

Hydro-Gain™ boosts barrier and skin hydration genes

Objective:

- To show that Hydro-Gain enhances the expression of genes that favor a better moisturization of the skin.

Technique:

- Gene expression analysis by qRT-PCR assay: After treatment of reconstituted human epidermis, total RNA was extracted followed by cDNA synthesis by reverse transcription and eventually gene expression analysis by quantitative PCR.

Study Details:

Design	Cell-culture assay
Test Panel	Reconstituted human epidermis
Test Substance	1% Hydro-Gain
Application Frequency	Incubation for 24 h
Endpoint	96 key genes involved in epidermal biology

Result:

- Hydro-Gain did not down-regulate any genes but activated a dozen of genes playing a pivotal role in epidermal barrier formation and prevention of water loss, e.g. SPRR1A, LOR, CASP14 and KLK7 (Fig. 3).

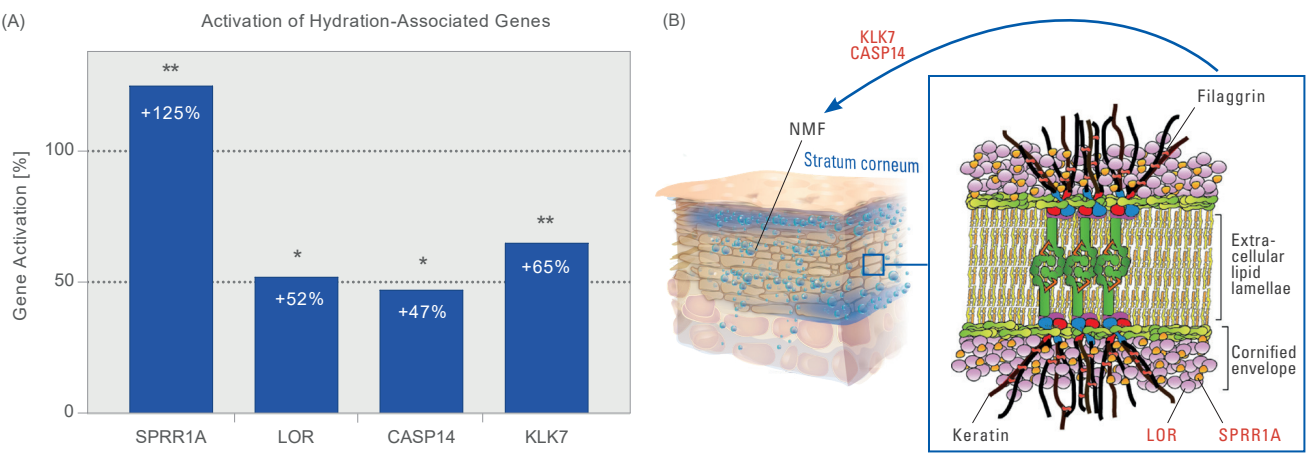


Fig. 3: Hydro-Gain activates key genes for proper skin hydration. (A) Selected genes that were upregulated by Hydro-Gain, and (B) their schematic role in the skin. Small proline rich protein 1A (SPRR1A) and Loricrin (LOR) are formed in terminally differentiating human keratinocytes and are part of the cornified envelope, which forms a physical barrier against water loss and extrinsic hazards ^[20-22]. Caspase-14 (CASP14) and Kallikrin-related peptidase 7 (KLK7) play a key role in the formation of natural moisturizing factors (NMFs), which are responsible for retaining water in the stratum corneum ^[4,23,24]. N = 3 independent cultures; * = p < 0.05; ** p = < 0.01. Figure adapted from ^[25].

Conclusion:

- Hydro-Gain activates genes that are linked to a reinforcement of the stratum corneum and a strengthening of the skin's water-impermeability function. This will eventually help to increase skin hydration levels.

***in vivo* Activity**

Hydro-Gain™, the barrier strengthener

Objective:

- To show that Hydro-Gain strengthens or ‘seals’ the skin barrier. Notably, skin hydration is closely linked to a proper barrier function of the skin.

Technique:

- Confocal Raman spectroscopy was used to measure skin barrier composition and stratum corneum thickness: active ingredients that strengthen the barrier cause an increase of the water content which correlates with water accumulation and hence with the hydration efficiency of a moisturizer ^[23].

Study Details:

Design	Double-blind, placebo-controlled, randomized <i>in vivo</i> study
Test Panel	10 Caucasian female volunteers, 20-65 years old
Test Substances	<ul style="list-style-type: none">• Cream formulation without active ingredient (placebo)• Same formulation with 4% glycerin (benchmark)• Same formulation with 5% Hydro-Gain
Application Site	Forearm
Application Frequency	Twice daily for 7 days
Endpoints	Raman spectroscopy was used to evaluate: <ul style="list-style-type: none">• Stratum corneum composition, a marker for barrier functionality• Stratum corneum thickness, a marker for skin hydration

Results:

- The application of Hydro-Gain for 7 days increased skin lipids and proteins in the upper part of the stratum corneum (Fig. 4A). The application of glycerin, in contrast, did not cause any changes in the skin barrier composition (Fig. 4B). Elevated lipids and proteins will help to seal the water barrier tightly.
- Indeed, after 7 days of treatment, Hydro-Gain increased the stratum corneum thickness by roughly 10% suggesting improved water accumulation and hydration. Treatment with placebo or glycerin was less effective (Fig. 5).

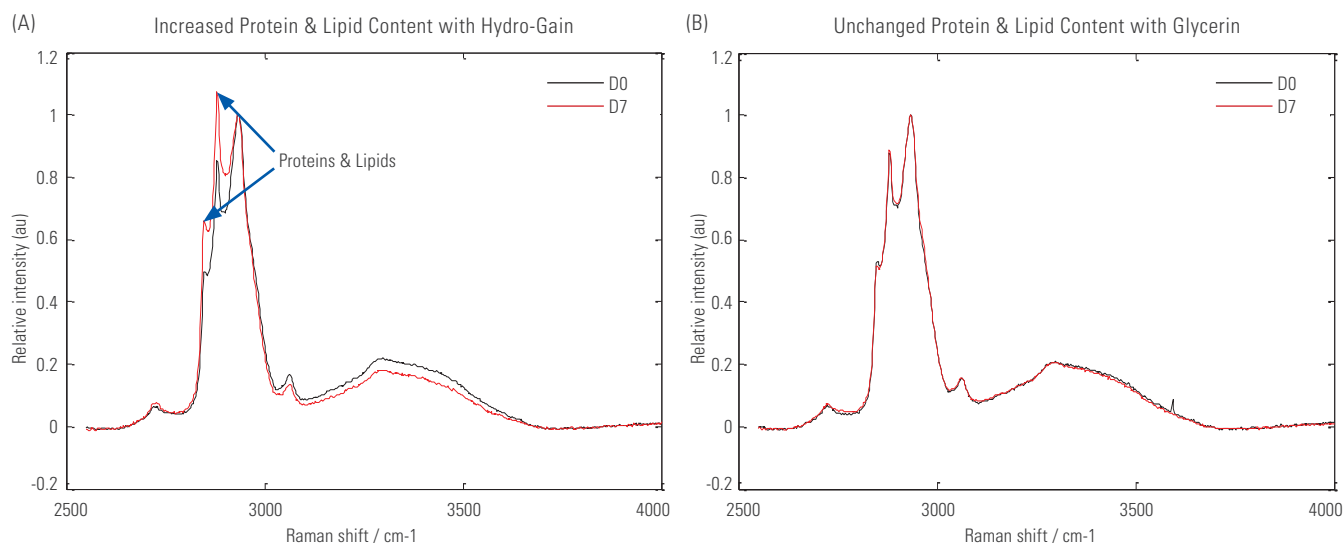


Fig. 4: Hydro-Gain replenishes the skin barrier. Treatment with Hydro-Gain (A) caused a strengthening of the skin barrier indicated by an increase of skin lipids and proteins, whereas glycerin (B) did not. Overlaid Raman spectra at a depth of 2 μM before (D0) and after a 7 day treatment (D7) with 5% Hydro-Gain or 4% glycerin.

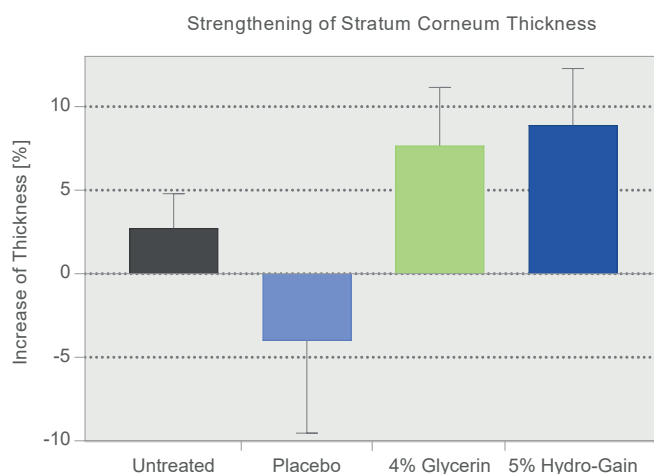


Fig. 5: A strong barrier entraps water effectively. Increased stratum corneum thickness points to effective water accumulation and hydration. Glycerin served as benchmark. N = 10; Mean + SEM.

Conclusion:

- The increase in stratum corneum thickness caused by Hydro-Gain and glycerin were rather similar but only Hydro-Gain caused an increase in proteins and lipids. We thus conclude that only Hydro-Gain but not glycerin caused an actual improvement of the skin barrier composition. Replenishing the skin barrier is thought to prevent water loss and to enable efficient long-term water retention.

***in vivo* Activity**

Hydro-Gain™, the 24-hour moisturizer

Objective:

- To show that long-term treatment with Hydro-Gain increases the skin moisture content and enhances the skin barrier.

Techniques:

- Skin hydration was measured by corneometry.
- Skin barrier function was evaluated by measuring transepidermal water loss (TEWL) using a tewameter.

Study Details:

Design	Double-blind, placebo-controlled, randomized <i>in vivo</i> study
Test Panel	23 Caucasian female volunteers, 20 – 65 years old, with dry skin
Test Substances	<ul style="list-style-type: none">• Cream formulation containing 4% glycerin without active ingredient (placebo)• Same formulation with 0.1% hyaluronic acid (benchmark ^[5,8])• Same formulation with 5% Hydro-Gain
Application Site	Forearm
Application Frequency	Twice daily for 2 weeks
Endpoints	<ul style="list-style-type: none">• Skin hydration, corneometry• Skin barrier function (TEWL), tewametry <p>Measurements were done 24 h after the last application</p>

Results:

- 24 h after the last application, skin hydration improved by ~40% already after a 1 week of Hydro-Gain application. Placebo and hyaluronic acid (benchmark), in contrast, were unable to substantially increase hydration levels (Fig. 6A).
- Skin barrier function improved by ~25% and ~35% after 1 and 2 weeks of Hydro-Gain application, respectively. Of note, measurements were done 24 h after the last application, and Hydro-Gain was much more effective than placebo or the benchmark (Fig. 6B).

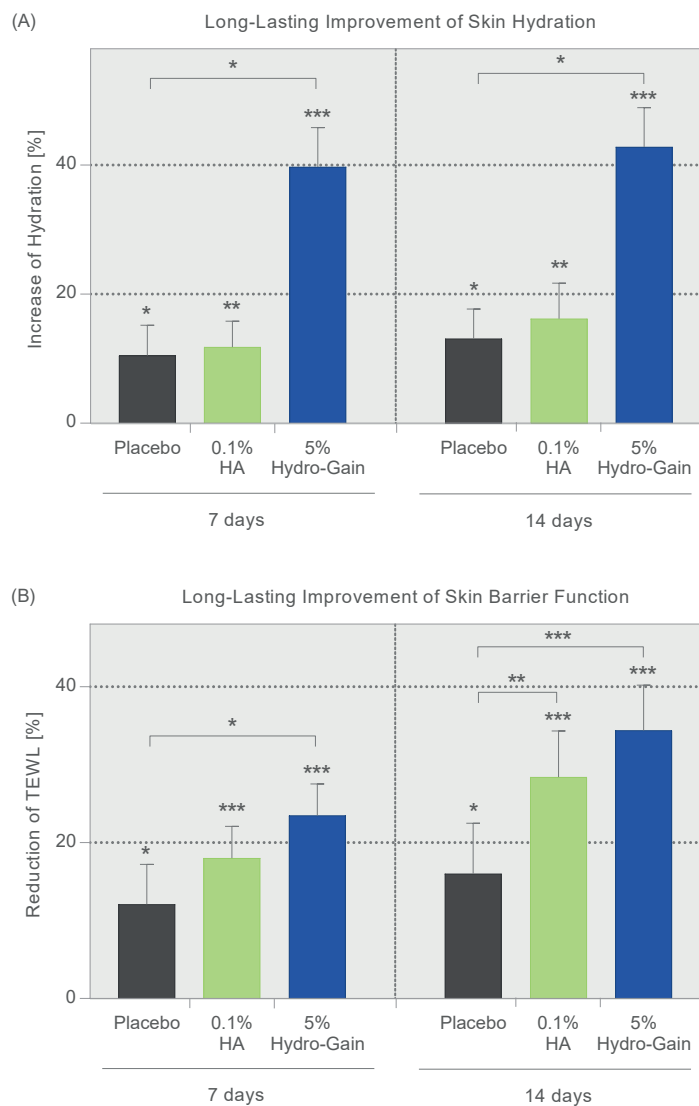


Fig. 6: Hydro-Gain is a powerful long-term moisturizer. Relative improvement of skin hydration (A) and barrier function (B) 24 h after the last application. Hyaluronic acid (HA) served as benchmark. N = 23; Mean + SEM; non-parametric permutation test versus baseline and between treatments; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Conclusion:

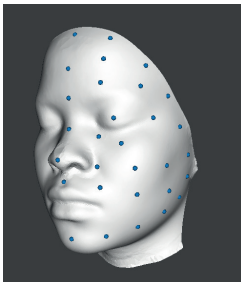
- Hydro-Gain is a very strong moisturizer with excellent barrier strengthening properties. It provides long-lasting skin hydration being present even 24 h after the last application.

Hydro-Gain™ provides short- and long-term hydration right down to the deepest layers

Objectives:

To demonstrate that an economical concentration of only 1% Hydro-Gain provides:

- instant moisture supply (after 1 h)
- daylong hydration (for 24 h)
- deep-down moisturization
- increased hydration for face and arms.



Techniques:

- **Short- and long-term** efficacy was evaluated using corneometry by measuring hydration 1 h after a single application (short-term) and 24 h after 2 weeks repeated applications (long-term). Skin hydration was measured on 30 distinct facial sites (see illustration on the right) allowing us to compute continuous 3D skin hydration maps.
- **Deep-down** efficacy was evaluated on the forearm using corneometry by measuring hydration on areas stripped 6 times with sticky tapes.

Study Details:

Design	Double-blind, placebo-controlled, randomized <i>in vivo</i> study
Test Panel	2 x 15 Caucasian female volunteers (face), respectively 1 x 30 (forearm), 21 – 65 years old, with dry skin
Test Substances	<ul style="list-style-type: none">• Cream formulation containing 3% glycerin without active ingredient (placebo)• Same formulation with 1% Hydro-Gain
Application Site	Face and forearm
Application Frequency	Twice daily for 2 weeks
Primary Endpoints	<ul style="list-style-type: none">• Short- and long-term moisturization, corneometry Measurements on 30 facial sites were done at baseline, 1 h after single application and 24 h after 2 weeks regular application• Deep-down hydration, corneometry Measurements on forearms stripped with sticky tape
Secondary Endpoint	3D skin hydration maps

Results:

- Only 1% Hydro-Gain significantly increased facial skin hydration 1 h after single application (Fig. 7A) and after 2 weeks of regular application (24 h after the last application; Fig. 7B). Of note, Hydro-Gain showed stronger and longer-lasting moisturizing properties than placebo, which showed some short-term efficacy but completely lacked long-term efficacy.
- Hydro-Gain effectively moisturized all areas of the face (Fig. 8) as well as very dry skin (Fig. 9).

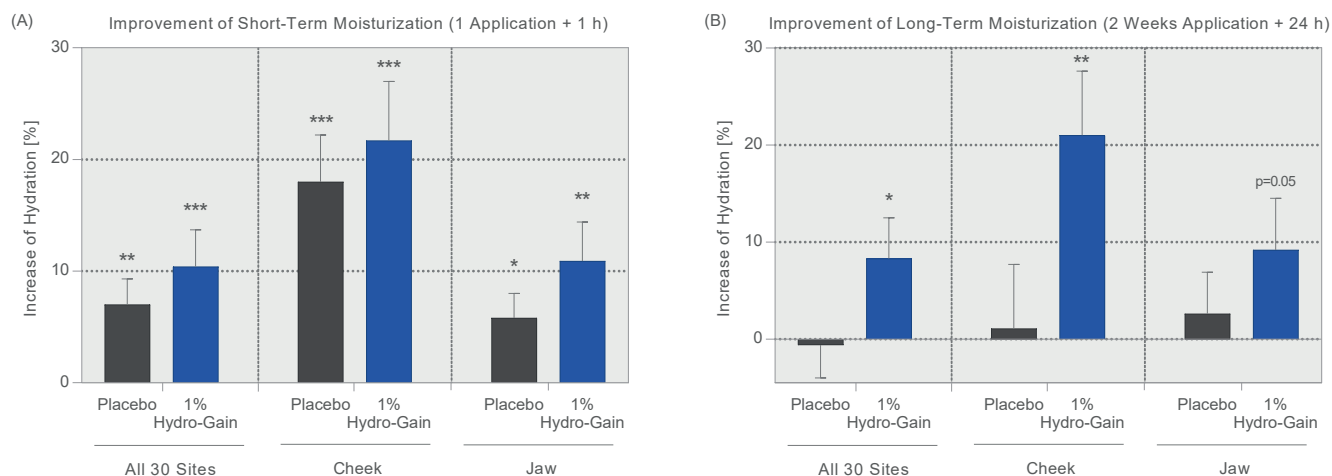


Fig. 7: Hydro-Gain provides short- and long-term moisturization. Relative improvement of skin hydration 1 h after single application (A) and 24 h after 2 weeks regular application (B). 1% Hydro-Gain showed long-lasting hydration efficacy, whereas placebo did not. N = 15; Mean + SEM; non-parametric permutation test versus baseline; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

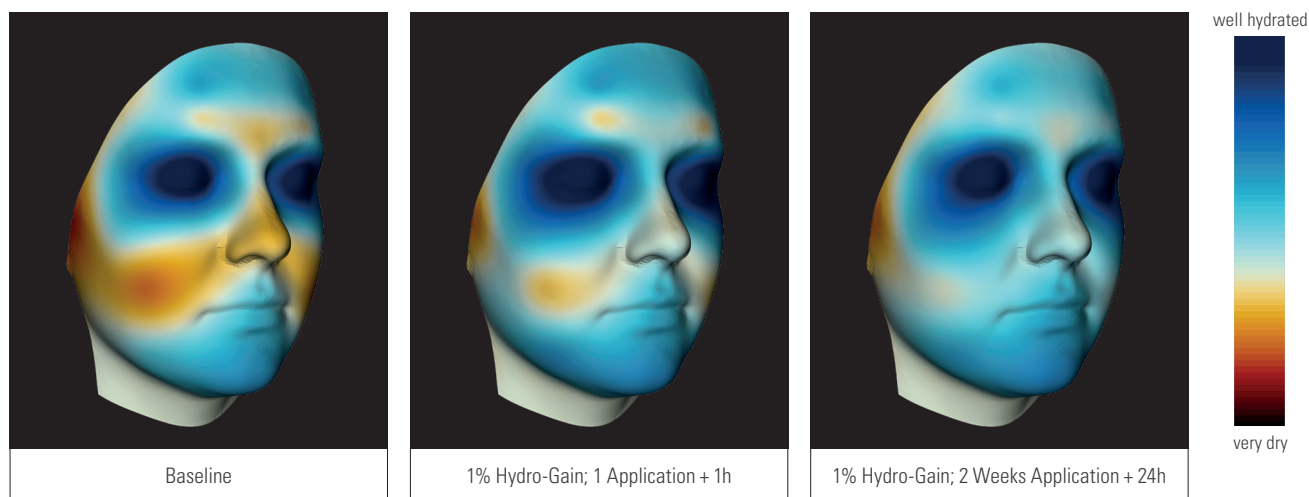


Fig. 8: Hydro-Gain moisturizes even the driest areas of the face. Averaged skin hydration color map at baseline (left), 1 h after single application of 1% Hydro-Gain (middle), and 24 h after 2 weeks regular application (right). The color code for corneometry units is shown on the scale at the right (blue = hydrated skin; grey, orange, red = dry skin).

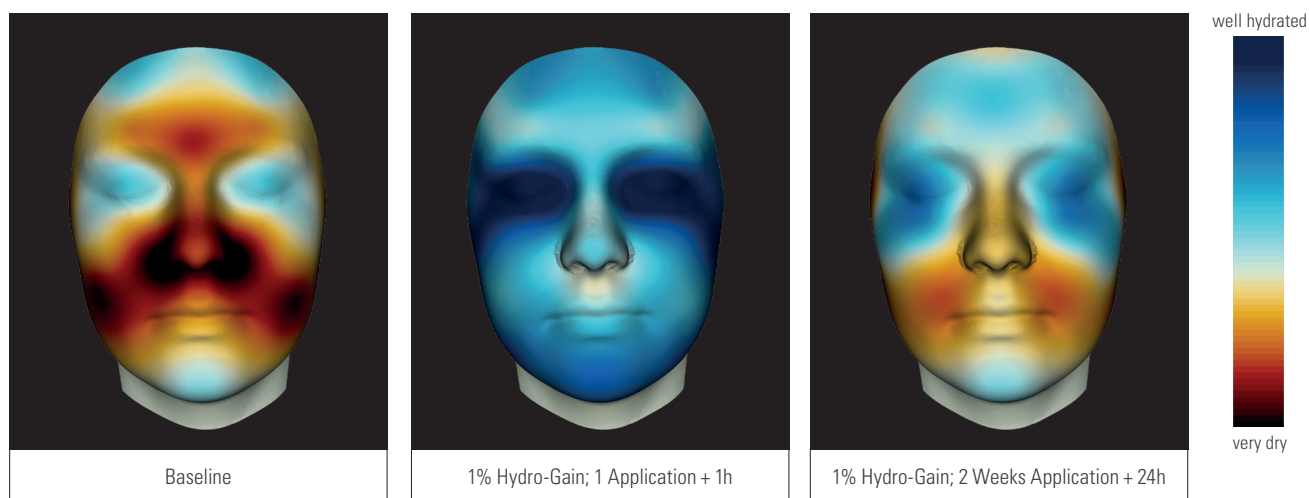


Fig. 9: Hydro-Gain provides extremely dry skin with quick and long-lasting moisture. Example of a color hydration map from an individual with very dry skin at baseline (left), 1 h after single application of 1% Hydro-Gain (middle), and 24 h after 2 weeks regular application (right). The color code for corneometry units is shown on the scale at the right (blue = hydrated skin; grey, orange, red = dry skin).

Results:

- Hydro-Gain not only moisturized the upper layers (Fig. 7-9) but also the deeper layers of the skin, as seen on stripped skin (deep moisture; Fig. 10). Of note, Hydro-Gain showed stronger moisturizing properties than placebo.

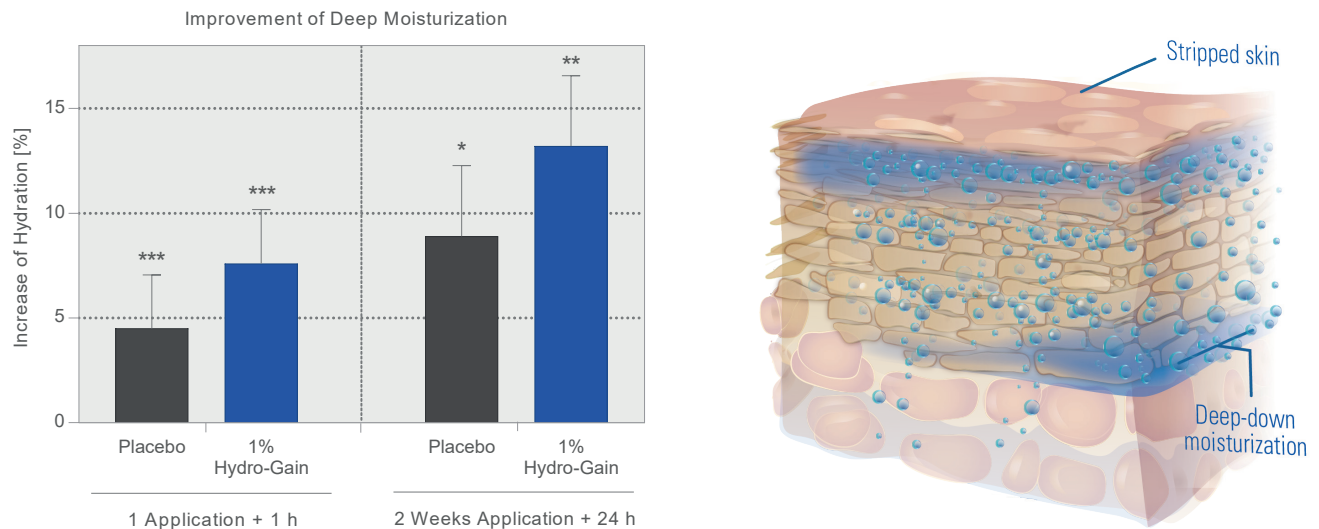


Fig. 10: Hydro-Gain moisturizes all layers of skin, from top to bottom. Skin was stripped 6 times with sticky tape. Hydro-Gain showed significant moisturizing performance on the deep stratum corneum levels. At each time point Hydro-Gain moisturized better than placebo. N = 30; Mean + SEM; non-parametric permutation test versus baseline; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

Conclusion:

- As few as 1% Hydro-Gain provides immediate (1 h) and long-lasting (24 h) skin hydration. It perfectly moisturizes dry and driest skin right down to the bottom layers - be it on the arms, on dry cheeks or the face in general.