

AMPLIFYING
BEAUTY,
SIMPLIFYING
ROUTINES



Argireline®
Amplified
peptide

Contents

- Minimalism applied to beauty routines
- Argireline® Amplified peptide**
- Efficacy
- Conclusions
- Applications and claims
- Technical data



Minimalist beauty

The end of more

A shift toward a more minimalist lifestyle, less driven by accumulation and consumption and more by multifunctionality and simplicity.

Skip care: achieve more while doing less

Skin care trend based on using fewer, but harder-working multifunctional products, in order to shrink the daily beauty routine, while maintaining its effectiveness.

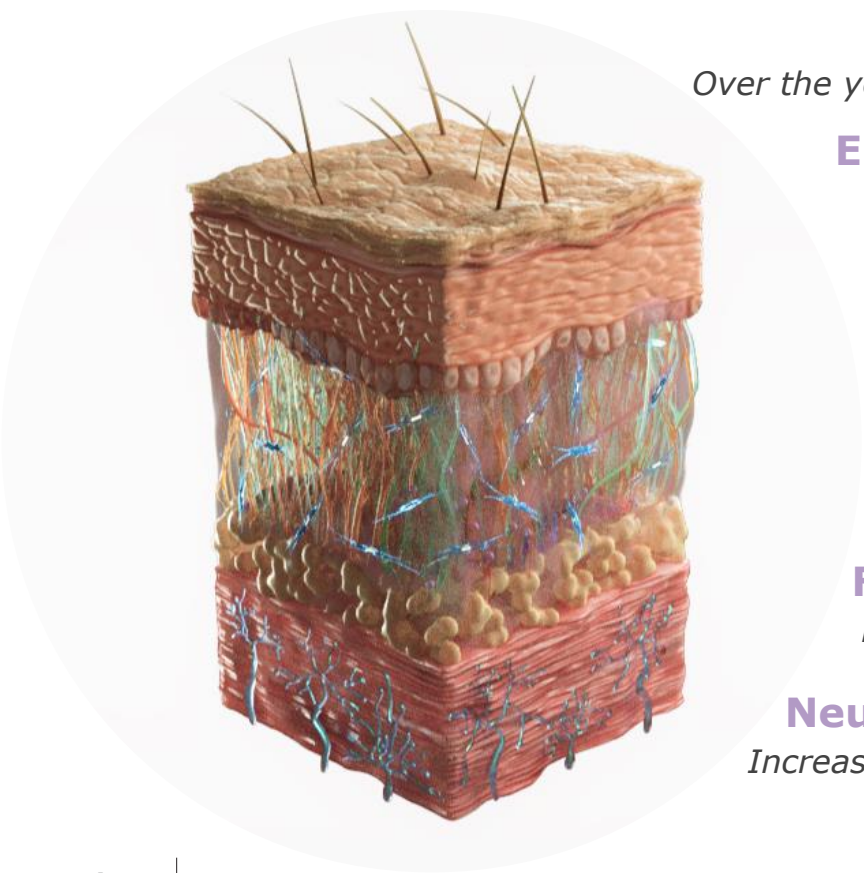


Increased demand for simpler, more convenient, results-driven beauty routines

Skip-care without skipping any skin layer

Beauty routines should be minimized, but the skin should be treated in an **integral manner**.

- In order to offer **multiple benefits** to the skin, each layer has to be targeted in an intelligent way.



Over the years...

Epidermis

Barrier dysfunction

Dermis

Decreased firmness and appearance of wrinkles

Adipose tissue

Lower volume

Facial muscles

Loss of physical support

Neuron

Increased expression wrinkles

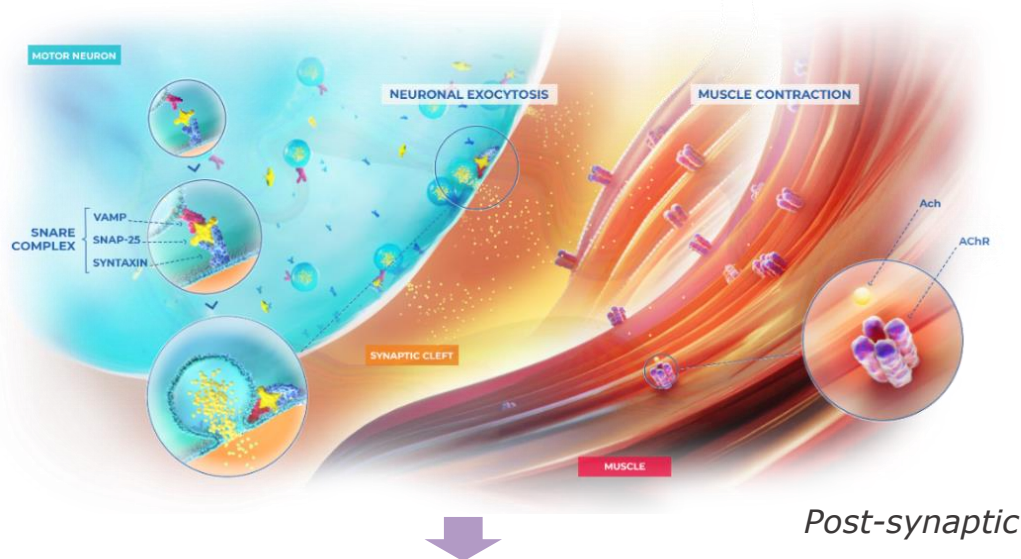
A **multilayer care** is needed to achieve an **overall younger looking skin** complexion

Increased interest for essential beauty ingredients packed with all the efficacy to simplify the skincare regimen

Aging starts as early as in your 30s with expression wrinkles...

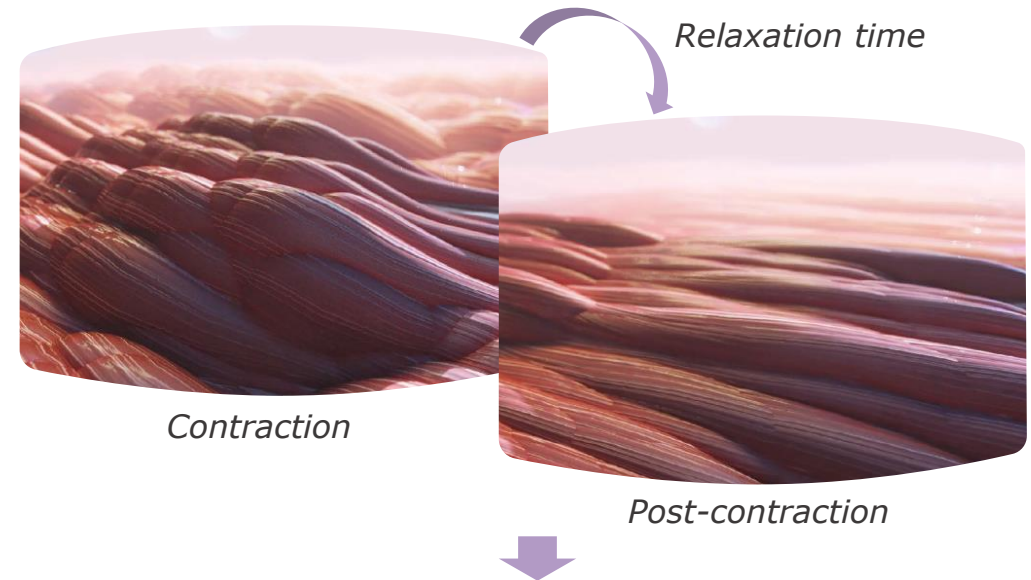
Neuron-muscle interaction

Pre-synaptic



Repeated movements caused by the contraction of facial muscles lead to expression lines.

Muscle relaxation time



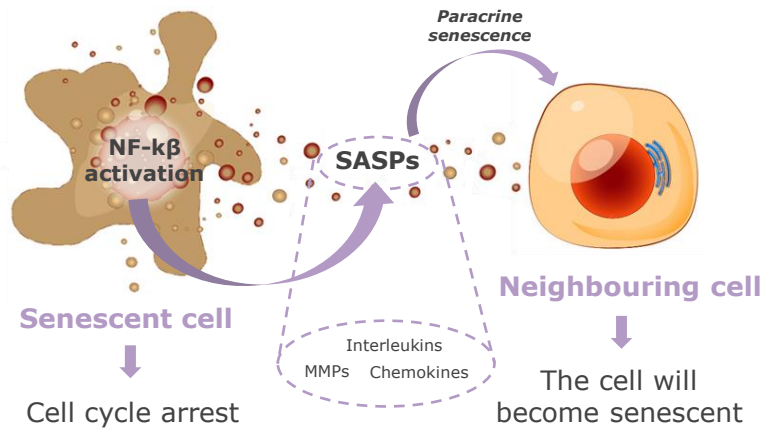
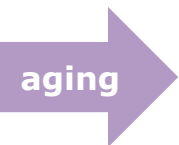
The time it takes muscles to relax after contracting increases with age, so wrinkles are visible for longer.

Weaker muscle contraction and faster relaxation time: the perfect the perfect combo for minimizing the appearance of expression wrinkles

... and it continues affecting all skin layers

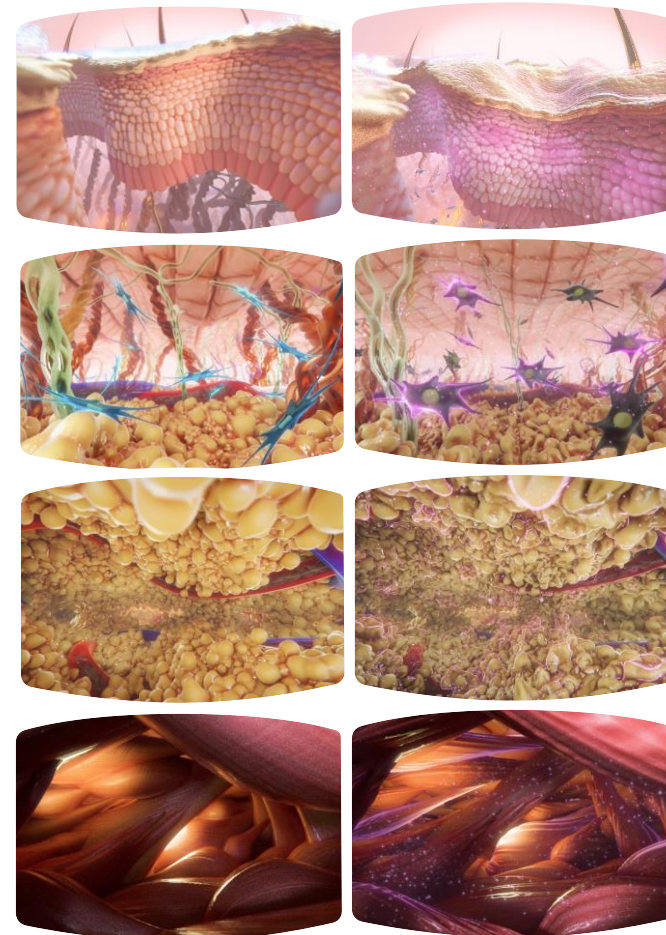
Senescence, a hallmark of aging

State of stable cell cycle arrest in which cells stop dividing and show morphological and metabolic changes.



SASPs (Senescence-associated secretory phenotypes)

Complex mixture of pro-inflammatory components secreted by senescent cells which induces senescence in neighboring cells.



Epidermis

- ↓ cell-to-cell connections
- ↓ water transport capacity

Lower protection and hydration (barrier dysfunction)

Dermis

- Bigger, flatter and less active fibroblasts
- ↓ Extracellular matrix components

**Loss of firmness and elasticity
Appearance of wrinkles and sagginess**

Adipose tissue

- ↓ lipid accumulation in adipocytes

Loss of tissue volume

Muscle

- ↓ muscle fiber diameter

Loss of muscle mass and support



Simpler buying, better buying



**A way to
make
beauty
more
sustainable**

Minimalism also represents a **positive impact on the environment.**

Those who choose to simplify their daily routine also contribute to **make beauty more sustainable**, by minimizing the amount of products and hence, the number of packages used.

Another way of making beauty more sustainable is making chemistry greener



Green chemistry applied to peptide ingredients

Green chemistry: “design of chemical products and processes to reduce or eliminate the use and generation of hazardous substances”.*

The **12 principles of green chemistry** provide an outline of how to reduce the negative impact that chemicals and their synthesis have on the environment and on our health.

Our philosophy:

Applying sustainable practices even in the synthesis of peptides to have cleaner and safer ingredients.

Green chemistry principles in the manufacturing process

ACS (American Chemical Society)
Green Chemistry Institute



-  WASTE PREVENTION
-  ATOM ECONOMY
-  LESS HAZARDOUS CHEMICAL SYNTHESIS
-  DESIGNING SAFER CHEMICALS
-  SAFER SOLVENTS & AUXILIARIES
-  DESIGN FOR ENERGY EFFICIENCY
-  USE OF RENEWABLE FEEDSTOCKS
-  REDUCE DERIVATIVES
-  CATALYSIS
-  DESIGN FOR DEGRADATION
-  REAL-TIME POLLUTION PREVENTION
-  SAFER CHEMISTRY FOR ACCIDENT PREVENTION

Amplifying beauty, simplifying routines



Hexapeptide produced according to the principles of green chemistry. The ingredient is an evolution of Argireline® peptide, with superior activity and added muscle relaxation effects. It also reduces the appearance of age related changes in all skin layers.

Multi-level action

IN VITRO

- ✓ Muscle-neuron interaction & muscle relaxation
- ✓ Delaying and recovering aging in:
 - ✓ Muscle
 - ✓ Adipose tissue
 - ✓ Dermis
 - ✓ Epidermis

All-in-one efficacy

IN VIVO

- ✓ Improving appearance of expression wrinkles
- ✓ Feel the expressions and forget about wrinkles
- ✓ Multi-level improvement in tissues functionality
- ✓ What do the volunteers think?

Argireline® Amplified peptide, produced following the principles of green chemistry



WASTE PREVENTION

- Use less organic solvents (-15%)



LESS HAZARDOUS CHEMICAL SYNTHESIS

- Use less hazardous acids (-20%)
- No thiols
- Use lower temperatures
- Replace explosive reagents



DESIGNING SAFER CHEMICALS

- Complete toxicity studies



USE OF RENEWABLE FEEDSTOCKS

- Use of amino acids from natural sources



REDUCE DERIVATIVES

- Reduce the number of protection/deprotection steps during the synthesis and the subsequent generated waste (-7%)



DESIGN FOR DEGRADATION

- Biodegradable peptide



REAL-TIME POLLUTION PREVENTION

- Installation of pressure valves to monitor/prevent the release of polluting substances



SAFER CHEMISTRY FOR ACCIDENT PREVENTION

- Automated delivery of solvents to avoid manual manipulation of solvents

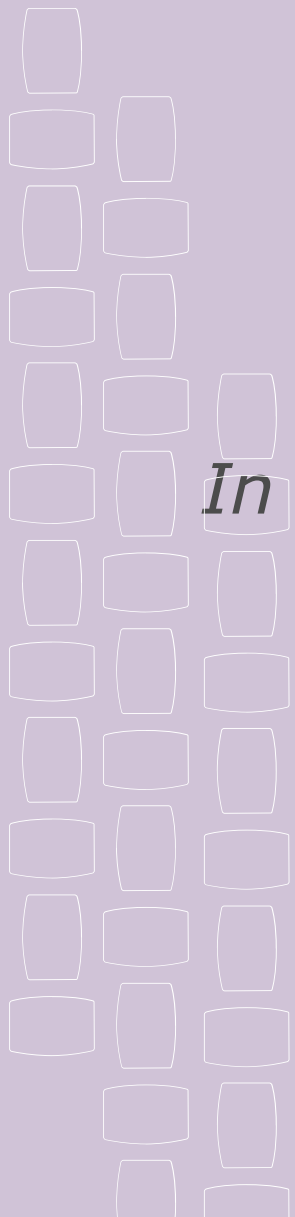
Peptide designed and produced following our commitment to apply the principles of green chemistry to the whole process.



**You know the destination
WE'LL HELP YOU REACH IT SUSTAINABLY**

Road to the 12 principles...





In vitro efficacy

1) Muscle-neuron interaction & muscle relaxation

Neuron

- Amplifying competition with SNAP-25
- Attenuating neuronal exocytosis

Muscle

- Attenuating the strength of muscle contraction and improving the relaxation of muscle contraction

2) Delaying and recovering aging in:

Muscle

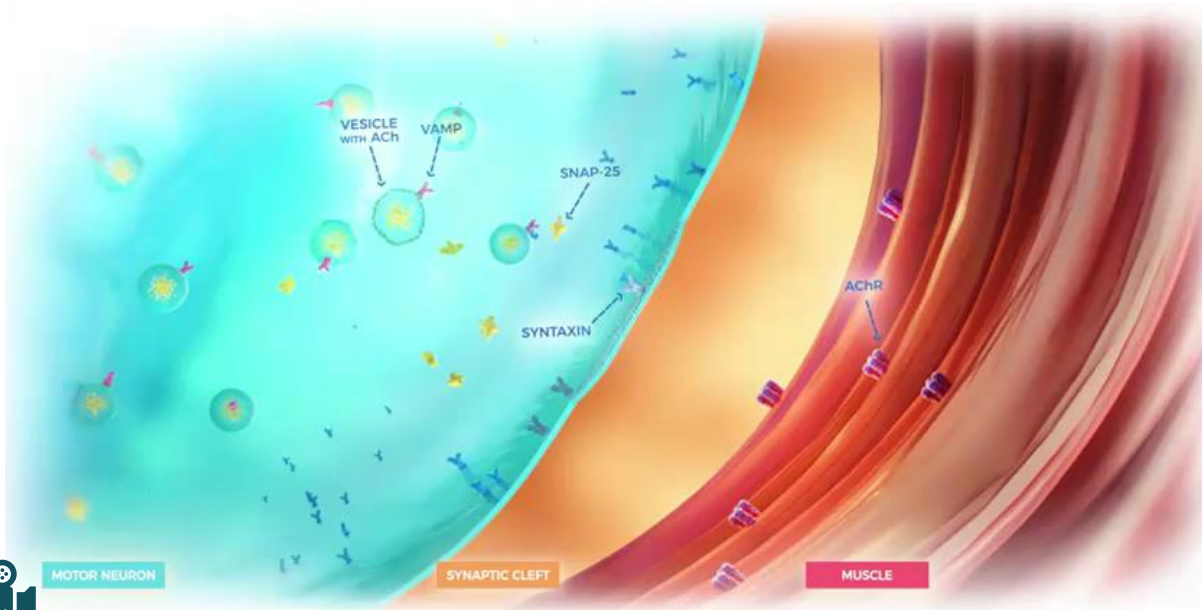
Adipose
tissue

Dermis

Epidermis



Facial muscles contraction mechanism



- 1) Assembly of the **SNARE complex** in the motor neuron.
- 2) Release of **neurotransmitter** to the synaptic cleft.
- 3) Muscle **contraction** followed by **relaxation**.

FUNCTIONAL CHANGES

Aging is associated with a **slowing of muscle relaxation** after contraction

Repeated contraction and lower relaxation causing the permanence of skin wrinkles

Amplifying competition with SNAP-25

Bioinformatic modeling of the **competition of the peptide with SNAP-25 for its position in the SNARE complex.**

Measurement of **Gibbs free energy of interaction** of the peptide with Syntaxin 1A (ΔG_{int}). A lower value corresponds to a tighter binding and thus, a higher expected efficacy.

ΔG_{int} vs. Argireline[®] peptide

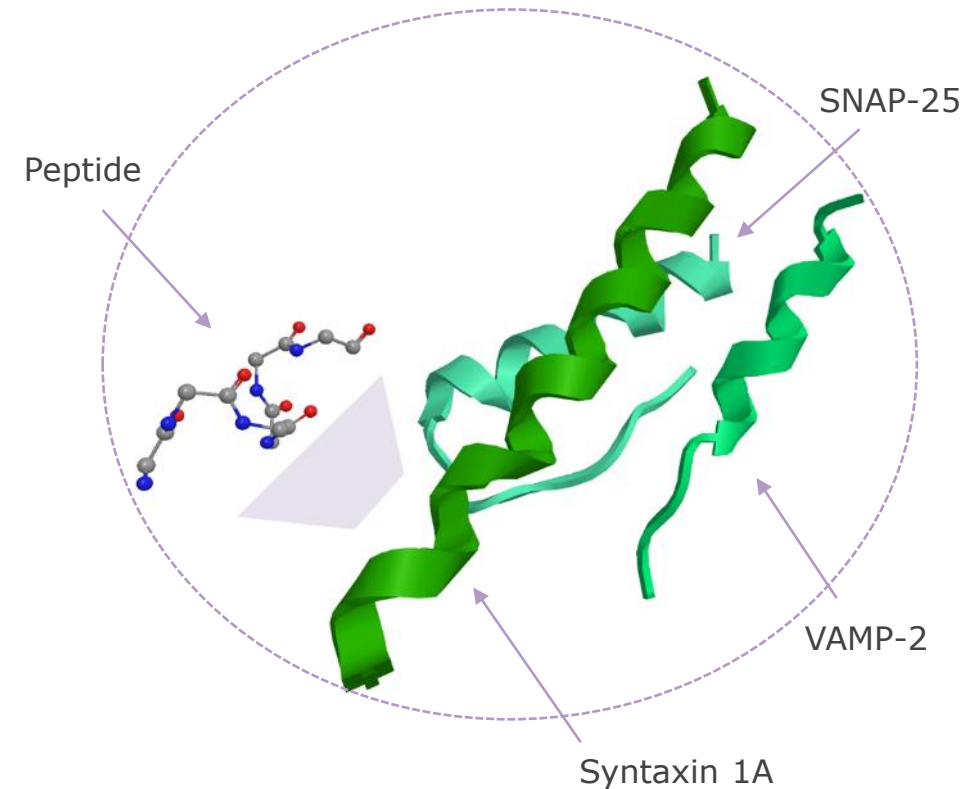
Argireline[®] Amplified peptide

-32.7%

Lower free energy of interaction than Argireline[®] peptide

The new peptide has a stronger affinity for syntaxin than Argireline[®] peptide and thus, it is more effective at inhibiting the SNARE complex formation.

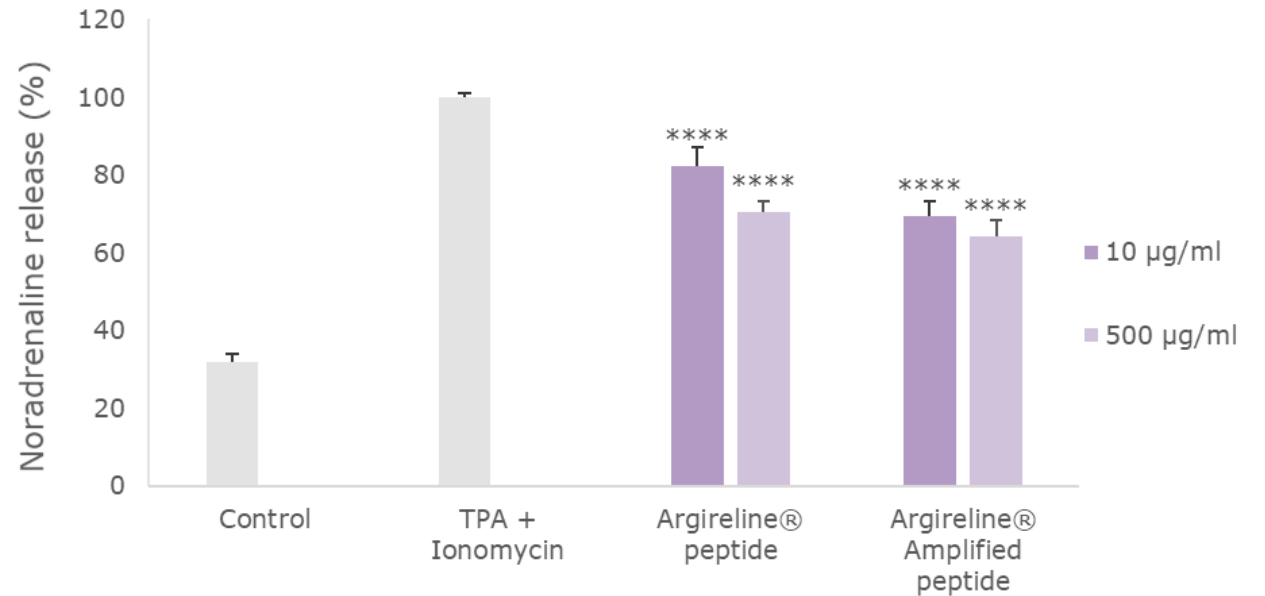
Amplifying the potency of binding by 33% vs. Argireline[®] peptide



Attenuating neuronal exocytosis

Human neuroblastoma cells were pre-treated with the peptides at different concentrations for 1 h and afterwards, exocytosis was induced by adding TPA and ionomycin.

Supernatants were collected and noradrenaline released determined by ELISA.



vs TPA + ionomycin: ****p<0.0001

Higher potency than Argireline® peptide in the inhibition of neuronal signaling

TPA: Tetradecanoylphorbol-13-acetate

Attenuating the strength of muscle contraction...

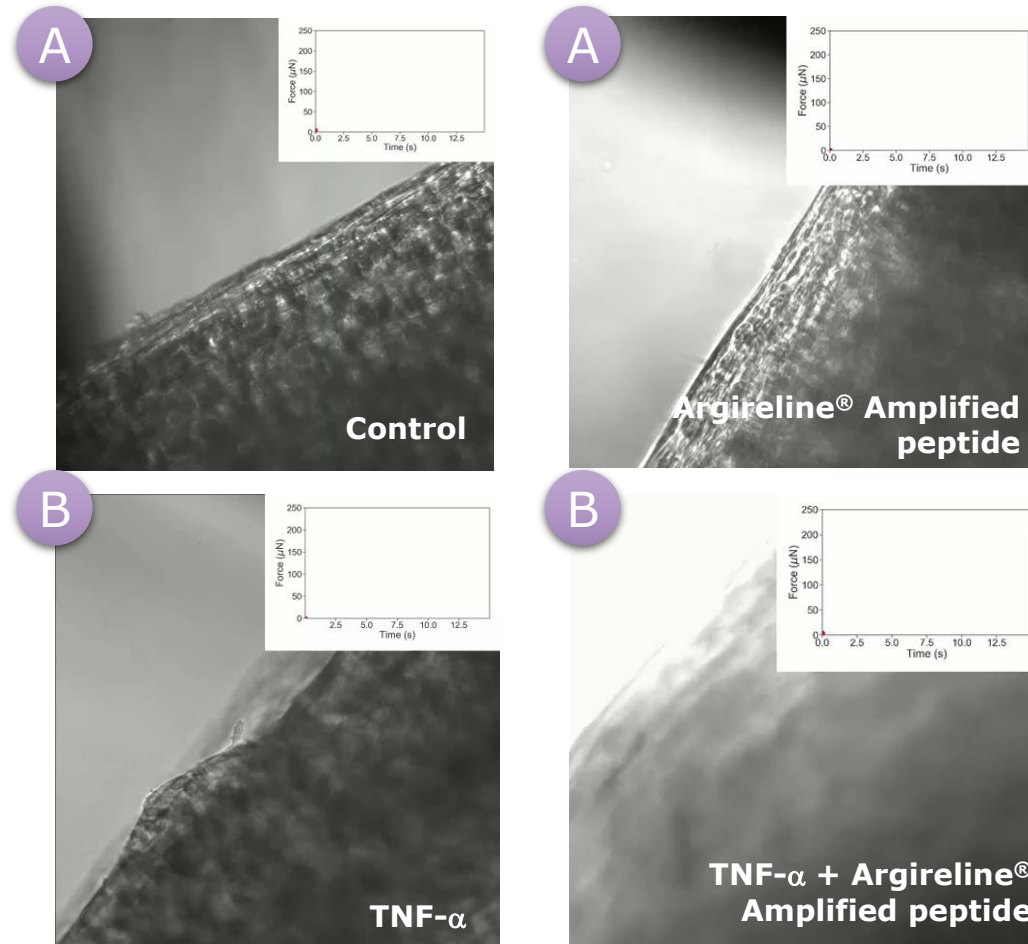
3D bioprinting of bioengineered human skeletal muscle tissue.

A 3D muscle model treated with 2 mg/mL **Argireline® Amplified peptide** for 48 hours or no treatment.

B Induction of muscle aging by incubation with **TNF-α** for 24 h followed by treatment with 2 mg/mL **Argireline® Amplified peptide** for 48 hours or no treatment.

Evaluation of **muscle activity**.

The peptide reduced the strength of **muscle contraction**



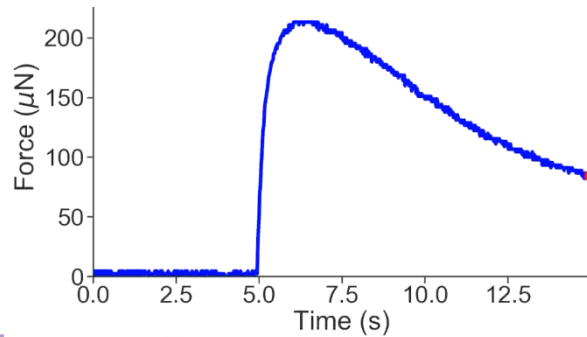
Young muscle

Aged muscle

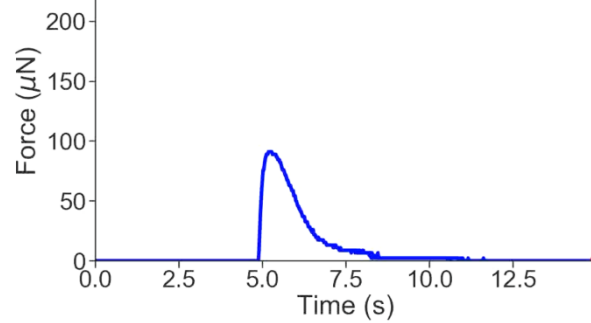
... and improving the relaxation of muscles

Young muscle

A Control



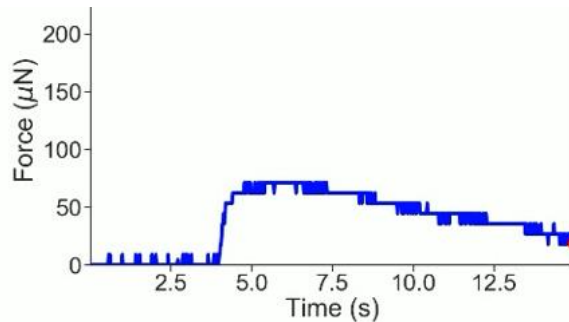
A Argireline® Amplified peptide



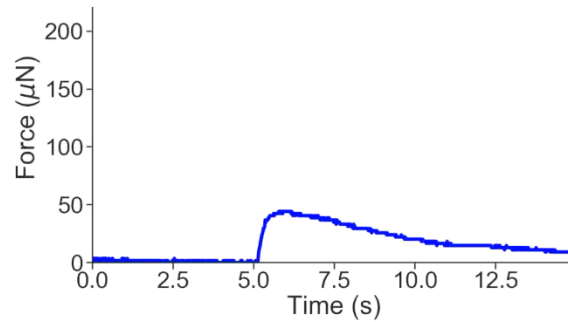
➔ 63% faster relaxation

Aged muscle

B TNF-α



B TNF-α + Argireline® Amplified peptide



➔ 31% faster relaxation

Faster relaxation in young and aged muscle thanks to Argireline® Amplified peptide, **helping to recover** the skin appearance after facial expressions

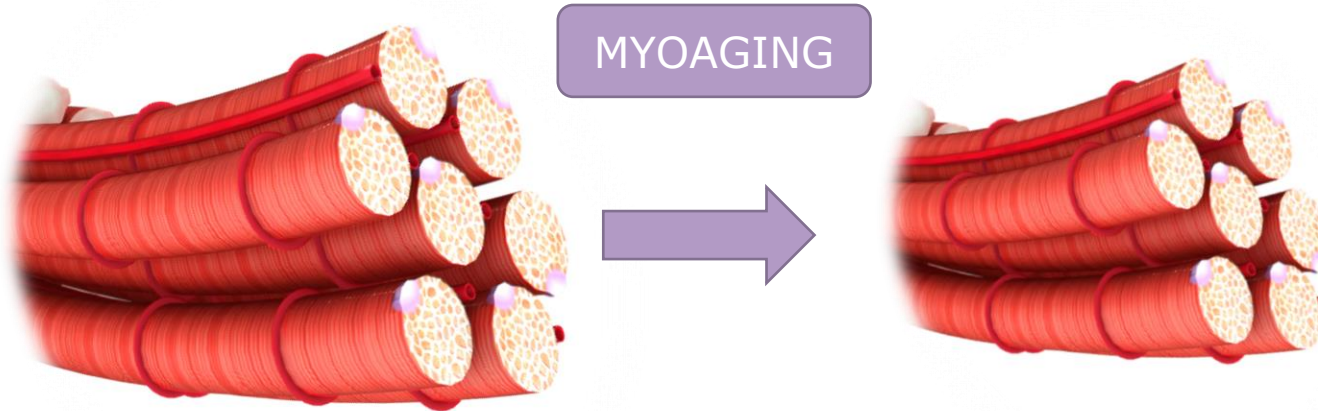




Aging of muscle

Morphological changes occurring with aging in muscle:

MORPHOLOGICAL CHANGES



Can be induced *in vitro* by the cytokine **TNF- α** (SASPs)

Increase of **senescent muscle cells** that negatively affect neighboring cells

- Aged muscle presents a reduced **diameter** of **myotubes** and a lower muscle mass and support (muscle **weakness**)

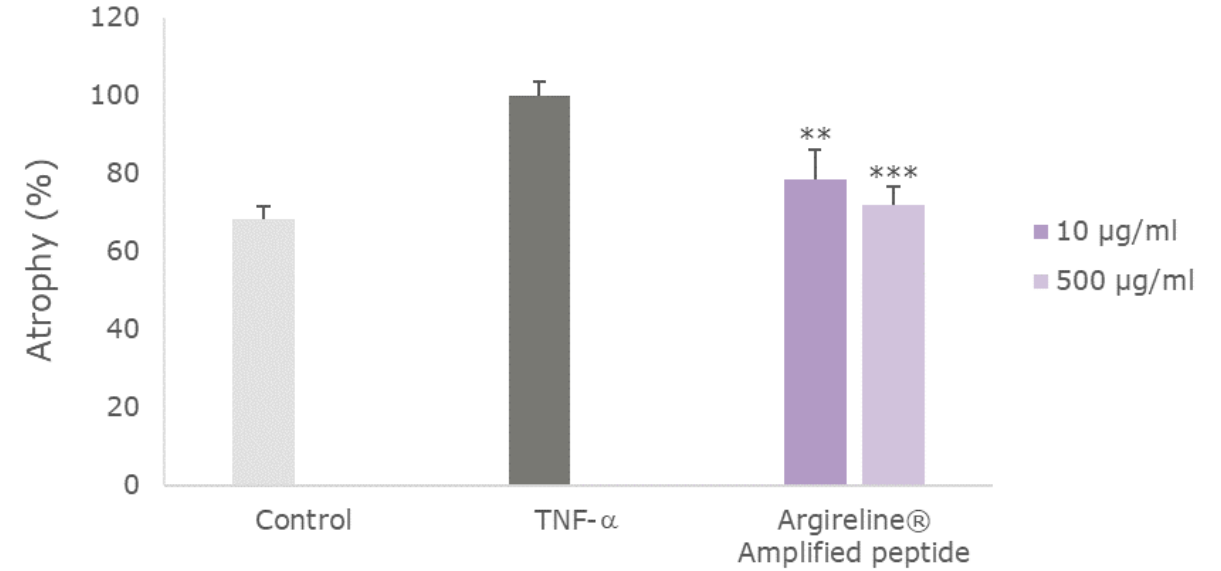
Altered morphology of muscle reducing the support to the skin and contributing to sagginess

Restoring myoaging

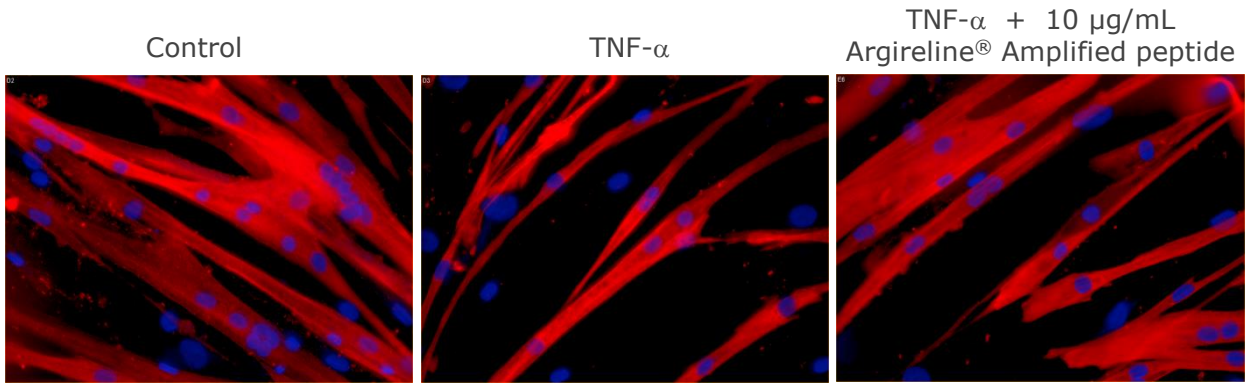
Differentiation of **human skeletal muscle cells**

Incubation with **TNF- α** (to induce **myoaging**) for 24 h. Afterwards, treatment with Argireline® Amplified peptide at different concentrations for 48 h.

Myotube diameter determined through imaging by means of microscopy after myosin heavy chain immunofluorescence. Percentage of atrophy was calculated.



vs TNF- α : **p<0.01, ***p<0.001

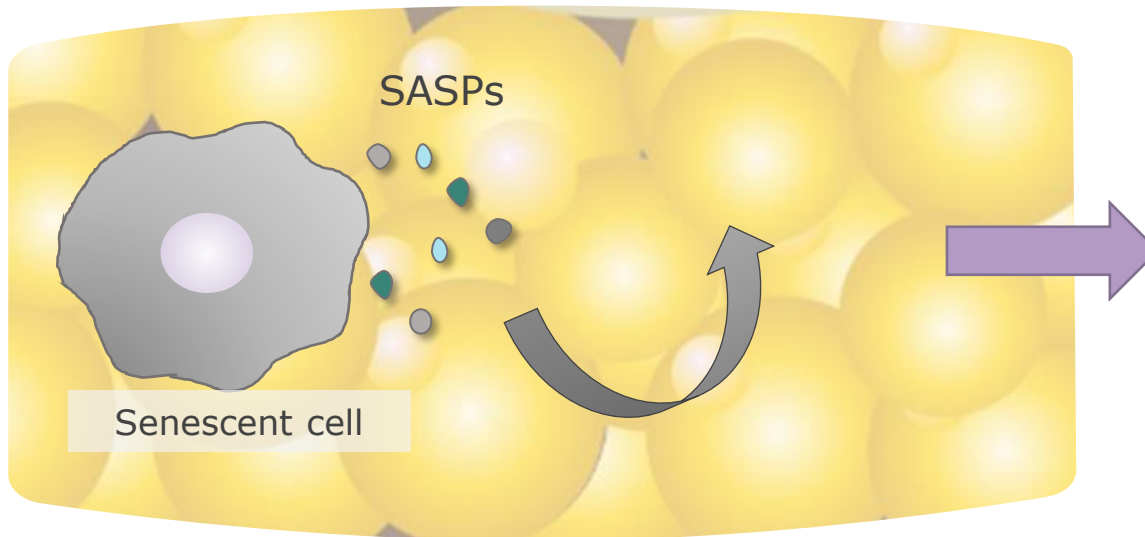


Myosin heavy chain staining in red; cell nuclei in blue.

Argireline® Amplified peptide helped to restore muscle loss induced by aging

Aging of adipose tissue

Senescent cells in the adipose tissue **secrete SASPs components**



Signaling of SASPs to non-senescent cells results in an **impairment of adipogenic functions**

Reduced accumulation of lipids by adipocytes

Age-dependent reduction in volume causing flattening of facial contours

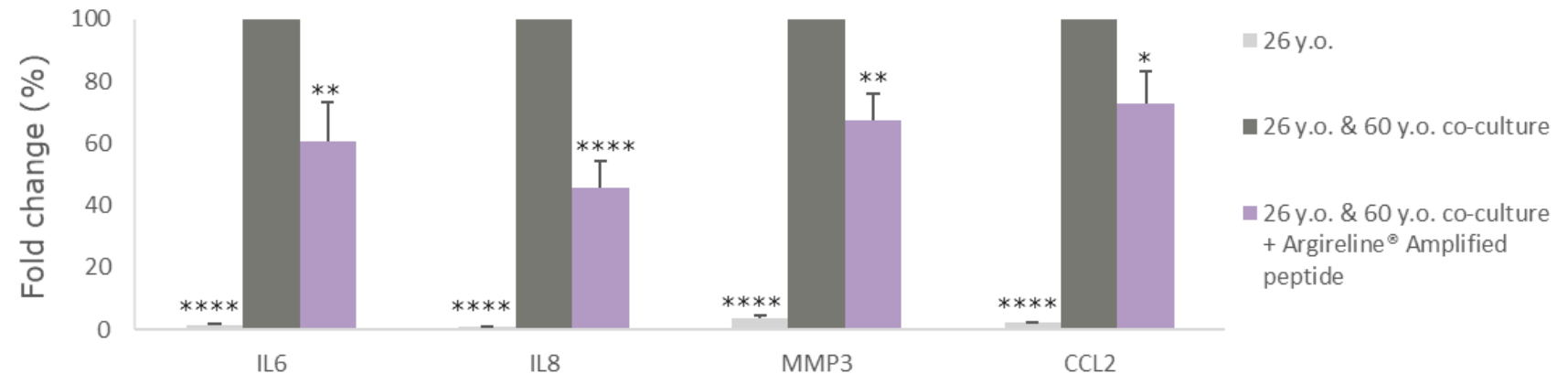
Delaying senescence of adipose tissue

Human preadipocytes from donors of **26 y.o.** and **60 y.o.** were cultured separately or together in **co-culture**.

Differentiation into adipocytes was induced during 13 days in the presence or the absence of 10 µg/mL Argireline® Amplified peptide.

Expression of genes involved in senescence was analyzed by **RT-PCR**.

• **SASPs**



26 y.o. vs co-culture: ****P<0.0001
 Co-culture + Argireline® Amplified peptide vs co-culture:
 *p<0.05, **p<0.01, ****p<0.0001

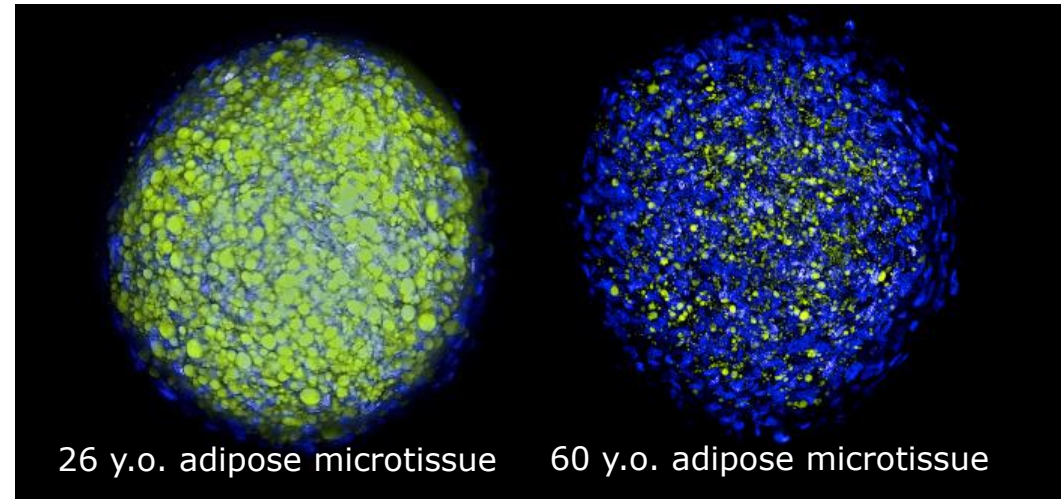
Preventing the release of SASPs due to aging

Recovery of adipogenesis

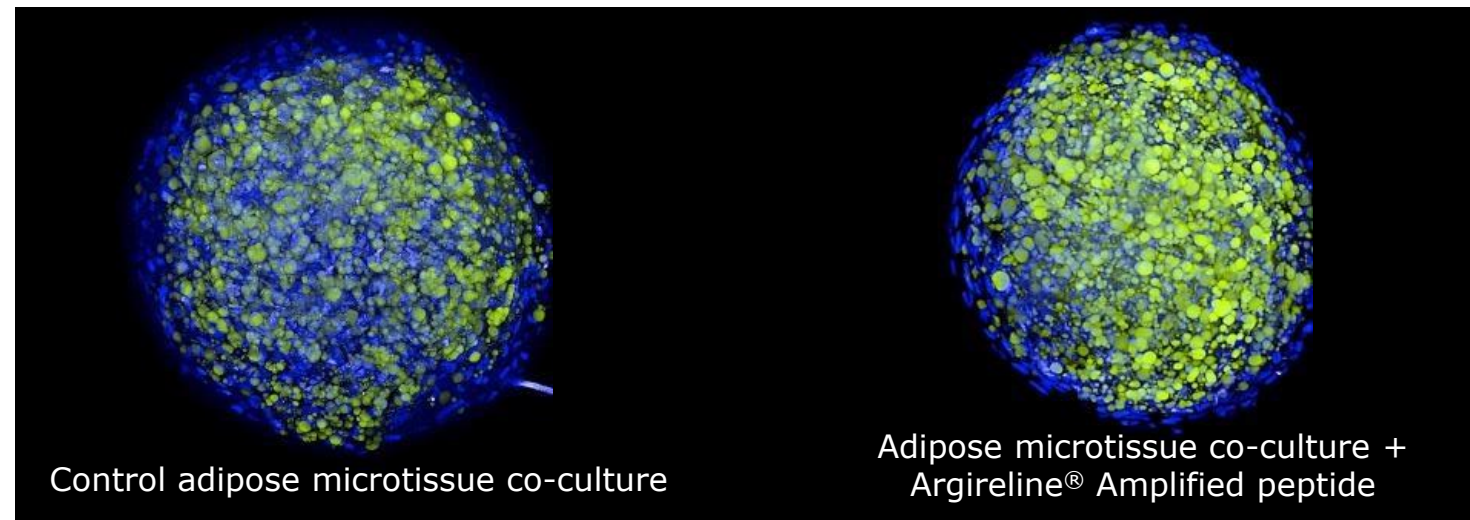
3D adipose microtissues were created from **human preadipocytes** from **26 y.o.** and **60 y.o.** donors cultured alone or in **co-culture**.

Differentiation into adipocytes was induced during 13 days in the presence or the absence of 10 µg/mL Argireline® Amplified peptide.

Lipid accumulation was quantified by means of fluorescence by treating the cells with the Adipored™ reagent.



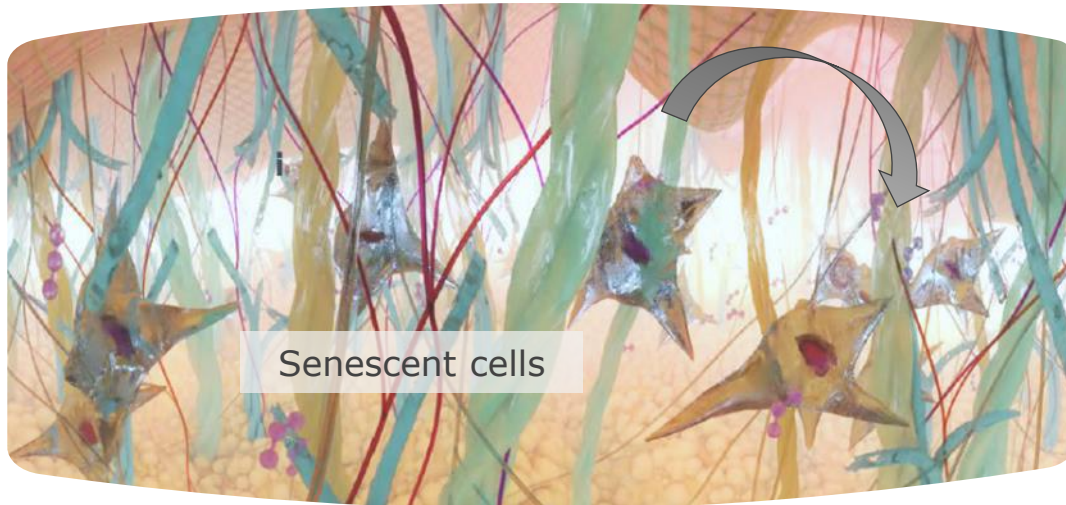
With the peptide, lipid accumulation was 18.8% higher despite the pro-senescence conditions



vs control adipose microtissue co-culture: *p<0.05

Dermal aging

The dermal aging process involves:



- Increase of **senescent fibroblasts** that can negatively influence nearby fibroblasts
- This is correlated with a **decreased production** of **ECM** components
- Deterioration of the dermal layer and its biomechanical properties

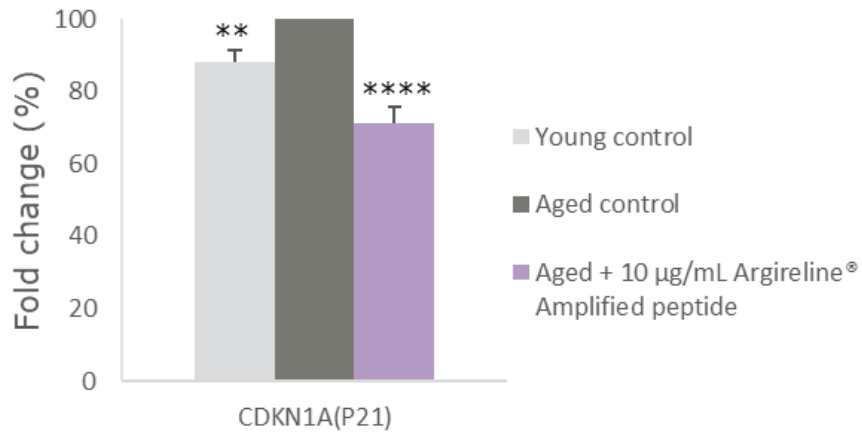
**Loss of essential properties of the skin,
such as firmness and elasticity**

Delaying and reverting dermal senescence

Replicative senescence was induced in **human dermal fibroblasts** while treated with 10 µg/mL Argireline® Amplified peptide during passaging.

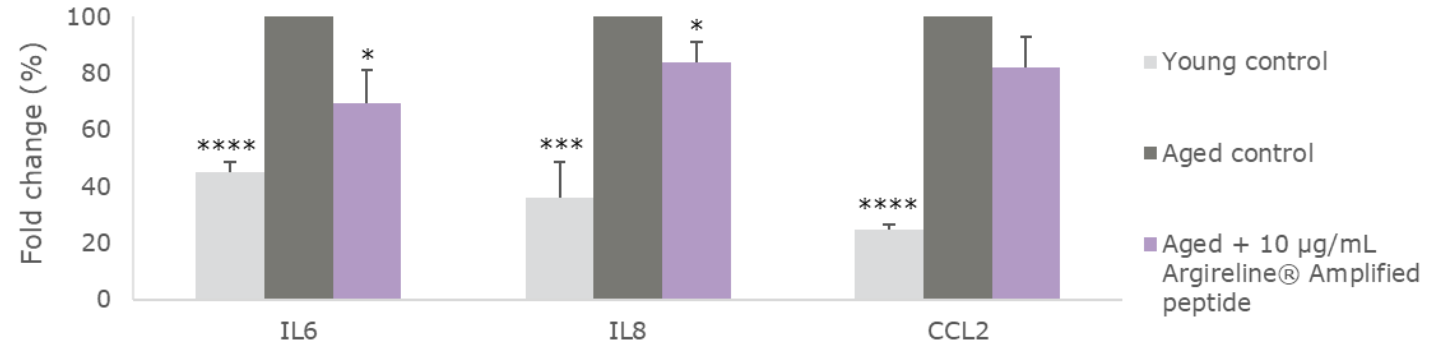
Expression of genes involved in **senescence and SASPs** was analyzed by RT-PCR.

• **Senescence marker**



vs aged control: **p<0.01, ****p<0.0001

• **SASPs**



Control: non-treated cells
vs aged control: *p<0.05, ***p<0.001, ****p<0.0001

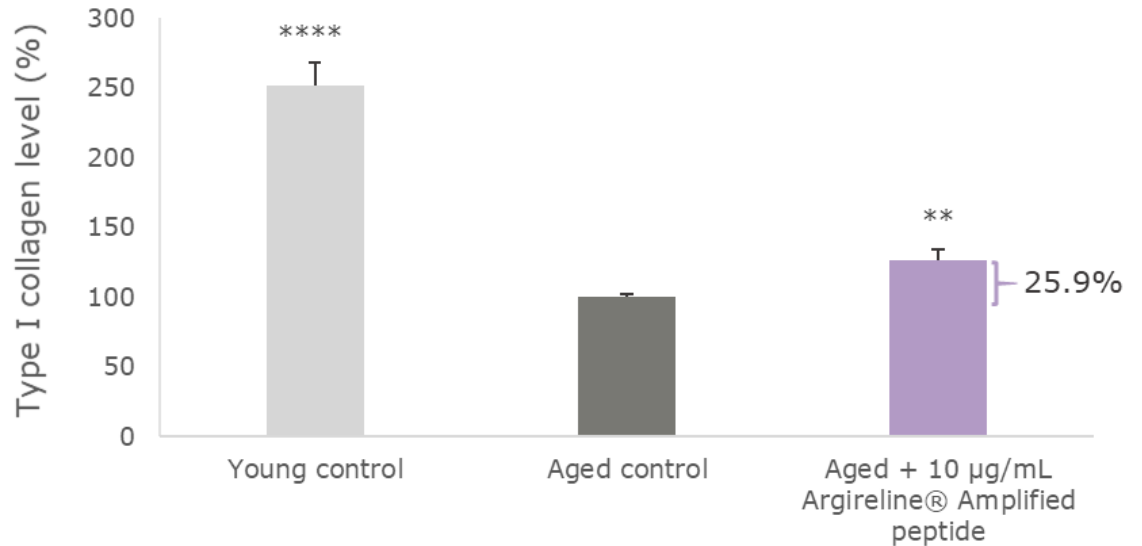
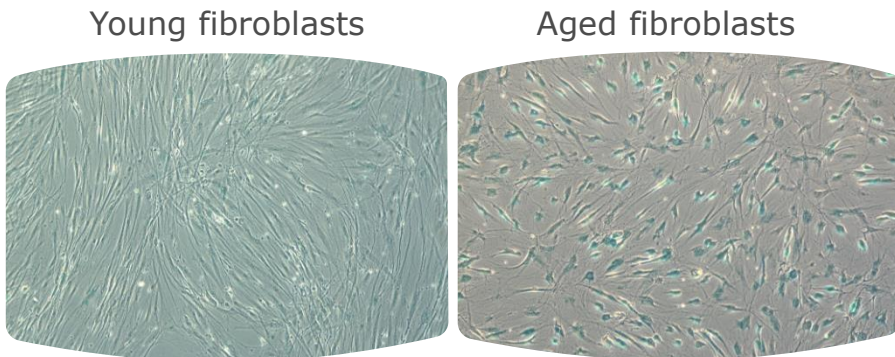
Argireline® Amplified peptide helps reduce the release of SASPs for lower dermal senescence

Collagen boosting in aged conditions

Replicative senescence was induced in **human dermal fibroblasts** while treated with 10 µg/mL Argireline® Amplified peptide during passaging.

Type I collagen levels evaluated with alphaLISA assay.

- **Senescence marker (β-galactosidase)**



Control: non-treated cells
vs aged control: **p<0.01, ****p<0.0001

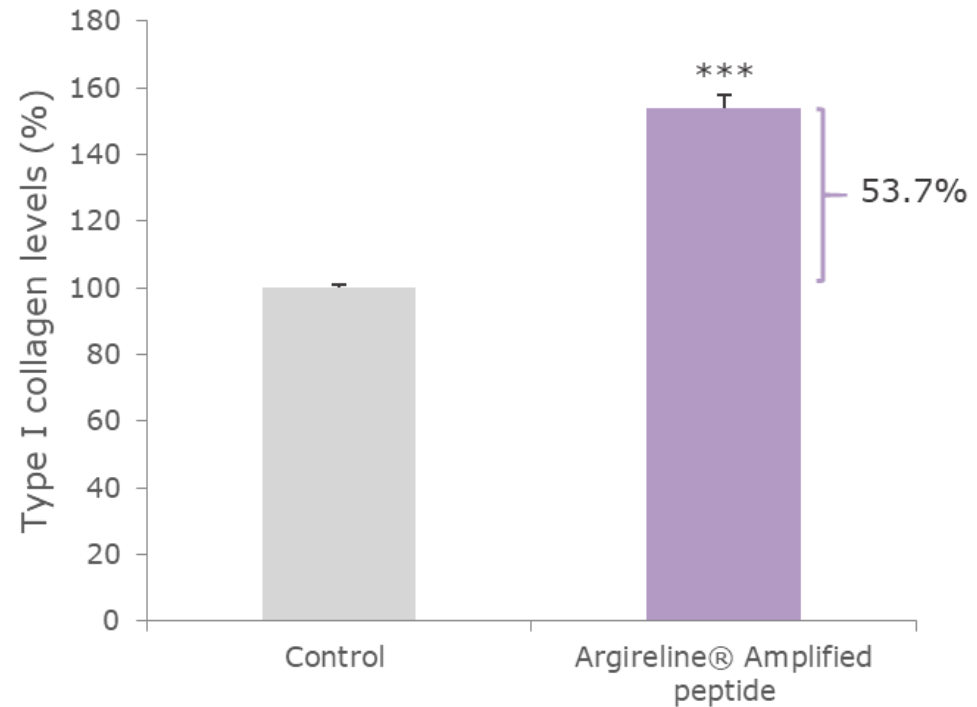
25.9% higher type I collagen levels even under aging conditions

New collagen boosting

Human dermal fibroblasts co-cultured with human keratinocytes were treated with 0.5 µg/mL Argireline® Amplified peptide for 48 h.

Type I collagen levels evaluated with an alphaLISA assay.

Peptide has the ability to enhance the synthesis of new collagen by 53.7%



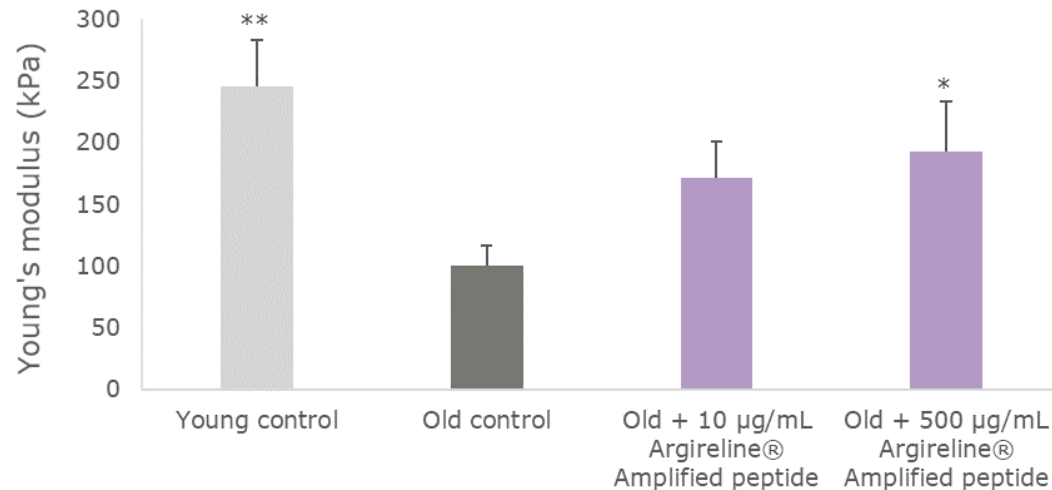
Control: non-treated cells

vs control: ***p<0.001

Restoring skin firmness

Human **skin biopsies** of **younger** donors (between 25 and 39 y.o.) and **older** donors (between 52-63 y.o.) were cut in small pieces and cultured in the presence of 10 or 500 µg/mL Argireline® Amplified peptide for 40 h.

Young's modulus (kPa) was determined by evaluating **tensile stretching**.

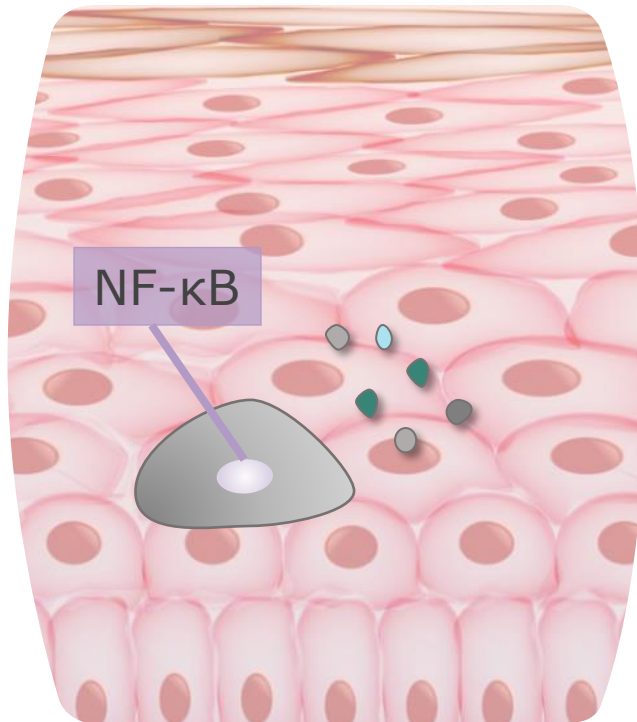


In collaboration with  **Bosch i Gimpera**
UNIVERSITAT DE BARCELONA

Argireline® Amplified peptide helped to recover firmness of the skin lost with aging

Young vs old controls: **p<0.01
Old + Argireline® Amplified peptide vs old control: *p<0.05

Epidermal aging



Increase in **cellular senescence**

- The nuclear factor κ B (**NF- κ B**) modulates **senescence** in keratinocytes and is a major inducer of the **SASPs**

Aged epidermis shows a **compromised barrier function**, with

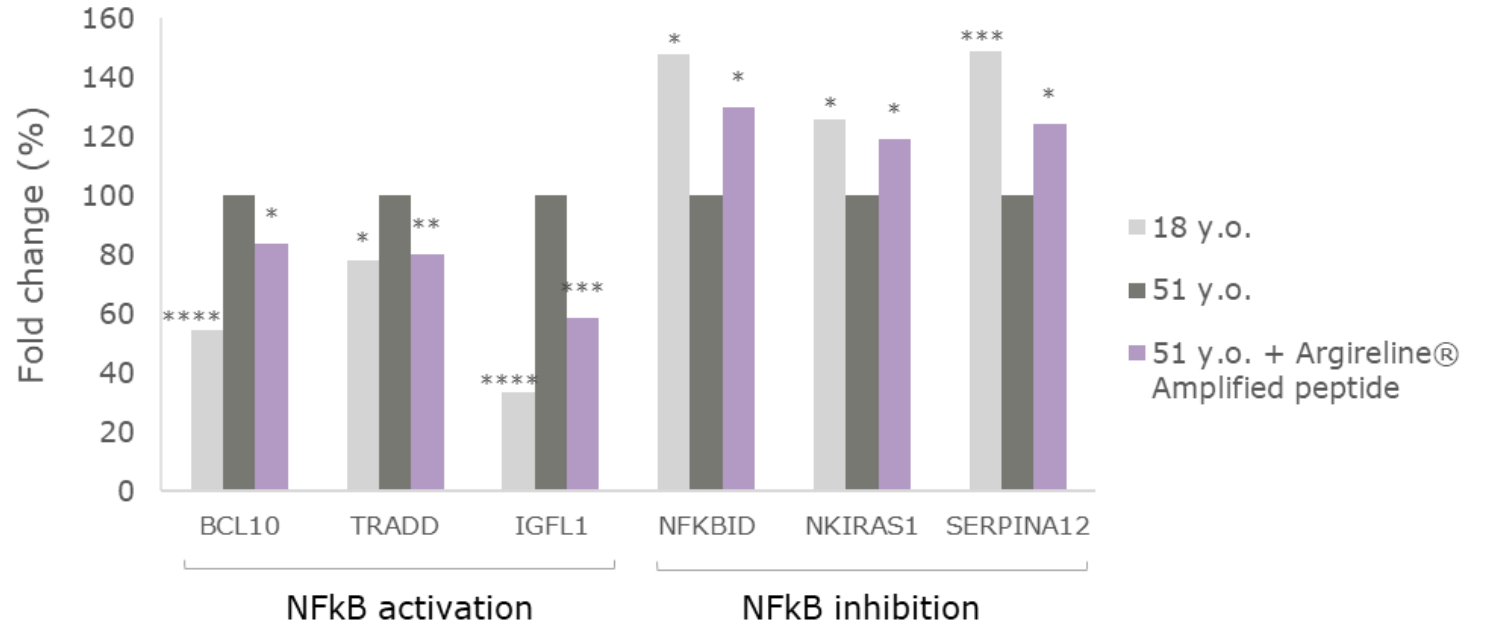
- reduced number of lamellar bilayers and decreased lipid content in the stratum corneum
- decreased expression of tight junction components

More fragile skin, with increased susceptibility to irritants or to water loss

Targeting the senescence cascade

Epidermal skin models from a **51 y.o.** donor treated with 10 µg/mL Argireline® Amplified peptide for 24 h or untreated. Models from **18 y.o.** donor used as control.

Expression of genes involved in senescence was analyzed by **RNA sequencing**.



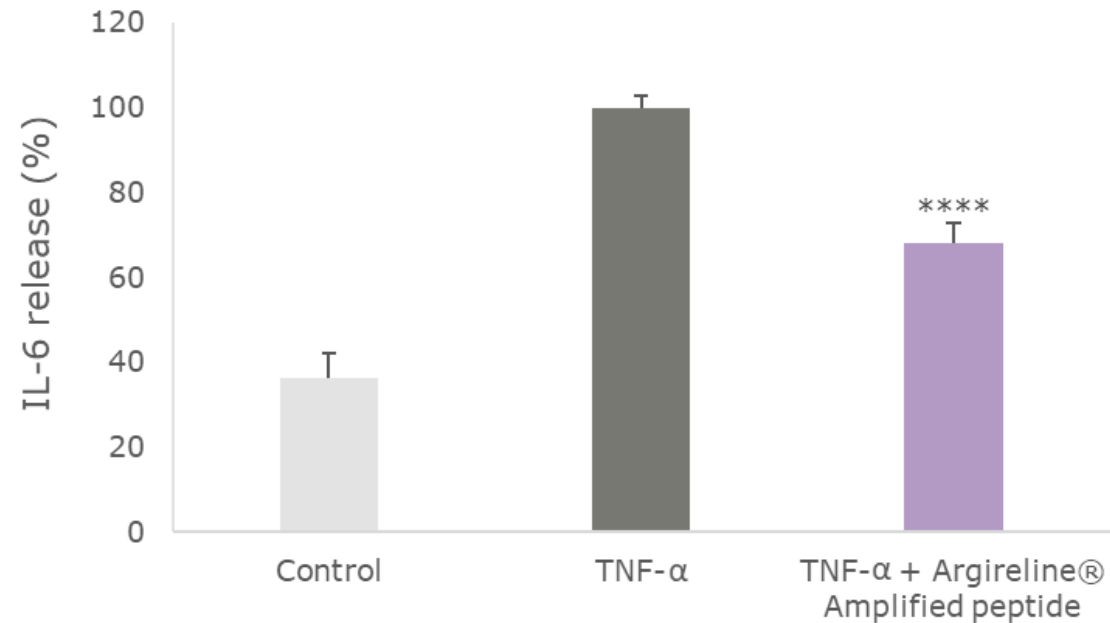
vs 51 y.o.: *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Gene expression regulation in favor of restricting the activity of NF-κB, a master regulator of the SASPs

Limiting the propagation of senescence

Epidermal keratinocytes were incubated with TNF- α , for the induction of IL-6 release, alone or in combination with 10 μ g/mL Argireline[®] Amplified peptide for 48 h.

IL-6 release was measured by ELISA.



Control: non-treated cells

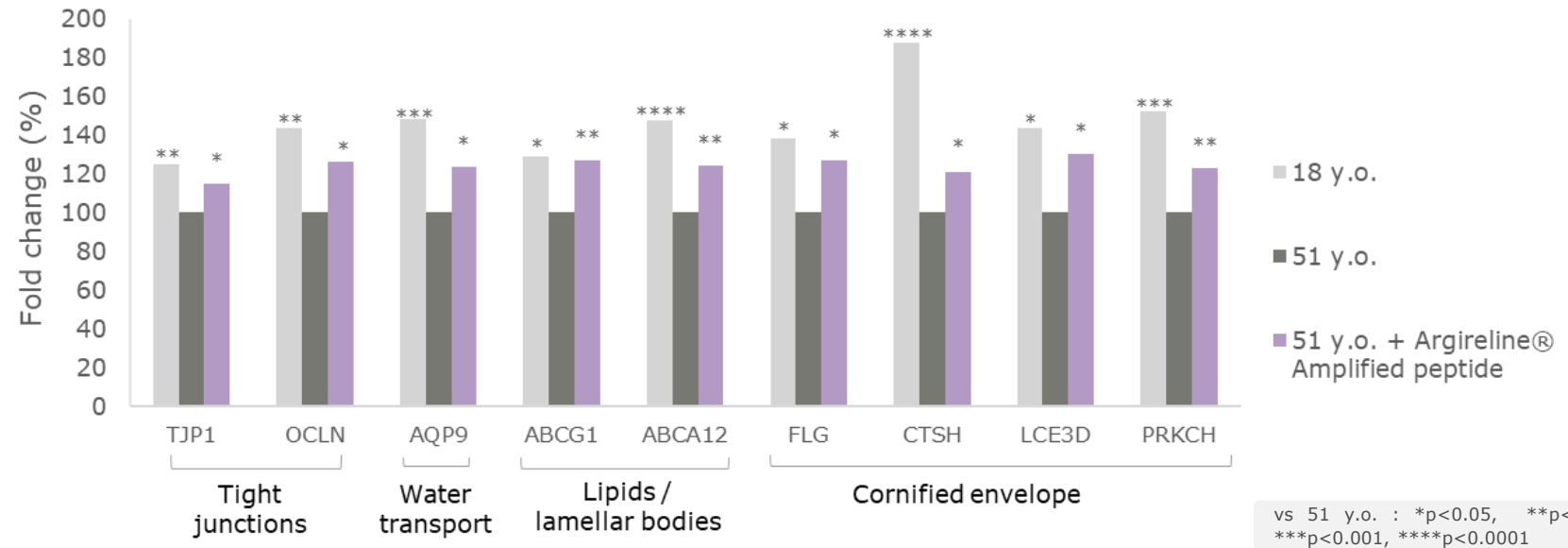
vs TNF- α : ****p<0.0001

Peptide could inhibit the release of cytokines of the SASPs that can damage the epidermal tissue

Counteracting the decline of barrier function

Epidermal skin models from a **51 y.o.** donor treated with 10 µg/mL Argireline® Amplified peptide for 24 h or untreated. Models from **18 y.o.** donor used as control.

Expression of genes involved in the barrier function was analyzed by **RNA sequencing**.



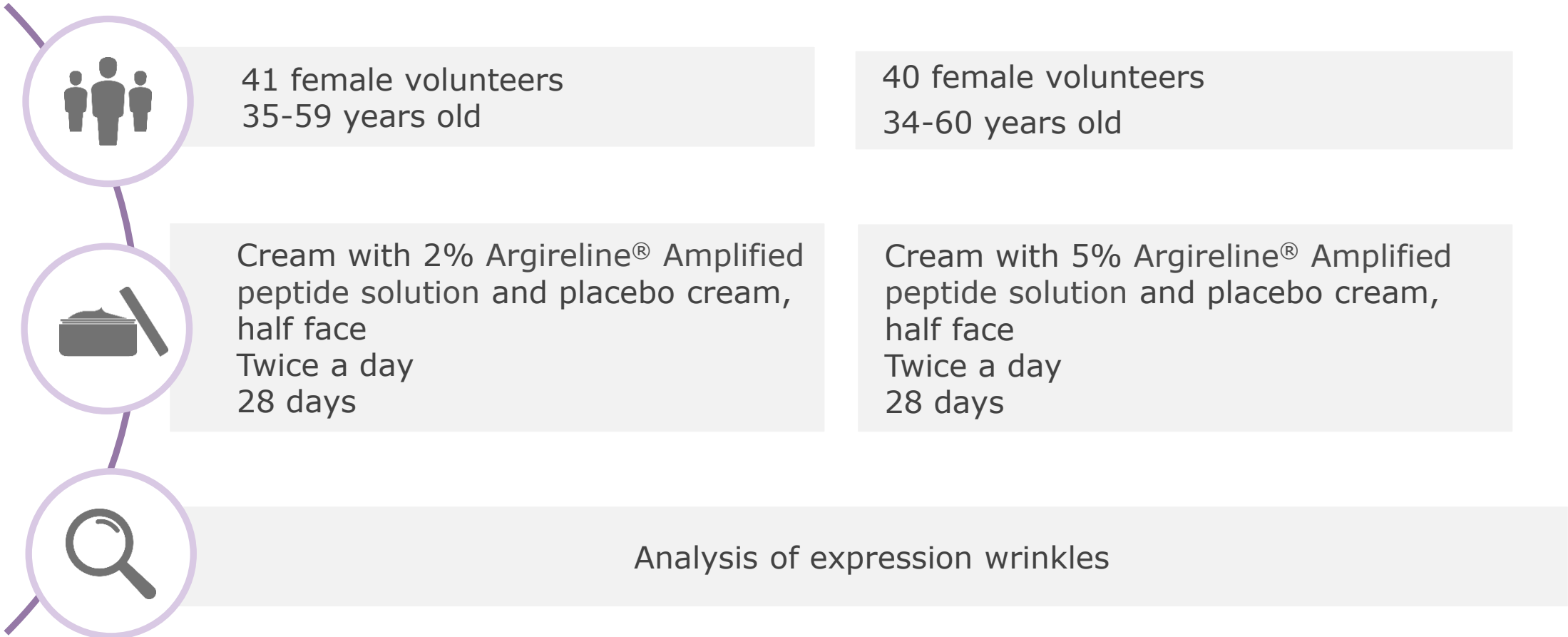
Argireline® Amplified peptide counteracts the dysfunction of epidermal barrier function that takes place with age



In vivo efficacy

- Improving appearance of expression wrinkles
- Feel the expressions and forget about wrinkles
- Multi-level improvement in tissue functionality
- What do the volunteers think?

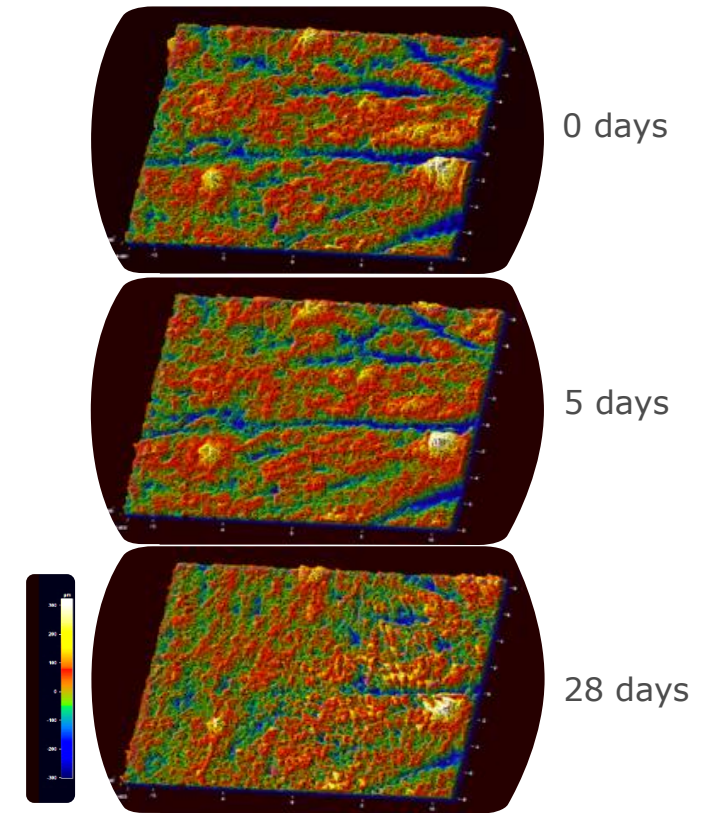
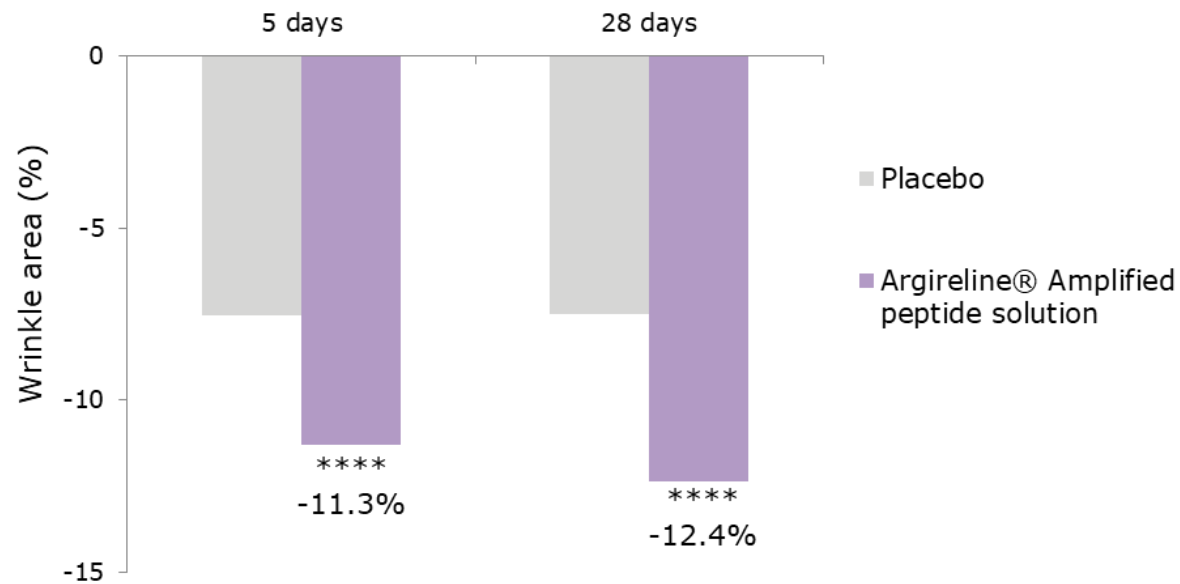
Improving appearance of expression wrinkles (I)



Improving appearance of expression wrinkles (II)

2% Argireline® Amplified peptide solution

The area of wrinkles on the crow's feet area was evaluated by means of real 3D microtopography imaging system based on fringe projection (PRIMOS) after 5 and 28 days.



Volunteer 6, 55 years old

Diminished area of wrinkles by an average of 11% in 5 days

vs initial time: ****p<0.0001 (5 and 28 days)

vs placebo: *p<0.05 (28 days)

Improving appearance of expression wrinkles (III)

0 days



5 days



28 days

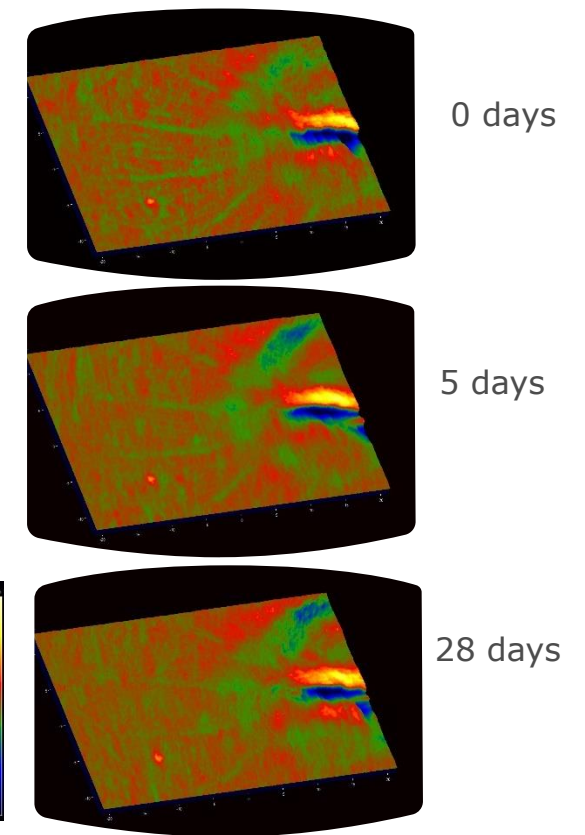
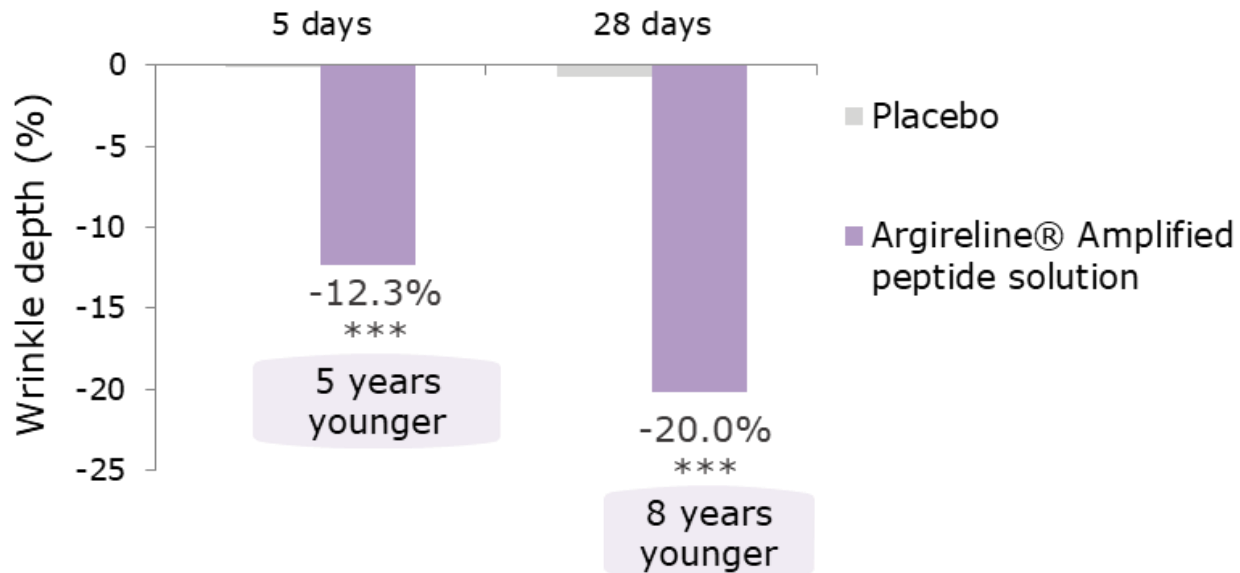


Volunteer 6, 55 years old

Improving appearance of expression wrinkles (IV)

5% Argireline® Amplified peptide solution

Wrinkle depth was measured on the crow's feet area by means of PRIMOS 3D after 5 and 28 days. By comparing with reference data on periorcular wrinkle depth and biological age, the "x years less" effect was calculated.



Volunteer 11, 43 years old

5 years younger-looking skin in only 5 days with 5% Argireline® Amplified peptide

vs initial time: ***p<0.001 (5 and 28 days)

vs placebo: ***p<0.001 (5 and 28 days)

Improving appearance of expression wrinkles (V)

5% Argireline® Amplified peptide solution

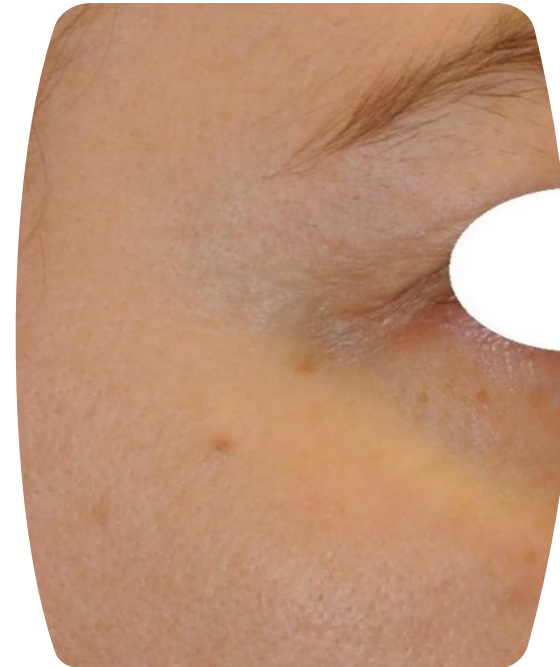
0 days



5 days

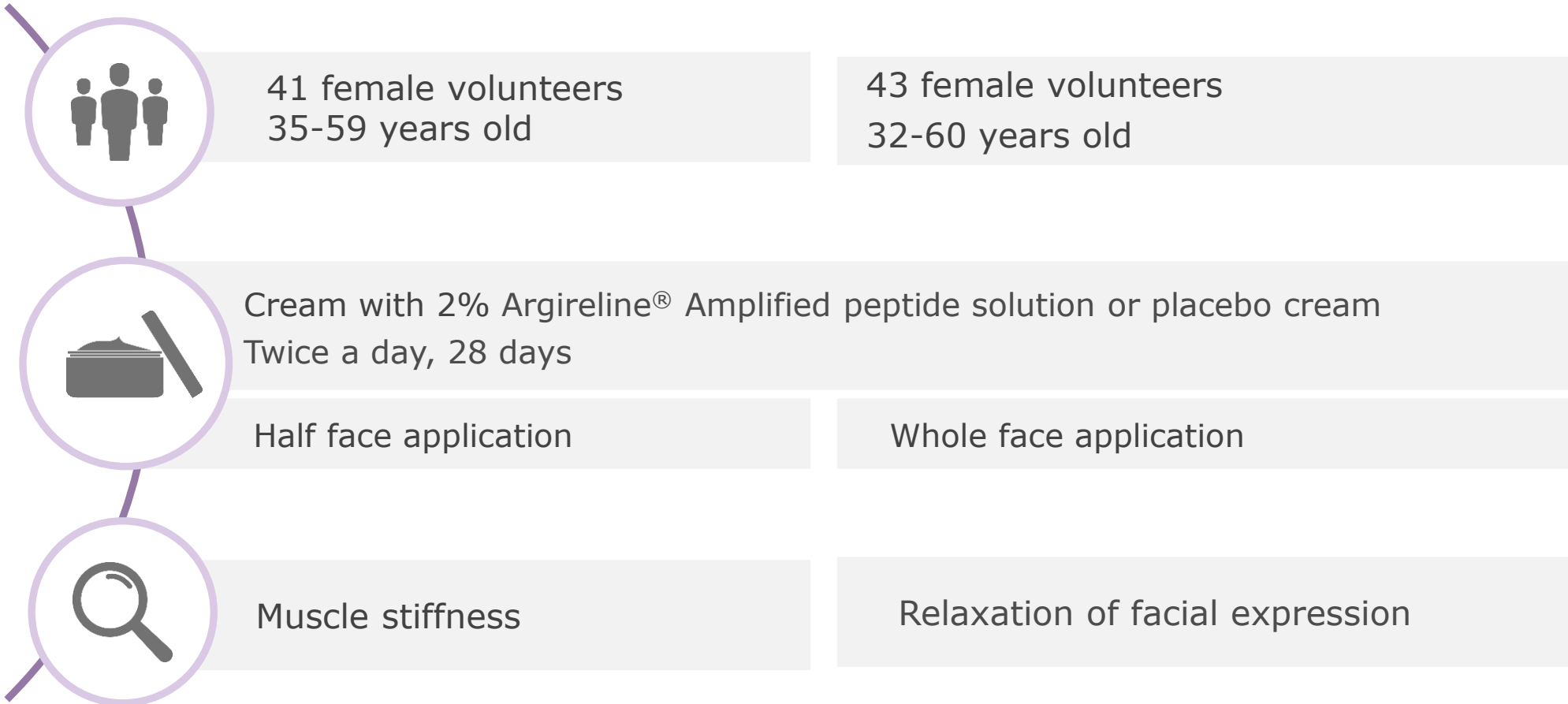


28 days



Volunteer 23, 48 years old

Feel the expressions and forget about wrinkles (I)



Feel the expressions and forget about wrinkles (II)

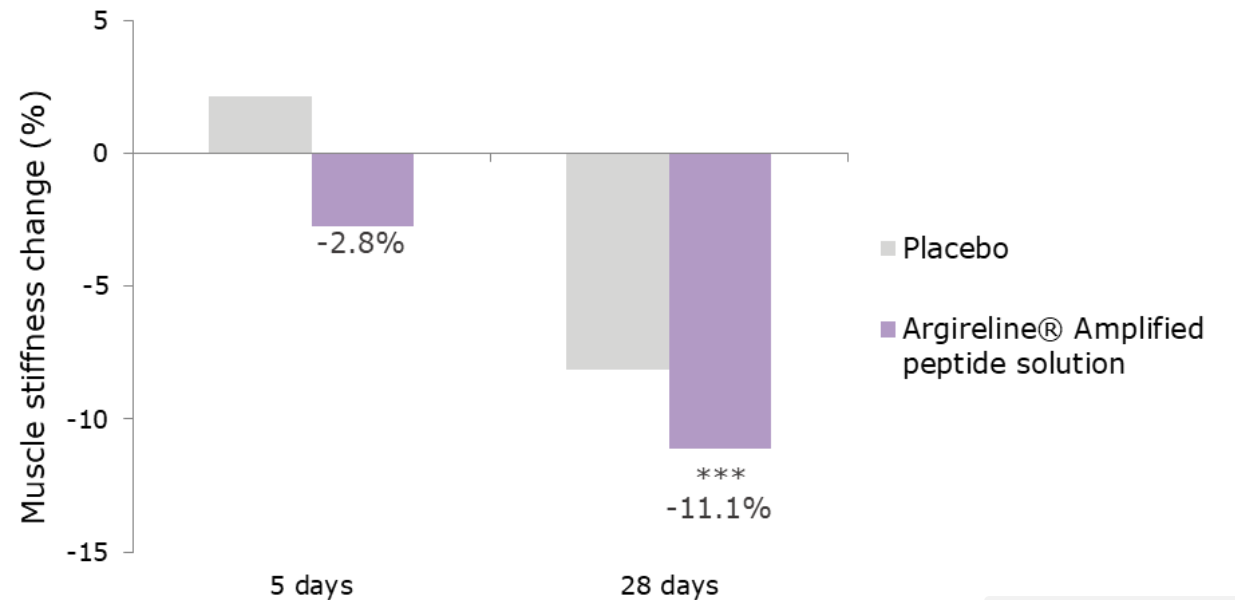
● Muscle stiffness

The stiffness of facial muscles in the cheek area was measured by myotonometry after 5 and 28 days of treatment.



MYOTONOMETER: provides an objective measure of the mechanical properties of muscles

MUSCLE STIFFNESS: increases with aging, reflecting the loss in the capacity to relax



Less stiff and more relaxed facial muscles, which are linked to a youthful state with less expression wrinkles

Feel the expressions and forget about wrinkles (III)

Relaxation of facial expression

The reduction in skin roughness was analyzed by means of 3D microtopography imaging system based on fringe projection (PRIMOS) 60 seconds after relaxing smiling facial expressions. The same evaluation was performed before and after 28 days of treatment.

Smile



Post-smile



Decrease of roughness (%) of the evolution between smile and post-smile

Crow's feet

-5.9% roughness
p<0.1 (vs initial time)

Placebo: +4.81% roughness

Nasogenian fold

-7.4% roughness
p<0.05 (vs initial time)

Placebo: -0.7% roughness

Improved post-expression relaxation, so you won't stop smiling

0 days (60 sec. post-expression) 28 days (60 sec. post-expression)



Volunteer 41

0 days (60 sec. post-expression) 28 days (60 sec. post-expression)



Volunteer 27

Multi-level improvement in tissue functionality (I)



41 female volunteers
35-59 years old



40 female volunteers
37-60 years old



Cream containing 2% Argireline® Amplified peptide solution and placebo cream, half face
Twice a day
28 days

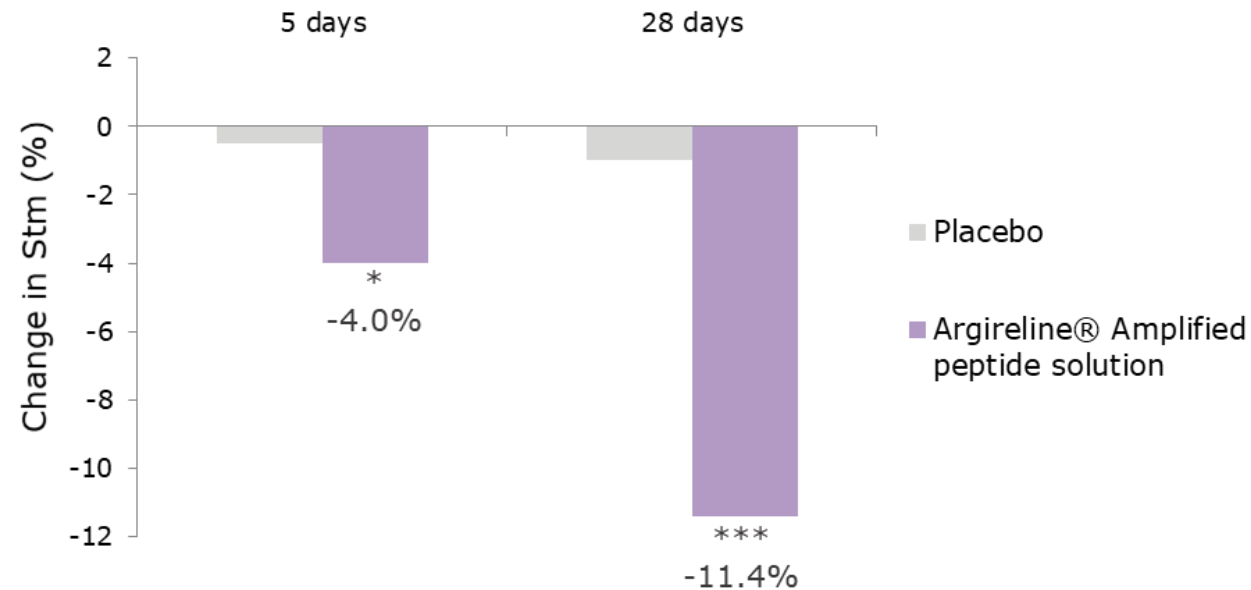


Skin roughness	Fine lines	Skin isotropy	Skin radiance
Firmness and elasticity	Skin fatigue	Volumizing effect	Lifting effect

Multi-level improvement in tissue functionality (II)

● Skin surface roughness

The profilometry of the skin surface was evaluated by means of 3D microtopography imaging system based on fringe projection (PRIMOS). Changes in the average maximum height (Stm) of the skin profile were calculated.



Smoothing effect on the profile of the skin surface

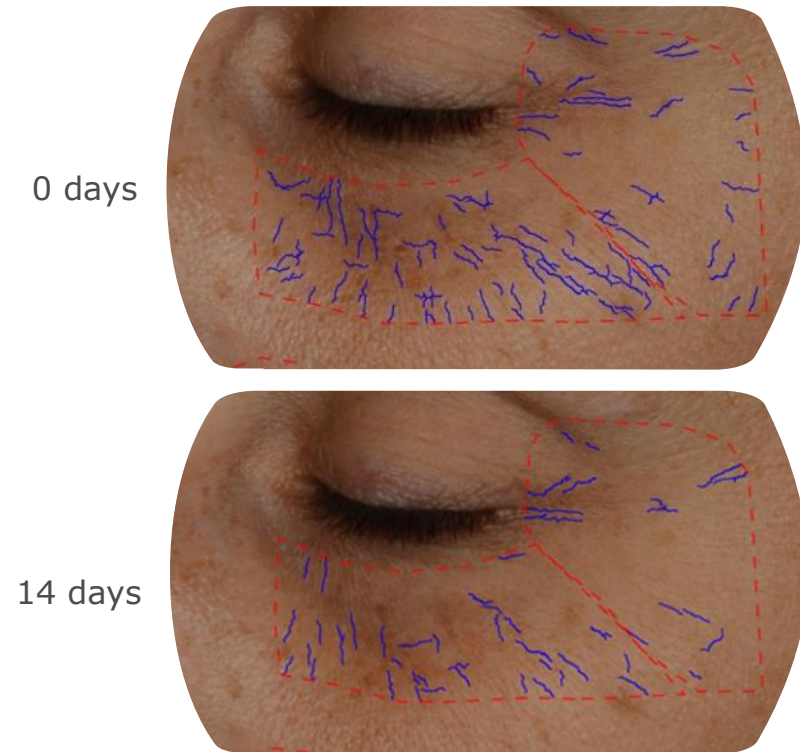
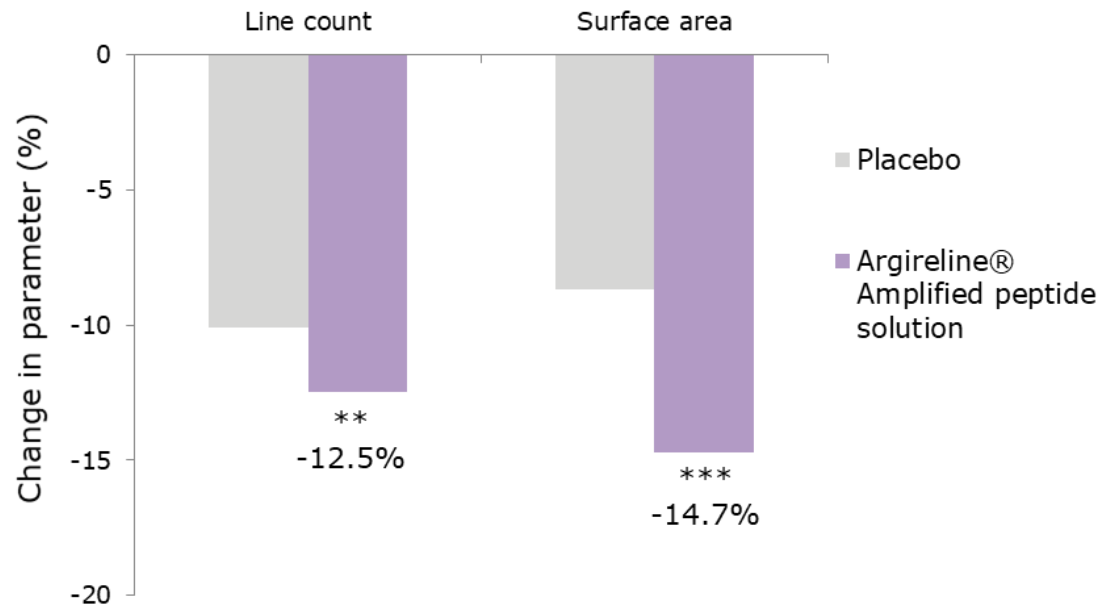
vs initial time: *p<0.05 (5 days), ***p<0.001 (28 days)

vs placebo: *p<0.05 (5 days), ***p<0.001 (28 days)

Multi-level improvement in tissue functionality (III)

Fine lines

The presence of fine lines on the skin surface was measured after 14 days by means of image analysis software.



Global reduction of visible fine lines

Volunteer 51, 38 years old

vs initial time: **p<0.01, ***p<0.001

Multi-level improvement in tissue functionality (IV)

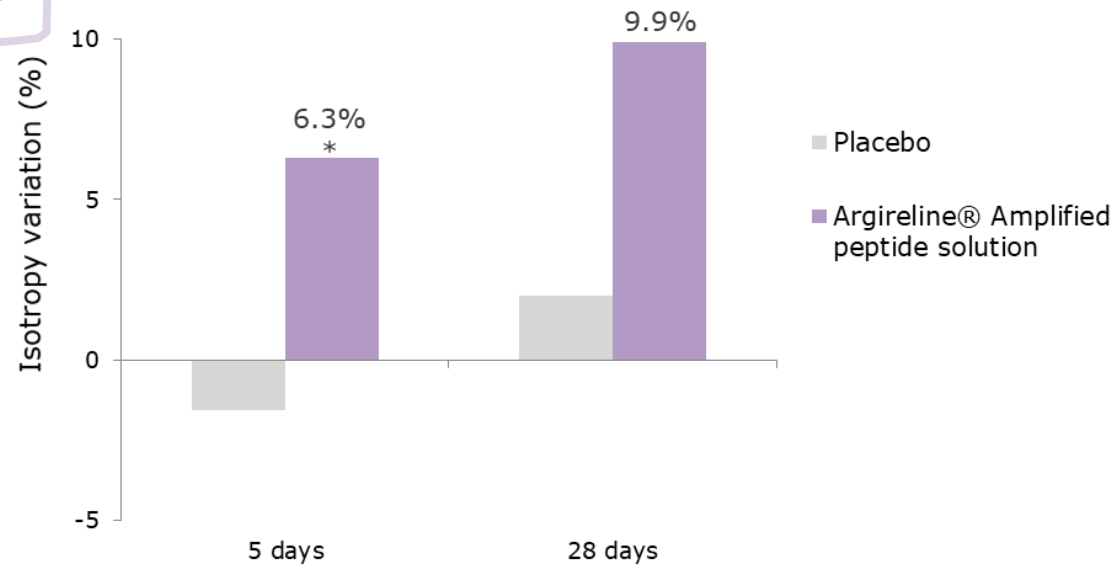
● Skin isotropy

Parameter measured in the crow's feet area by means of real 3D microtopography imaging system based on fringe projection (PRIMOS) after 5 and 28 days.

ISOTROPY: orientation of superficial lines in all directions

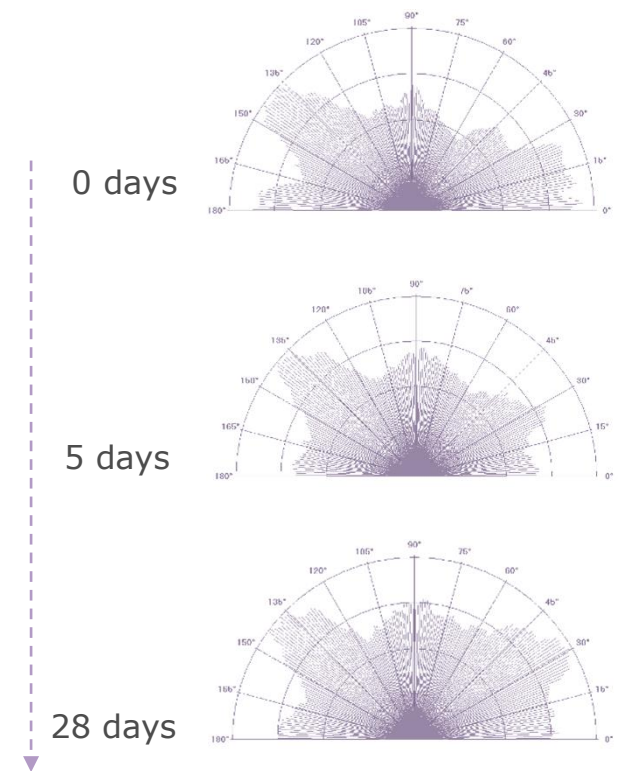
Younger skin has higher isotropy, meaning that all the superficial lines have different orientations. With time, lines become oriented in a certain direction, altering skin's biomechanical properties.

vs initial time: *p<0.05 (5 days)
vs placebo: *p<0.05 (5 days)



Improved directionality of superficial lines

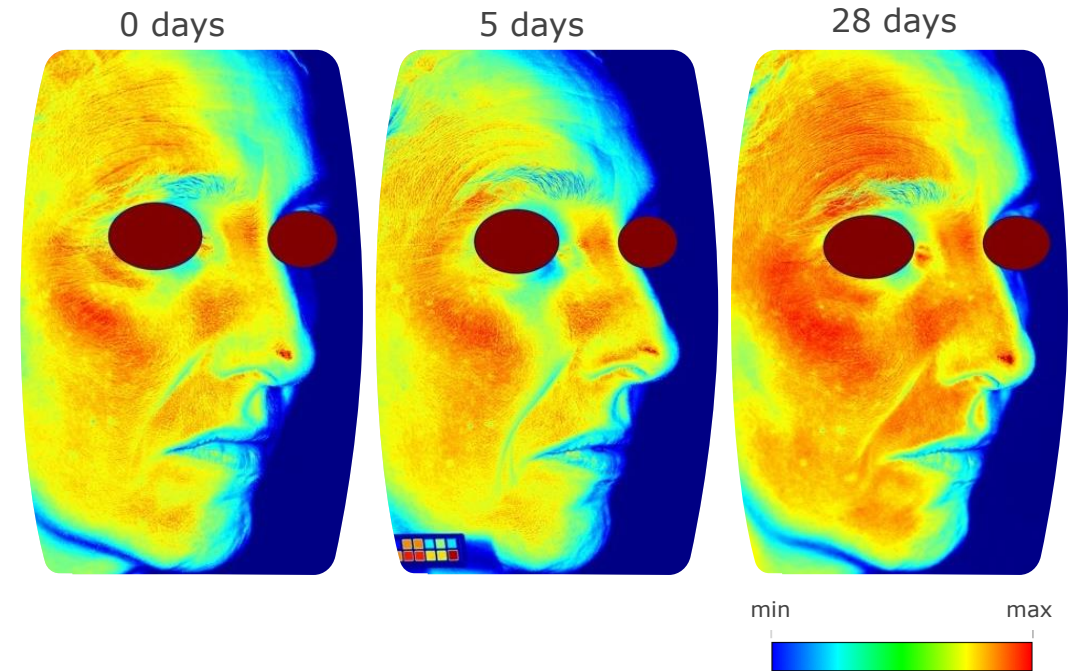
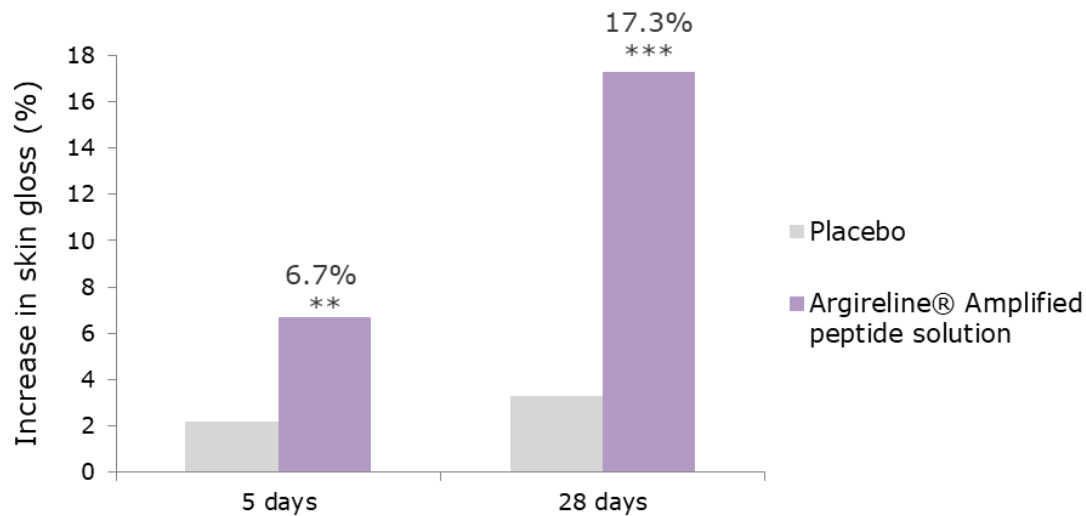
Volunteer 11, 52 years old



Multi-level improvement in tissue functionality (V)

● Radiance of the skin

The gloss parameter, corresponding to the ability to reflect light, was determined by spectrophotometry.



A more radiant complexion was detected after the application of the active ingredient

vs initial time: **p<0.01, ***p<0.001

vs placebo: *p<0.05 (5 days), ***p<0.001 (28 days)

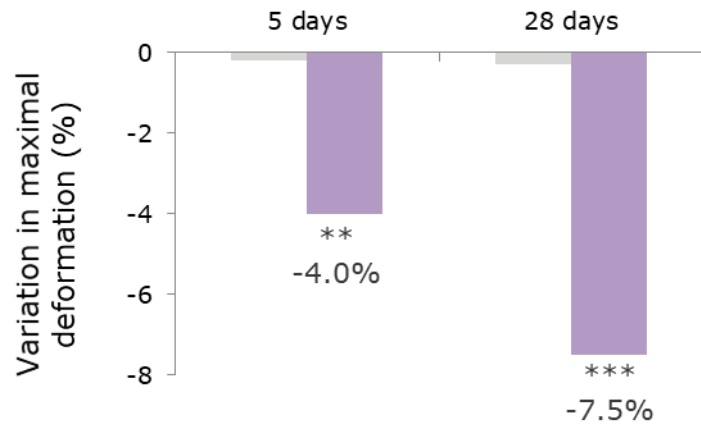
Multi-level improvement in tissue functionality (VI)

Firmness and elasticity

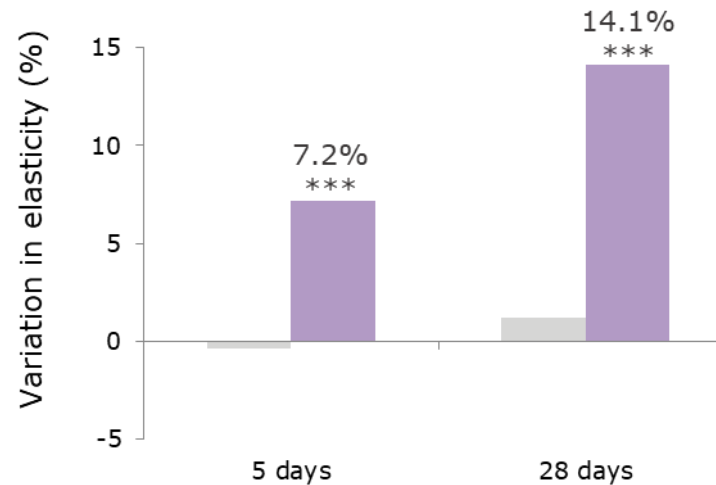
The biomechanical properties of the skin were measured by means of cutometry.

■ Placebo
■ Argireline® Amplified peptide solution

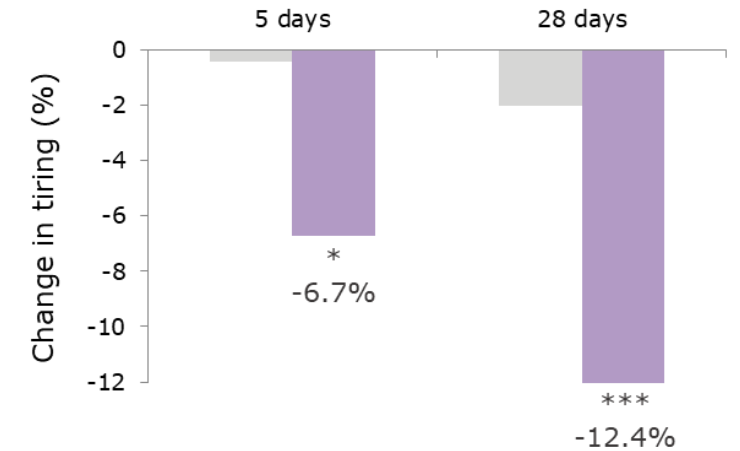
Maximal deformation (R0)



Elasticity (R2)



Tiring effect (R9)



vs initial time: **p<0.01, ***p<0.001

vs placebo: *p<0.05 (5 days), ***p<0.001 (28 days)

vs initial time: ***p<0.001 (5 and 28 days)

vs placebo: **p<0.01 (5 days); ***p<0.001 (28 days)

vs initial time: *p<0.05; ***p<0.001

vs placebo: **p<0.01 (28 days)

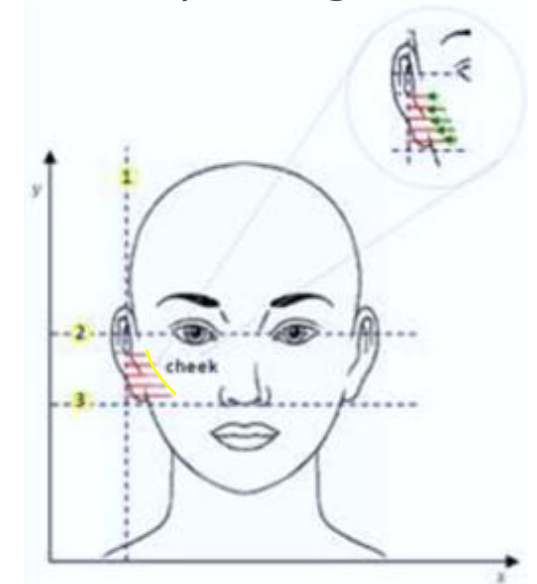
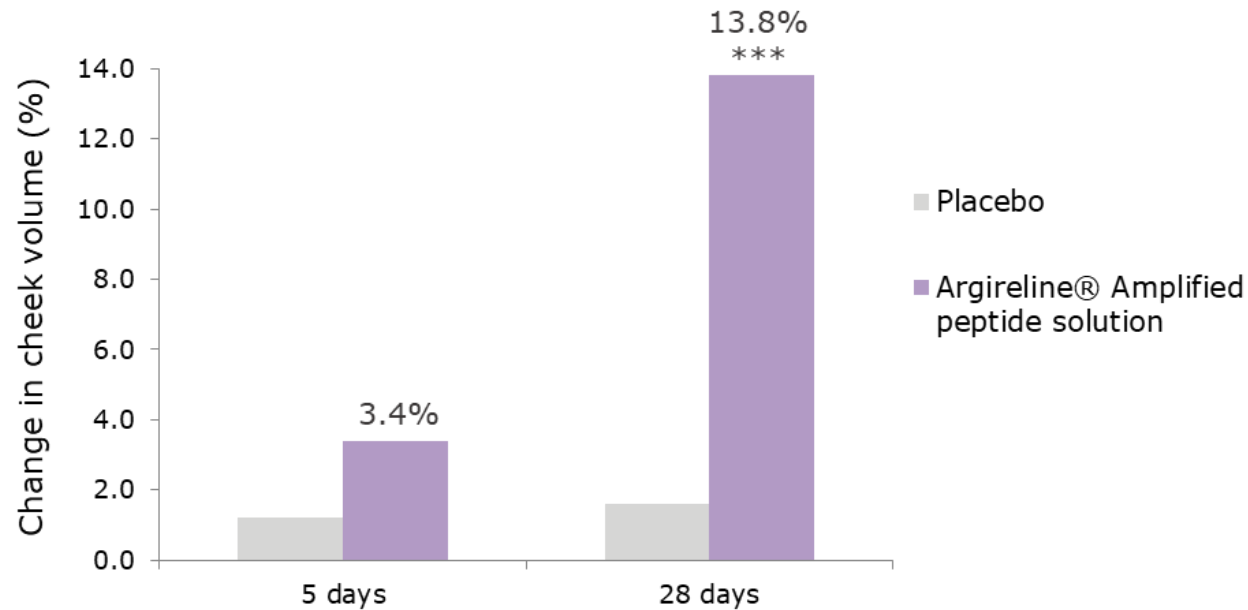
Increase in firmness and elasticity for a tensor effect

Less fatigued skin after treatment with the peptide

Multi-level improvement in tissue functionality (VII)

Volumizing effect

Changes in volume were evaluated on the cheeks by means of image analysis, involving the measurement of the distance between the cheekbone profile and a line passing vertically through the ear.



A decrease in cheek distances (mm) corresponds to an increase in volume (%)

Volume increase on the cheeks area, leading to a more youthful facial appearance

vs initial time: ***p<0.001 (28 days)
vs placebo: ***p<0.001 (28 days)

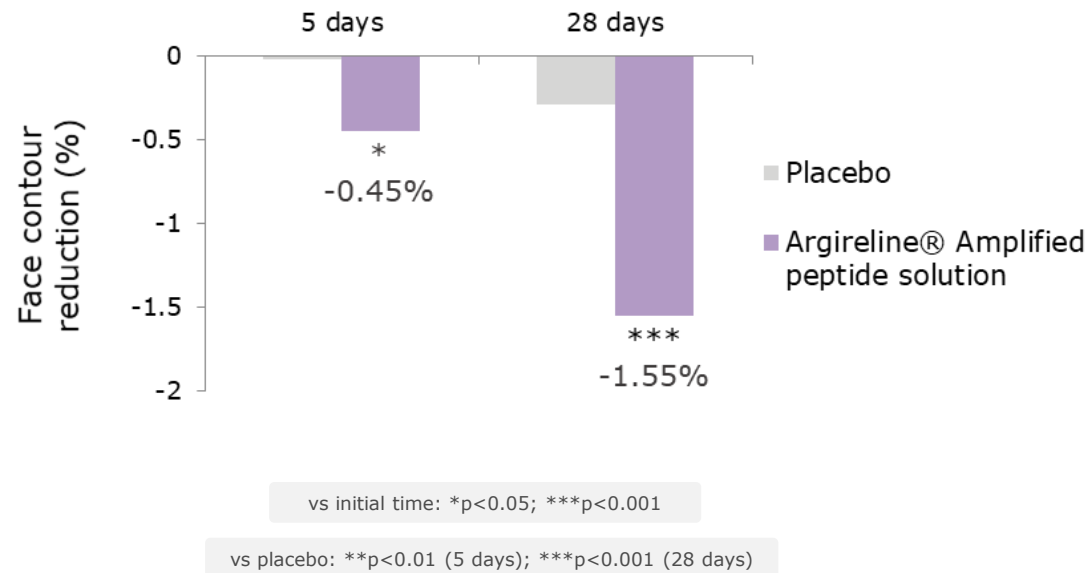
Multi-level improvement in tissue functionality (VIII)

Lifting effect

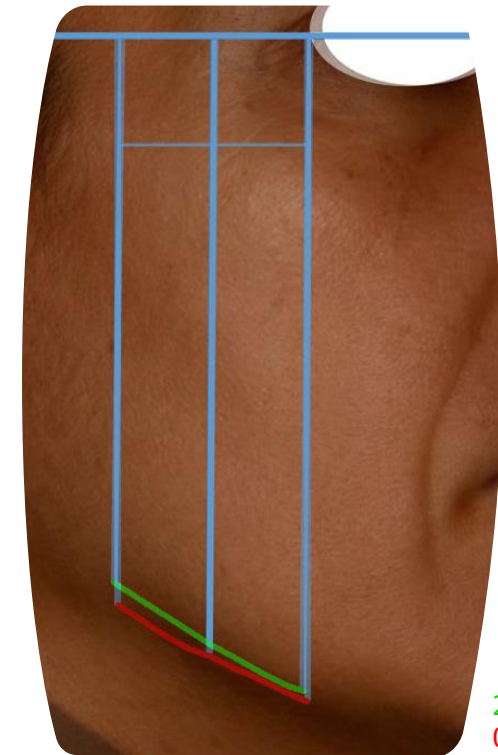
The analysis of the three vertical lines is performed by means of a specific software as reported in the picture below.



The shorter the distance of the 3 vertical lines, the bigger the lifting effect.



Argireline® Amplified peptide helps reduce skin sagginess



28 days
0 days

“ What do the volunteers think? ”

Day 4: First impressions

*I noticed that **my skin feels very tight**, so **I think it will remove my wrinkles** as more weeks go by. This sensation **makes me feel good**, and since I also feel that my skin is more hydrated I believe it will go really well for me.*

- Carmen

Day 13

*Well, I'm noticing that **my skin is much smoother**. It has a **little less wrinkles** and it's super hydrated. When I apply it, I notice how it has an **effect of stretching the skin**, especially in the **crow's feet**, also a little on the **forehead**, and I'm very happy.*

- Elisabet

Day 27: End of treatment

*My **wrinkles have faded around my eyes and my forehead**. Of course, the wrinkles around my mouth are probably a lot more difficult to erase, but I have noticed that they have improved around my eyes and forehead. I'm not sure if they are more noticeable without glasses, but in truth, except for the smile lines around my mouth, everything else **has been a success**.*

- Chelo

*The **wrinkles have diminished**, I don't notice much difference on the expression lines on the side of the lips, but on the **side of the eyes** I notice that they **have softened**, they **have diminished** and on the **forehead** I also notice that they have diminished, they have softened.*

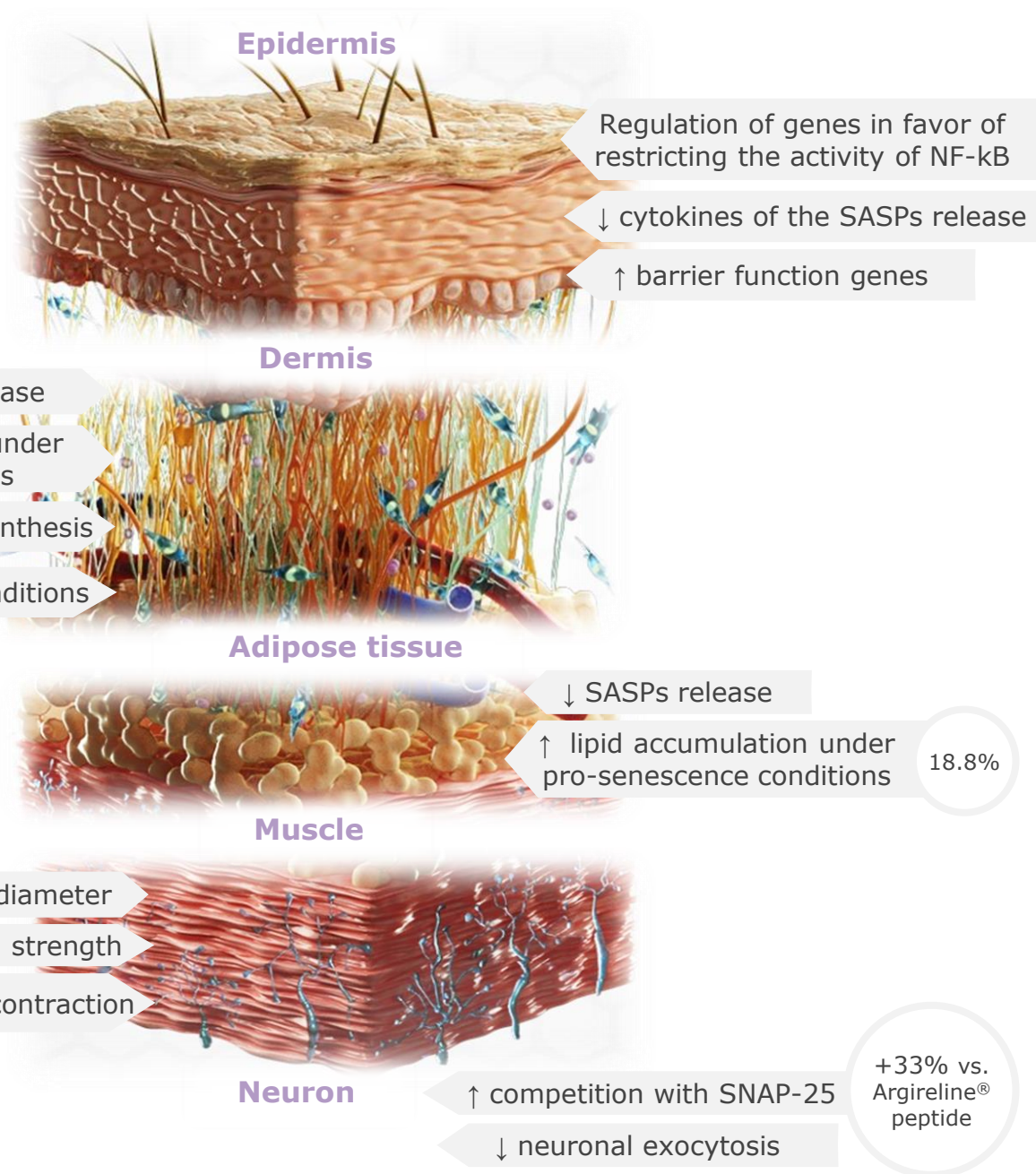
- Joana

*After using the cream for one month, I noticed my **skin becoming much more luminous and firm**, I can see a **big difference from before** and **I am very satisfied**.*

- Elena

”

Conclusions (I)



Conclusions (II)

Attenuated crow's feet



Improved post-expression relaxation

Multifunctional efficacy

at 2%



Applications and claims

Application ideas






- ✓ Skin care products intended to offer a complete care for expression wrinkles.
- ✓ Multifunctional products aimed to cut down the number of steps in beauty routines, without sacrificing efficacy.
- ✓ Cosmetic formulations intended to globally improve the skin appearance for a younger and better overall look.

Claim ideas

- ✓ All-in-one solution to your beauty routine
- ✓ Express yourself beautifully
- ✓ Simple, yet effective care for your skin
- ✓ Looking good even after smiling
- ✓ Multilayer care for a younger-looking skin



Technical information

Appearance	Transparent solution containing 0.05% Acetyl Hexapeptide-8
INCI	Water (Aqua), Acetyl Hexapeptide-8, Sodium Benzoate*   
Natural origin content	99.5% (according to ISO16128)
Solubility	Water soluble
Dosage	2-5%
Recommended pH	4.0 – 8.0

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