeneration effects
- water channeling & water sealing
- morphology & cohesion
- epidermal differentiation & SC plasticity
- skin hydration & skin glow

# water filling and regeneration

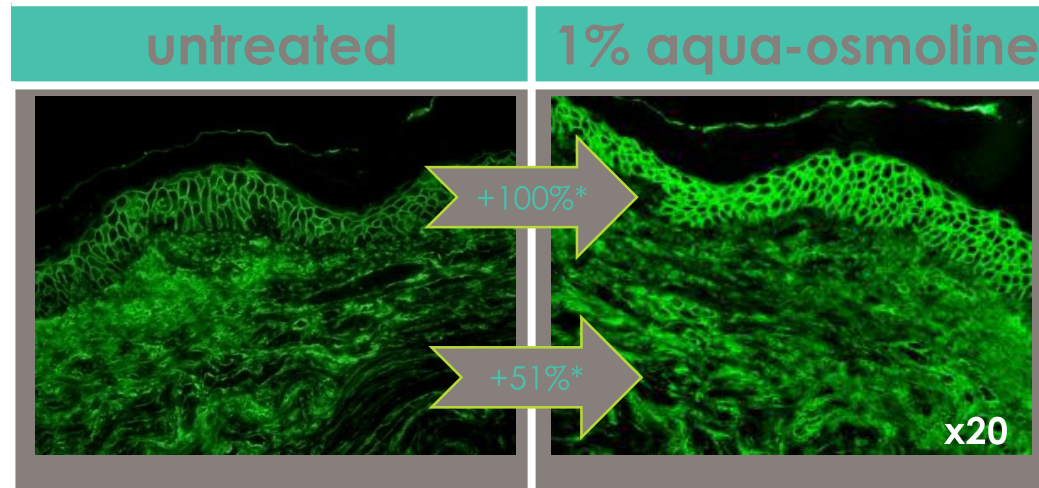
## evaluation of hyaluronic acid level in human skin (*ex vivo*)

**culture:** *ex vivo* human skin biopsies

**product application:** aqua-osmoline™ biofunctional 1%, once per day

**application time:** 48 hours

**evaluation:** hyaluronic acid staining using a specific marker HABP (hyaluronic acid binding protein), quantification by image analysis software



\*: significant with Student's *t* test (n=3)

results show that aqua-osmoline biofunctional was associated with an observed increase in hyaluronic acid expression in human skin, suggesting an improved water filling capacity.

# water filling and regeneration

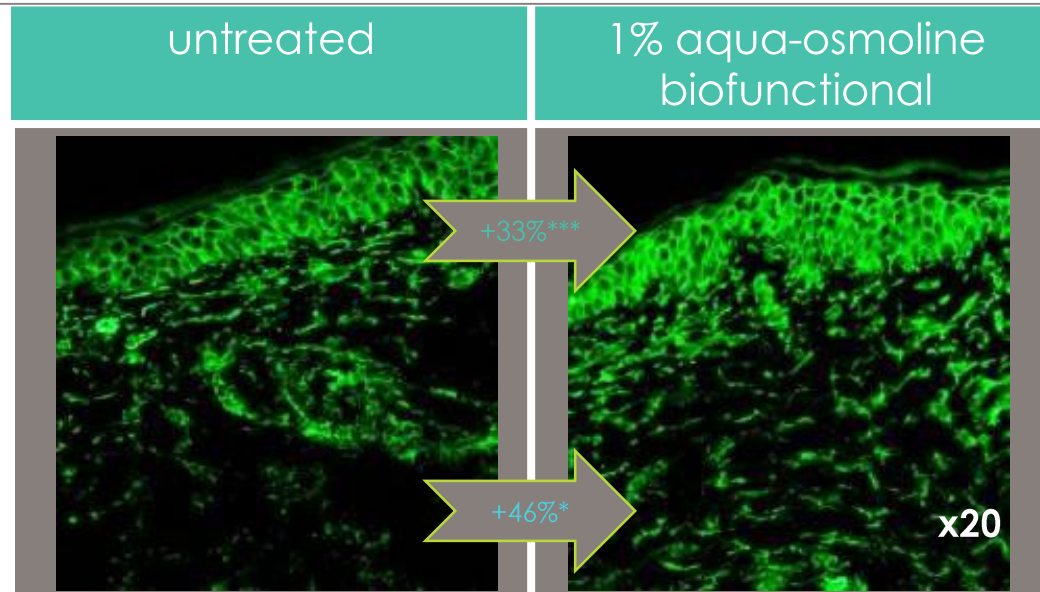
## evaluation of hyaluronic acid binding receptor level in human skin (*ex vivo*)

**culture:** *ex vivo* human skin biopsies

**product application:** aqua-osmoline™ biofunctional 1%, once per day

**application time:** 48 hours

**evaluation:** hyaluronic acid receptor CD44 immunofluorescent staining, quantification by image analysis software



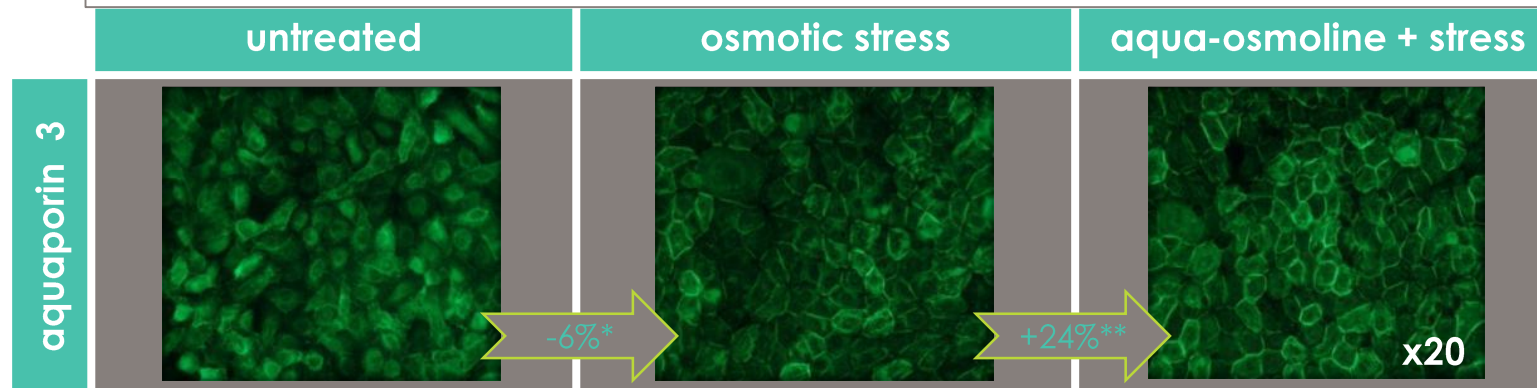
\*\*\*: highly significant, \*: significant with Student's *t* test (n=3)

results show an increased hyaluronic acid receptor CD44 expression after aqua-osmoline biofunctional application on the skin.

# water channeling and water sealing

aquaporin-3 (AQP3) expression on cell membranes after osmotic stress (*in vitro*)

culture: normal human keratinocytes  
product application: aqua-osmoline™ biofunctional 1%  
application time: 24 hours  
osmotic stress: after treatment, sorbitol 200 mM for 2 hours  
evaluation: aquaporin-3 immunofluorescent staining and quantification by image analysis software



\*\* : very significant,  
\* : significant with Student's *t* test (n=3)

when keratinocytes are exposed to an osmotic stress, AQP3 is expressed at their plasma membrane. Aqua-osmoline biofunctional was associated with an observed increase of AQP3 localization at the plasma membrane, and an observed increase of the viability of keratinocytes (MTT test, results not shown) during hyperosmotic stress conditions.



# water channeling and water sealing

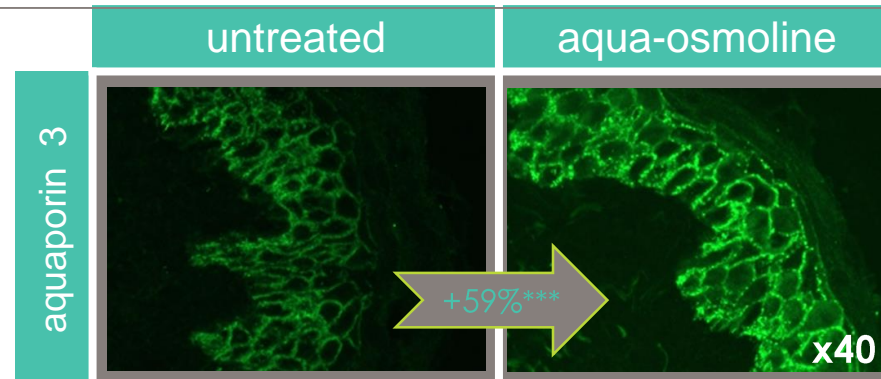
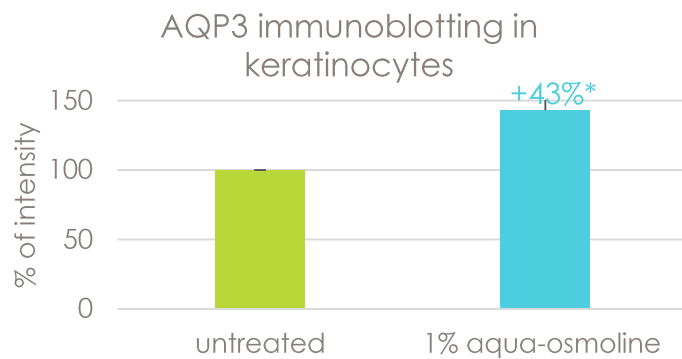
aquaporin-3 (AQP3) expression in keratinocytes (*in vitro*) and in human skin (*ex vivo*)

**culture:** normal human keratinocytes and *ex vivo* skin biopsies

**product application:** aqua-osmoline™ biofunctional 1%

**application time:** 24 hours on keratinocytes and 48 hours on *ex vivo* skin

**evaluation:** immunoblotting of AQP3 on keratinocytes and immunofluorescent staining of AQP3 on skin biopsies and quantification by image analysis software



\*\*\*: highly significant, \*: significant with Student's *t* test (n=3)

results show increased AQP3 level after aqua-osmoline biofunctional application on keratinocytes and human skin. in water-challenging conditions, other test results (not shown) suggest increased AQP3 expression in *ex vivo* skin after cold stress, mechanical stress (stripping) and UV stress.

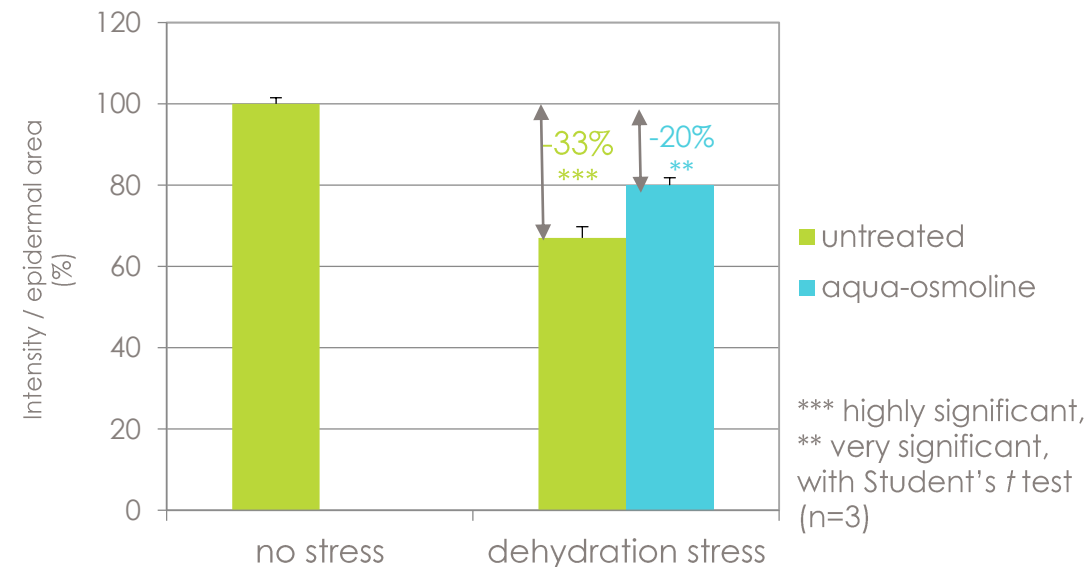
# water channeling and water sealing

evaluation of **claudin-1** (component of tight junctions) in human skin (*ex vivo*)

**culture:** *ex vivo* human skin biopsies  
**product application:** aqua-osmoline™ biofunctional 1%  
**stress:** desiccation with dry air flow for 2 hours  
**application time:** 24h → 2h dry air → 24h treatment  
**evaluation:** immunofluorescent staining of claudin-1 and quantification by image analysis software

other test results (not shown), suggest increased claudin-1 expression after cold stress (4°C) and after UV stress.

claudin-1 expression after dehydration stress



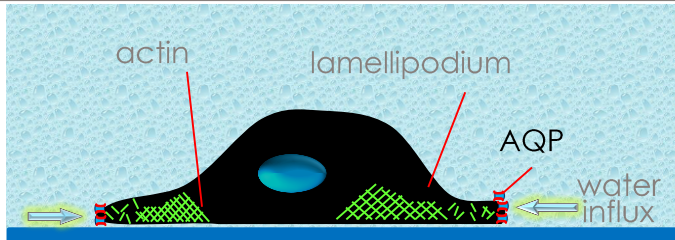
results suggest that aqua-osmoline biofunctional helps limit the decrease in claudin-1 induced by dehydration stress.

# morphology and cohesion

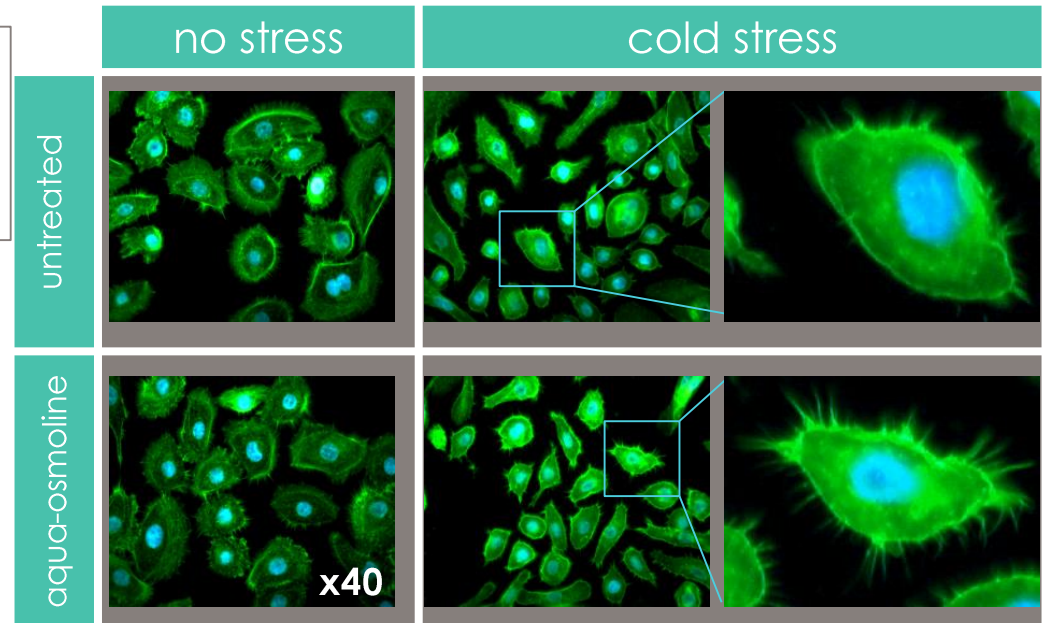
evaluation of **actin** expression after cold stress (*in vitro*)

as water content declines, i.e., during winter, the skin becomes prone to develop cracks and fissures, triggering a **re-epithelialization** process. actin and AQP3 are known to play a key role in cell movement.

**culture:** normal human keratinocytes  
**product application:** aqua-osmoline™ biofunctional 1%  
**stress:** cold stress 4 hours at 4°C  
**application time:** 24 hours  
**evaluation:** actin immunofluorescent staining



changes in cell shape involve actin cytoskeleton constant remodeling and water flows through aquaporins.

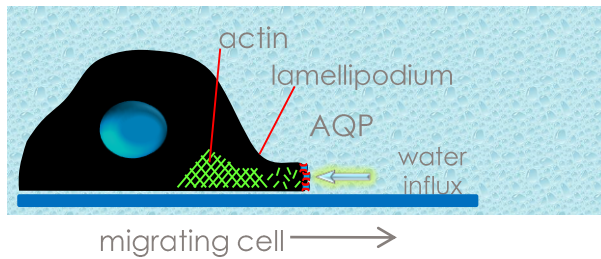


aqua-osmoline biofunctional application on keratinocytes may help preserve **cell shape** during stress conditions.

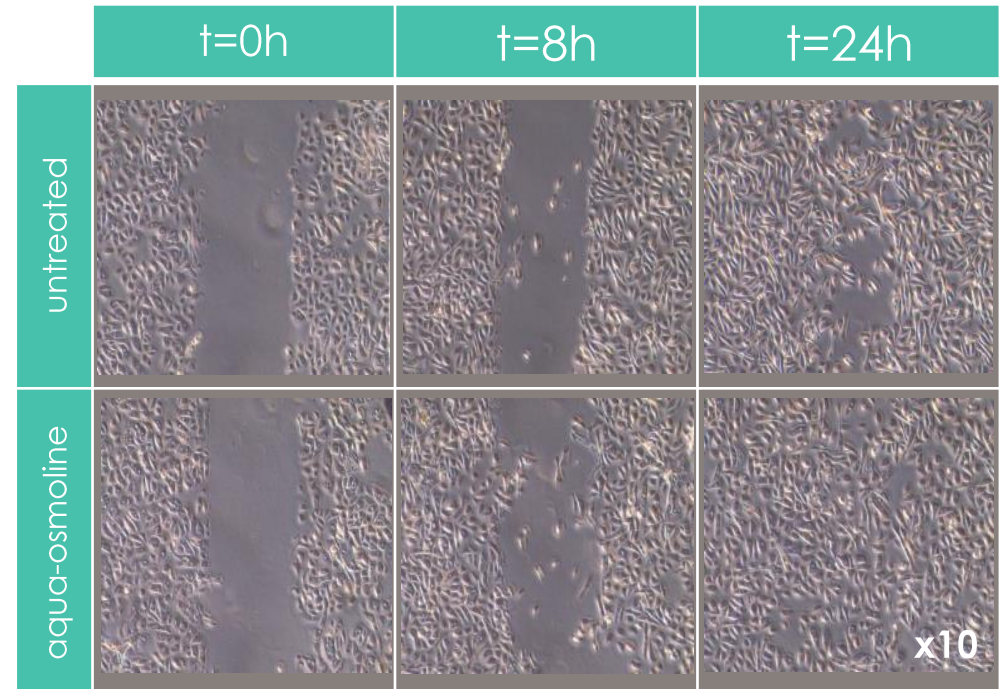
# morphology and cohesion

helps improve skin **regeneration** (*in vitro* experimental skin repair model)

**culture:** normal human keratinocytes  
**product application:** aqua-osmoline™ biofunctional 1%  
**application time:** 8h and 24h  
**evaluation:** culture insert (IBIDI) and contrast phase microscopy



*all cell movement is accompanied by changes in cell shape and water flows. the role of AQP3 in cell migration is attributed to its water transport properties.*



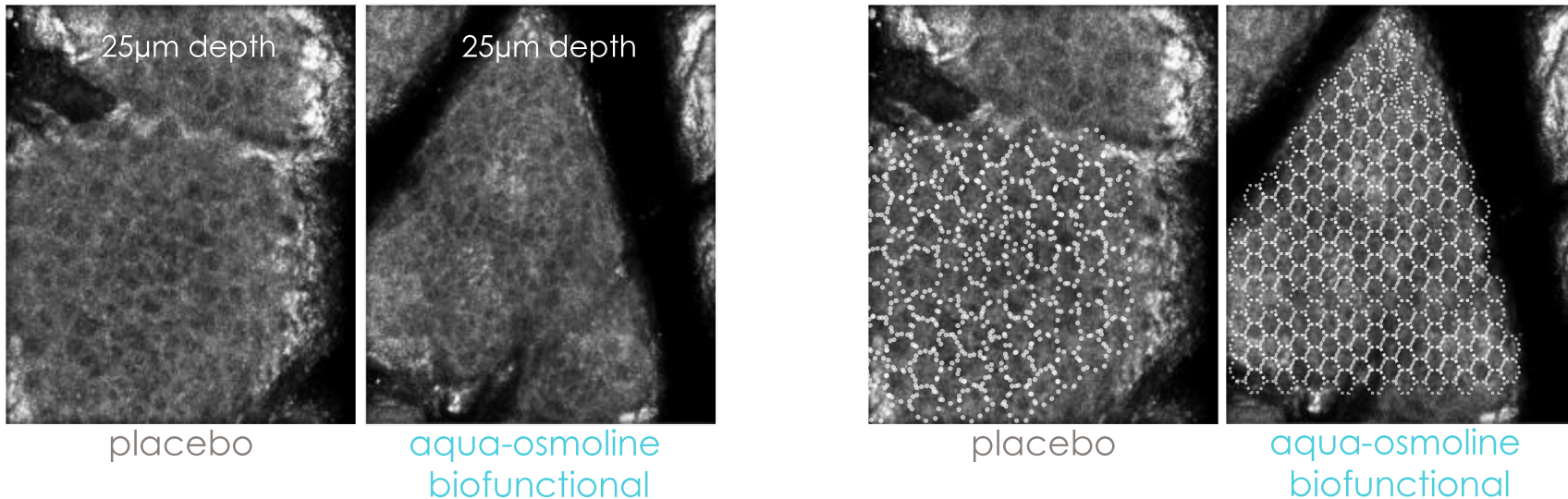
results show that aqua-osmoline biofunctional helps facilitate the movement of cells during *in vitro* skin regeneration.

# morphology and cohesion

vivascope\* evaluation of cell shape and cohesion (clinical study, protocol p. 25)

skin dryness is characterized by decreased cohesion between granular cells. claudin-1, a component of tight junctions, plays an essential role in preserving cell cohesion in the granular layer.

evaluation of cell shape and cohesion in the granular layer after 7 days of application



in the skin treated with placebo, granular cells appear with irregular cell borders, whereas in the skin treated with 1% aqua-osmoline™ biofunctional, the granular cells are arranged in a more cohesive pattern and are smaller in size for all volunteers, at D7 and D21

\*trademark owned by a third party

# morphology and cohesion

vivascope\* evaluation of cell shape and cohesion

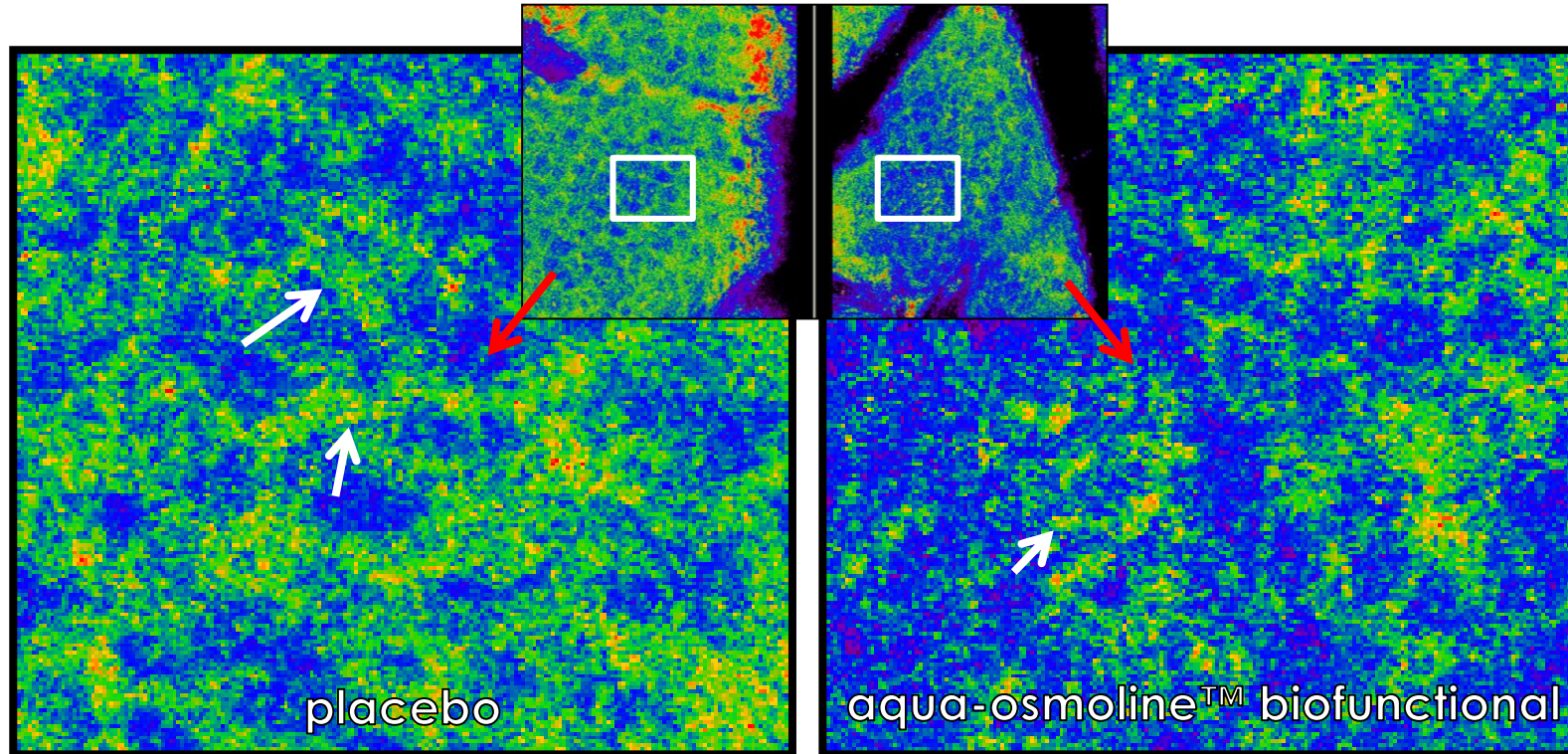


image transformation with Huygens – essentials software.  
in green: space between granular cells

\*trademark owned by a third party

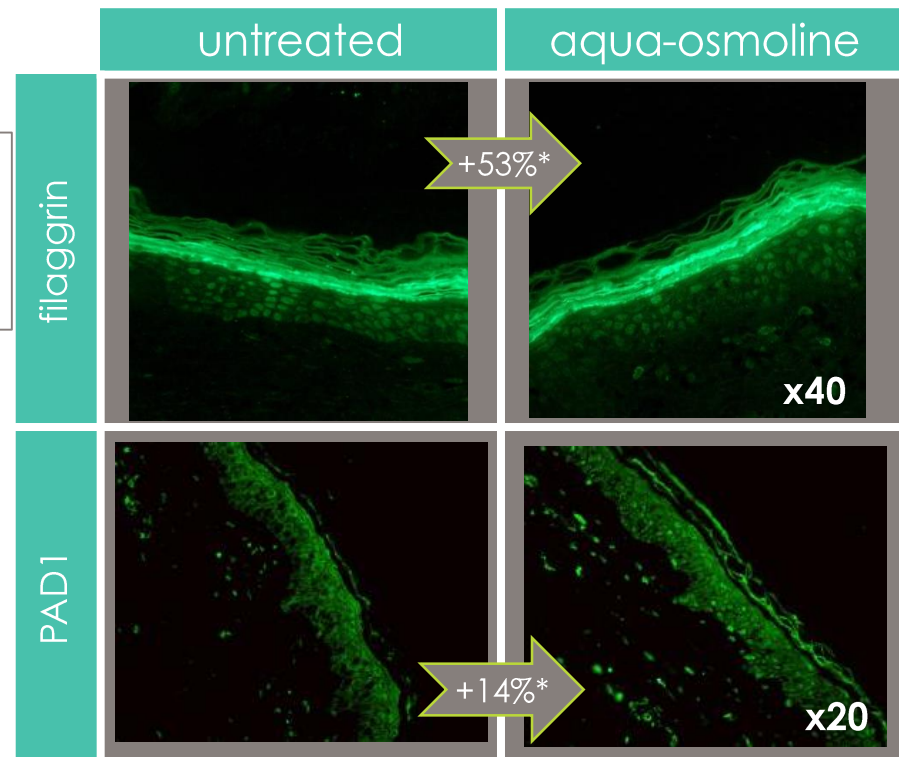
# epidermal differentiation and SC plasticity

evaluation of the natural moisturizing factor (NMF) precursor filaggrin and PAD1 expression in human skin, *ex vivo*

**culture:** *ex vivo* human skin biopsies  
**product application:** aqua-osmoline™ biofunctional 1%  
**application time:** 24h (filaggrin) 48h (PAD1)  
**evaluation:** immunofluorescence of filaggrin and PAD1;  
quantification by image analysis software

**filaggrins** are filament-associated proteins which bind to keratin fibers.

**PAD** (peptidylarginine deiminase) are enzymes involved in filaggrin processing. filaggrin is necessary for the generation of **hygroscopic amino acids** in the NMF.



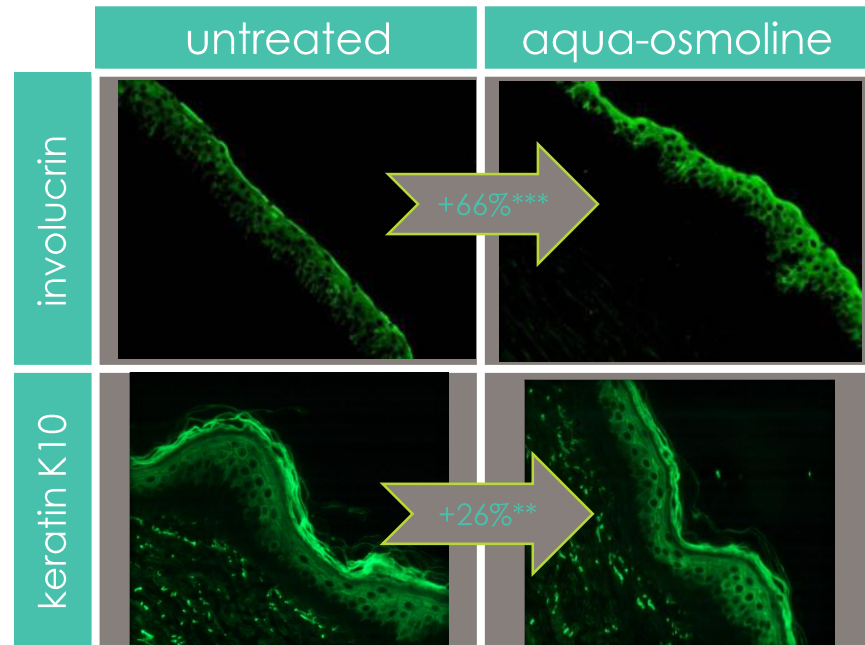
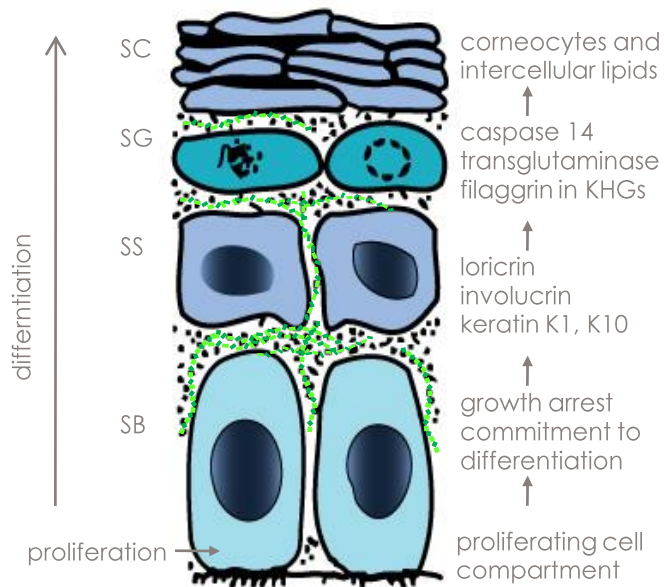
\*: significant with Student's *t* test (n=3)

observed results suggest that aqua-osmoline biofunctional increases filaggrin and PAD1 expression in skin.

# epidermal differentiation and SC plasticity

evaluation of keratin 10 and involucrin expression in human skin ex vivo

**culture:** ex vivo skin biopsies    **product application:** aqua-osmoline™ biofunctional 1%  
**application time:** keratin 10: 24h, involucrin: 48h  
**evaluation:** K10 (keratin 10) and involucrin immunofluorescent staining; quantification by image analysis software



\*\*\*: highly significant, \*\*: very significant with Student's *t* test (n=3)

results show that aqua-osmoline biofunctional was associated with an observed increase in keratin 10 and involucrin levels in human skin model



# skin hydration and skin glow

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double blind clinical study against placebo

**objective:** evaluate the benefits of aqua-osmoline™ biofunctional on skin hydration and perception of skin glow

**number of volunteers:** 10 volunteers with normal skin (age between 20 and 49)

**duration:** 3 weeks, control visits at D0, D7 and D21

**tested material:** cosmetic formulation containing aqua-osmoline biofunctional at 1%

**product application:** twice a day on the forearm, morning and evening at 2 mg/cm<sup>2</sup>

**evaluation:**

- measurements of skin hydration (capacitance, using a corneometer\* CM825)
- evaluation of cell size and cohesion using vivascope\* 1500
- perception of skin glow (expert scoring)

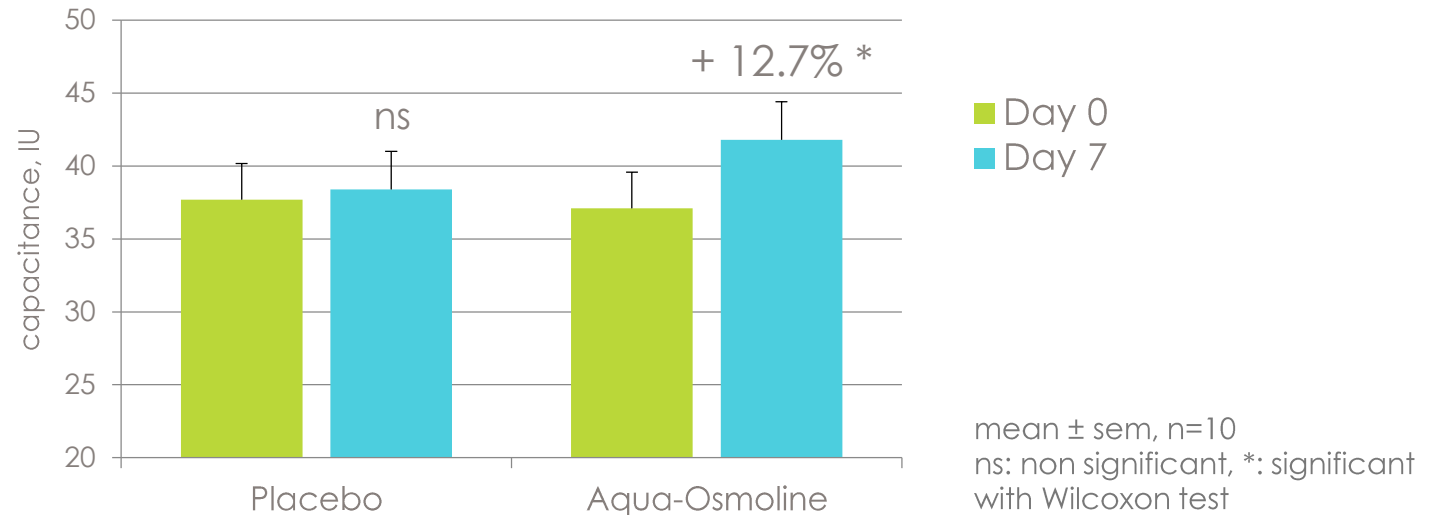
**statistical analysis:** non-parametric Wilcoxon matched pairs test

\*trademark owned by a third party

# skin hydration and skin glow

evaluation of skin hydration (*in vivo*)

skin hydration was measured as changes in electrical capacitance with a corneometer\*



Results show an increase in skin moisture content after aqua-osmoline™ biofunctional cream application.  
This increase is observed in 70% of the volunteers

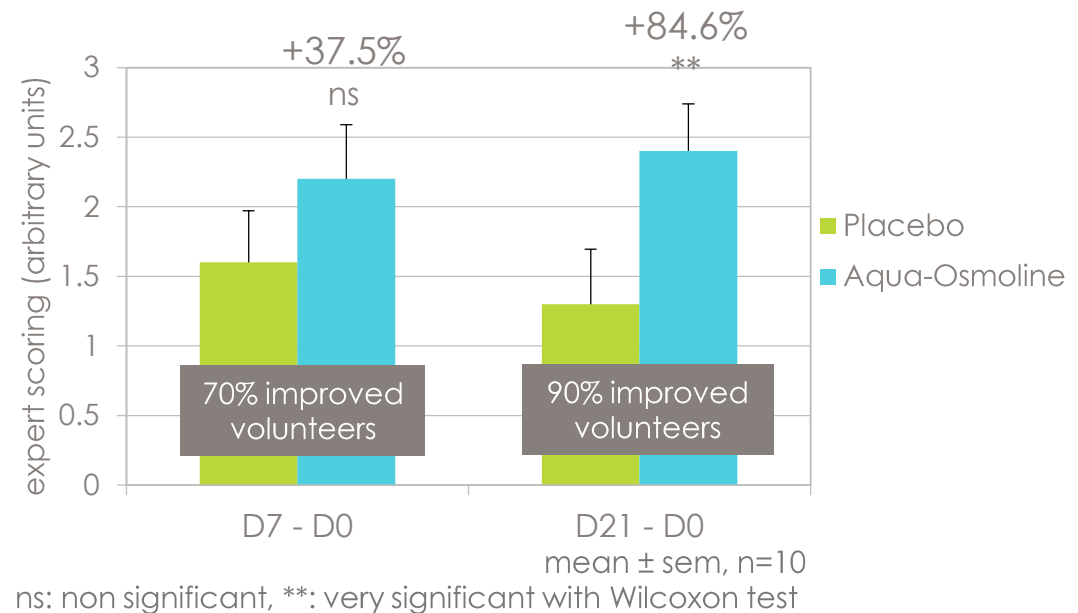
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# skin hydration and skin glow

evaluation of the perception of skin glow (expert scoring)

at D7 and at the end of the study, an observation based on the perception of skin glow was performed on each volunteer by a trained expert

the evaluation ranged from 0 to 10  
0 → very dull and dry skin  
10 → very radiant and hydrated skin



expert scoring revealed a significant increase in the perception of skin glow after 3 weeks application of a cream containing 1% aqua-osmoline™ biofunctional, compared to the placebo cream application.

# aqua-osmoline™ biofunctional



○ applications

# cosmetic applications

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moisturizing, anti-dehydrating skin care products

skin care products for dry and sensitive skin

skin care products for aging skin

skin care products to help skin regeneration

- *after peeling, after dermabrasion creams*
- *aftershave, post-depilation creams*

skin care products to help preserve against water-challenging conditions

- *winter creams*
- *protective skin care (against external environmental factors)*

recommended usage level: 1% (clinically tested at 1%)

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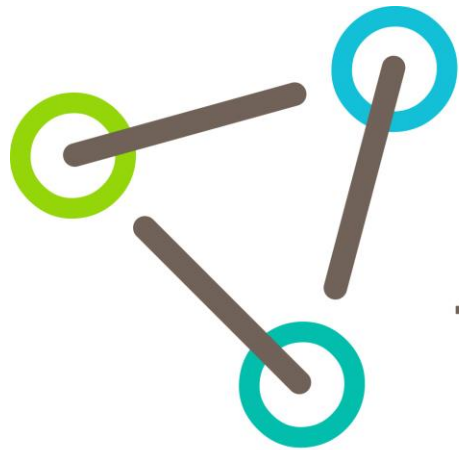
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