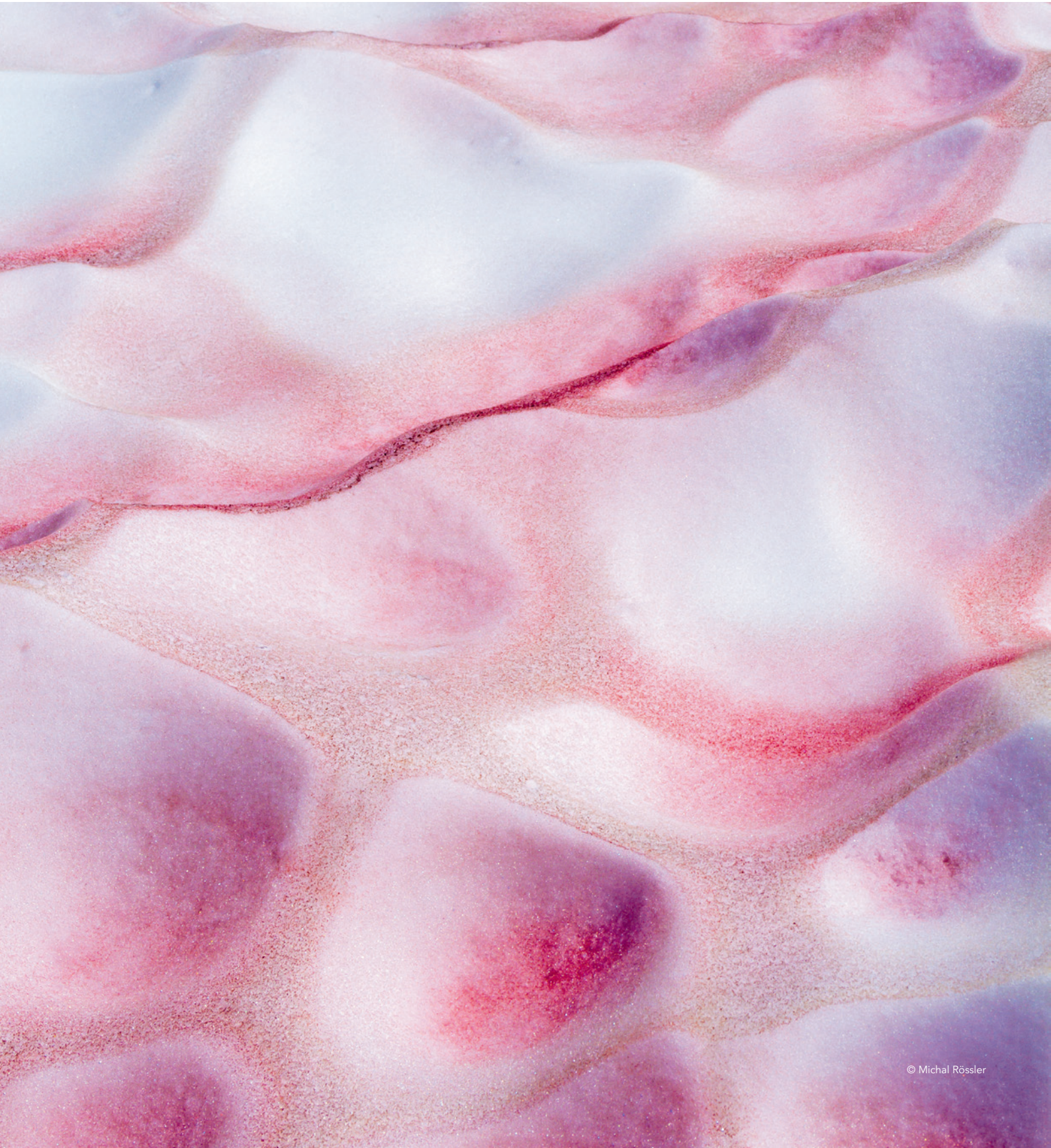




PRELIMINARY

Snow Algae Powder

Key to skin's longevity



Snow Algae Powder

Key to skin's longevity

Repairing the Effects of Time by Mimicking Calorie Restriction

With Snow Algae Powder, Mibelle Biochemistry is offering an anti-aging treatment that is based on a new mechanism: the calorie restriction.

Calorie restriction has been shown to improve both the healthspan and lifespan of individuals and is thus considered to be a promising new anti-aging pathway.

Snow Algae Powder mimics the effects of calorie restriction and in this way improves the longevity of the skin cells. Consequently, it offers the skin the benefits of a diet without the need to undergo a low-calorie regime.

This innovative anti-aging active ingredient is based on the extract of a unique extremophile algae species that has managed to develop survival strategies that enable it to grow on glaciers and in eternal snow.

At the cellular level, Snow Algae Powder protects and activates two key factors of the calorie restriction pathway: the Klotho longevity gene and the AMPK energy sensor that together lead to improved cellular defenses, oxidative stress resistance, cell detoxification and repair.

The results in the skin are the production of collagen starting again and a rejuvenation of the dermal epidermal junction.

Consequently, the skin barrier is reinforced while the skin appears fresher and detoxified as age spots are less visible.

Claim Ideas for Snow Algae Powder

- Protects and activates longevity factors in skin cells
- Rejuvenates and protects skin at cellular level
- Safeguards skin's youthfulness by activating Klotho
- Strengthens cellular defense mechanisms through calorie restriction mimetic activity

Applications

- Rejuvenating and repair formulas
- Age-defense products
- Youth protecting and promoting skin care
- Formulas to increase skin's longevity – face, body, hand

Formulating with Snow Algae Powder

- Recommended use level: 2–3%
- Incorporation: Dissolve Snow Algae Powder into the aqueous phase or add pre-solved, during the cooling phase (<60 °C). Snow Algae Powder is dissolvable at up to 20% in water.
- Thermostability: Temperatures of up to 60 °C for a short time do not affect the stability of Snow Algae Powder.

INCI/CTFA-Declaration

Chlamydocapsa sp.-101 Extract* (and) Maltodextrin (and) Lecithin (and) Aqua/Water

*INCI not yet confirmed

Additional Information

Preservative-free

Snow Algae

A unique extremophile algae from Swiss mountains

A Swiss Cryophilic Algae

- Snow algae are single-cell algae with the unique ability to actively live at a temperature of approximately 0 °C. In contrast, most other plants either reduce their metabolic activities or are simply unable to survive at this temperature.
- Snow algae grow in areas where there is either an Alpine or polar climate in which there is snow all year round (eternal snow). Therefore, these extremophile algae have the ability to thrive in freezing water with very low nutrient levels.
- *Chlamydomonas nivalis* is the most common type of snow algae.

From a Green to Red Color

Snow algae adapt to their environment by modifying their pigmentation:

- at the beginning of winter, the snow algae enter into a dormant phase, resting in the form of red spores under the snow. In fact, the algae turn red as they produce considerably more carotenoid pigments than chlorophyll ones in order to protect against UV rays.
- in spring, increased levels of nutrients and light, as well as melted ice, stimulate germination: snow algae cells appear green as they predominantly contain chlorophyll pigments. Chlorophyll absorbs energy from light in order to convert it into chemical energy (carbohydrates) that will fuel the activities of the algae. This is the photosynthesis process. In addition, the snow algae green cells have a pair of front-mounted flagella that enables them to ply the films of water found in melting snow. Consequently, the snow algae can travel towards the surface of the snow where they propagate.
- at the end of summer, this growth/reproduction stage alternates with a dormant one. As a result, snow algae green cells transform once again into red spores to survive until the following summer.

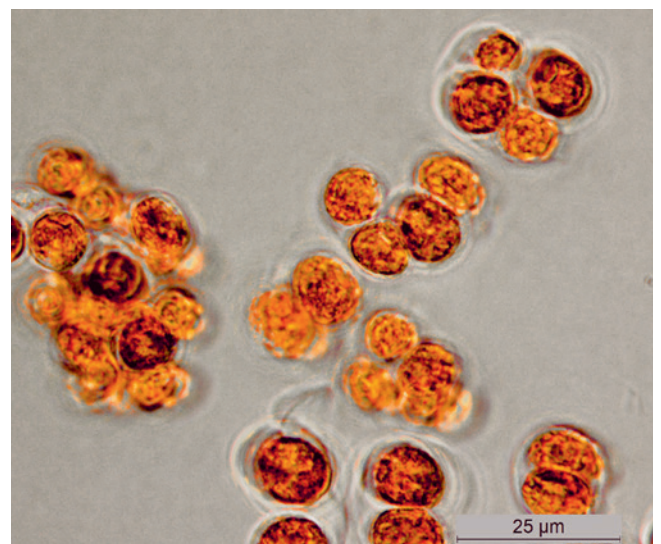
Pigment concentration change is one of the strategies developed by the snow algae to enable them to adapt to their extreme habitat. Production of other secondary metabolites such as biopolymers (gallerten), antifreeze glycoproteins (AFGPs) stress modifiers and osmotically active amino acids and sugars also help these extremophile algae to survive in their habitat.

Snow Algae Adapt their Pigmentation according to their Environment

Green color (favorable conditions)



Red color (unfavorable conditions)



Production of Snow Algae Powder

A sustainable process based on a tailor-made bioreactor

From the Snow Algae to the Active Ingredient

Mibelle Biochemistry has succeeded in cultivating snow algae cells in special reactors in conditions mimicking their natural environment:

- firstly, the snow algae grow in a medium supplied with light and air (including CO₂) to mimic favorable growth conditions. During this stage, the snow algae take on a green appearance due to the chlorophyll pigments that they contain.
- from there, the level of nutrients is reduced and light is strongly increased. As a consequence, the snow algae start to produce carotenoid pigments in a high concentration to protect against UV rays. Therefore, the snow algae turn red and they are then harvested.

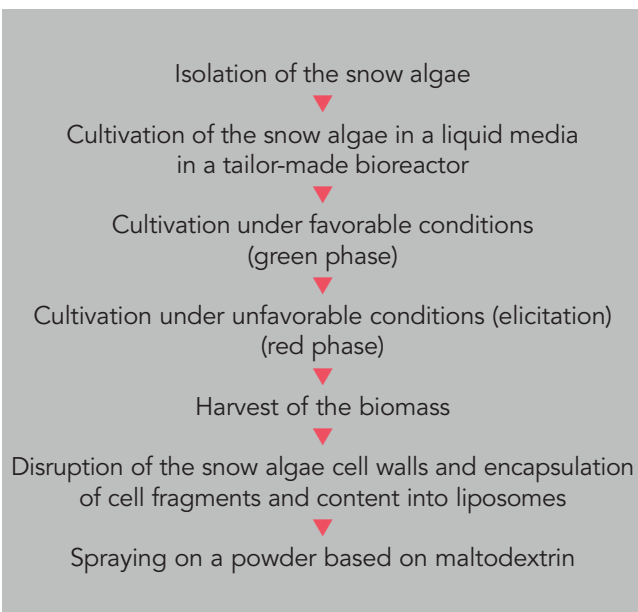
To obtain Snow Algae Powder, our cosmetic active ingredient, the cells of the snow algae are homogenized at 1200 bar together with phospholipids to encapsulate and stabilize their oil-soluble and water-soluble components into liposomes. The resulting suspension is carefully sprayed on a powder based on maltodextrin.

Advantages of the Technology

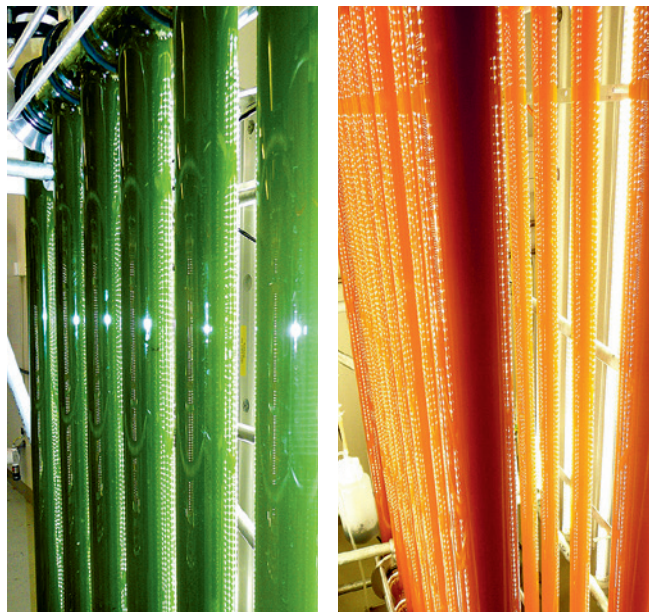
This innovative technology that Mibelle Biochemistry has developed offers the following advantages:

- preservation of the snow algae species (sustainable process based on biotechnology)
- availability of snow algae material regardless of the season and market demand
- snow algae material that is completely free of environmental pollutants and pesticides
- constant concentrations of metabolites in the extract through a defined and controlled process.

Production Process of Snow Algae Powder



A Tailor-Made Bioreactor



Calorie Restriction

A new and highly promising anti-aging strategy

Calorie Restriction Improves Longevity

Calorie restriction (CR) is a dietary regimen that involves reducing the calorie intake by between 25% and 65%, while maintaining optimal levels of protein, vitamins and minerals.

CR has been demonstrated in a variety of species, such as yeast, apes, mice and rats, to protect against stress and decelerate the biological aging process resulting in improved healthspan and extended lifespan.

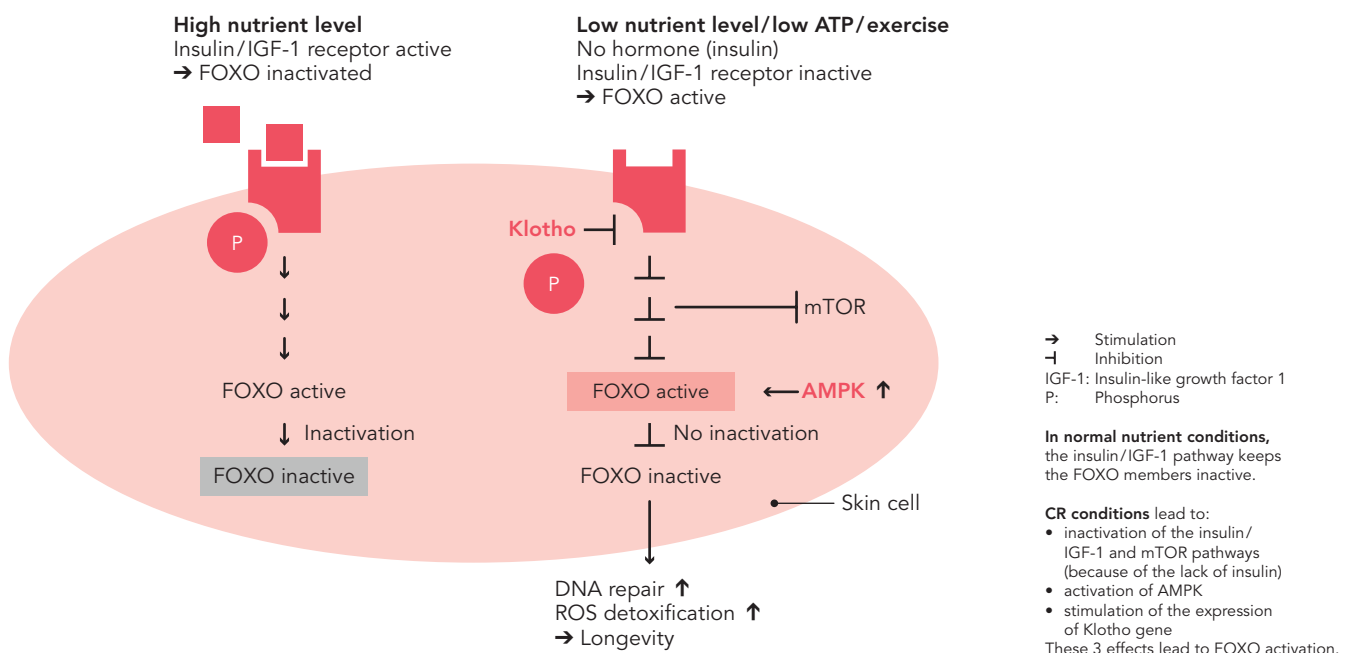
Therefore, CR is regarded as a promising pathway to slow down the signs of aging.

At the Cellular Level, CR Activates Detoxification and DNA Repair

CR causes a decrease in the nutrient (glucose and insulin) levels and ATP, which leads to a downregulation of the insulin/IGF-1 pathway and the nutrient sensor mammalian target of rapamycin (mTOR) and an upregulation of the AMP-activated protein kinase (AMPK).

This results in the activation of the forkhead transcription factors FOXO that control the response to different types of stress, regulating the cell cycle and promoting cell survival via DNA damage repair and free radical detoxification in the cells.

Mechanism of Calorie Restriction



Snow Algae Powder

Rejuvenates the skin by mimicking calorie restriction

Two Key Factors Mediate the CR Pathway

Discovered recently, the **Klotho** gene was found, in mice, to extend the lifespan when overexpressed, while it accelerates aging when its expression is disrupted. The name of this longevity gene comes from the Greek goddess Klotho. In Greek mythology, it was Klotho who controlled the lives and destinies of everyone.

- The Klotho gene codes for a transmembrane protein, the extracellular domain of which is shed and secreted.
- The secreted Klotho protein inhibits the phosphorylation of the IGF1 receptor which leads to the repression of the insulin/IGF-1 pathway. Therefore, Klotho activity results in an activation of FOXO.
- Activation of FOXO will induce anti-aging activity by stimulating detoxification and DNA-repair genes.

AMP-activated kinase (**AMPK**) is a sensor of energy that activates in response to low energy levels in order to maintain energy homeostasis. When activated, AMPK stimulates energy production from glucose and fatty acids and inhibits energy consumption.

- Exercise stimulates AMPK.
- CR also stimulates AMPK whereas nutritional overload impairs its activity.
- Activation of AMPK improves cellular antioxidant functions and resistance to stress.
- As we get older, the sensitivity of AMPK declines, which leads to an impaired metabolic regulation and increased oxidative stress.

Mechanism of Snow Algae Powder

Snow Algae Powder improves the longevity of the skin cells by mimicking the effects of CR and therefore by optimizing skin metabolism. As a result, Snow Algae Powder offers the benefits of a diet to the skin without the need to undergo a low-calorie regime.

In fact, Snow Algae Powder protects and activates two key factors of the CR pathway: the Klotho longevity gene and the AMPK energy sensor that together lead to improved cellular defenses, oxidative stress resistance, cell detoxification and repair.

Results:

- the age-induced decrease in collagen production is stopped and even reversed. In addition, the activity of the MMP is strongly reduced.
- the dermal epidermal junction recovers its original wavy architecture and is therefore rejuvenated.
- the skin barrier is reinforced.
- skin appears fresher and detoxified as age spots are less visible.

Snow Algae Powder

Study results



Stimulation of Klotho in Aged Skin Cells

The effect of Snow Algae Powder on the expression of the Klotho gene was evaluated in aged human dermal fibroblasts in a replicative aging model (Hayflick model).

Aged fibroblasts (passage 17) were cultured either with or without 0.1% Snow Algae extract. A control with normal fibroblasts (passage 8) was also performed in parallel. From there, the expression of the Klotho gene was analyzed by quantitative PCR.

Replicative senescence of fibroblasts induced a down-regulation of the anti-aging Klotho gene. However, Snow Algae Powder was shown to neutralize this decrease and even overcompensate for it as Klotho expression was stronger in aged fibroblasts treated with the active ingredient than in young and untreated ones.



Protection and Activation of AMPK

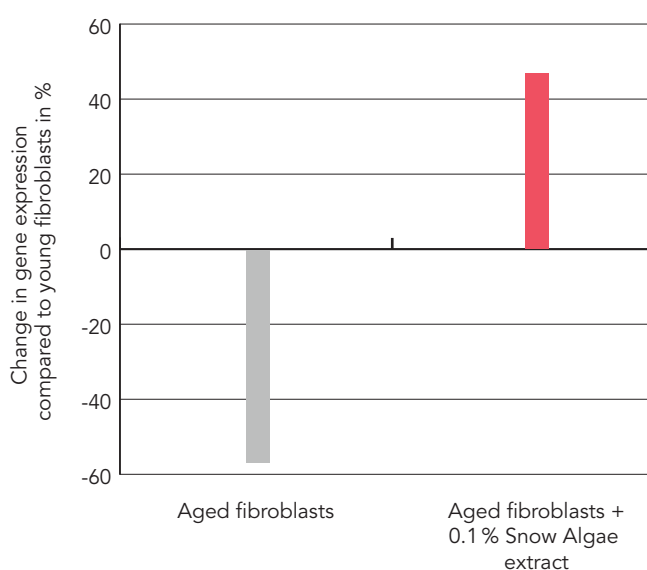
Normal human epidermal keratinocytes were cultivated either with or without Snow Algae extract under normal or calorie excess conditions (with 60ng/mL of insulin). The amounts of total AMPK and activated AMPK were determined by Western blot analysis.

Results showed that Snow Algae extract stimulated phosphorylation of AMPK in a dose-dependent way and in both types of condition. However, the stimulation was much stronger in calorie excess conditions.

Consequently, Snow Algae extract:

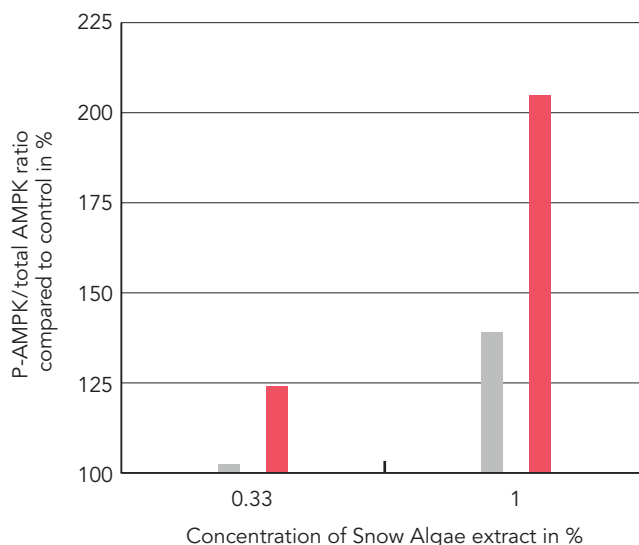
- Stimulates activation of AMPK under normal and calorie excess conditions
- Protects the skin cells from calorie excess
- Maintains the cellular defense systems in spite of calorie excess
- Prevents the age-related decline of AMPK.

Upregulation of Klotho in Aged Fibroblasts



AMPK Activation in Calorie Excess Conditions

■ Control medium ■ Calorie excess medium





Stimulation of Collagens in Aged Skin Cells

The effect of Snow Algae Powder on the expression of collagen I and III genes was evaluated in aged human dermal fibroblasts in a replicative aging model (Hayflick model).

Aged fibroblasts (passage 17) were cultured either with or without 0.1% Snow Algae extract. A control with normal fibroblasts (passage 8) was also performed in parallel. From there, the expression of collagen I and III genes was analyzed by quantitative PCR.

Replicative senescence of fibroblasts induced a downregulation of the collagen I and III genes. However, Snow Algae extract was shown to neutralize this decrease for the collagen I gene and in the case of the collagen III gene overcompensate for it.

Snow Algae Powder therefore helps to repair the effects of aging by stimulating the most abundant collagens in the skin.



Anti-MMP Effect in Aged Skin Cells

Senescence of normal human dermal fibroblasts was induced by incubating them with H₂O₂ (H₂O₂-induced oxidative stress model).

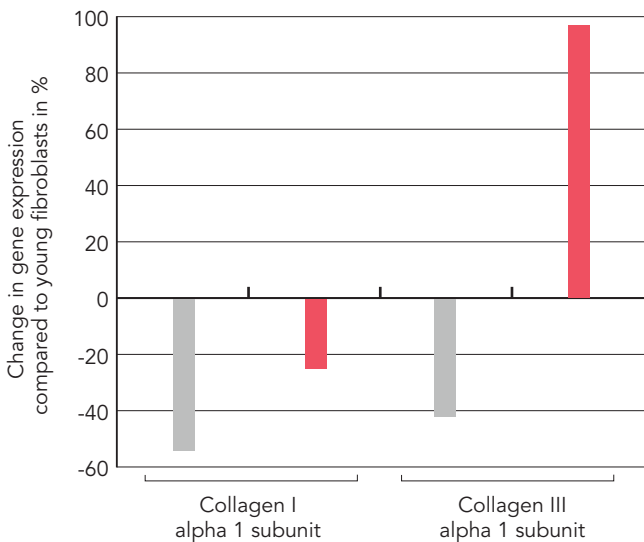
From there, these cells were cultured either with or without 0.1% Snow Algae extract and the expression of the MMP-1 and MMP-3 genes was analyzed by quantitative PCR.

In the control culture, H₂O₂-induced senescence led to the stimulation of the MMP-1 and MMP-3 genes, which code respectively for the MMP-1 and MMP-3 enzymes. These two enzymes are involved in the breakdown of several types of collagens including collagens I and III. However, Snow Algae extract was found to reduce this effect.

This confirms the capacity of Snow Algae Powder to counteract the effects of aging by preserving the collagens in the skin.

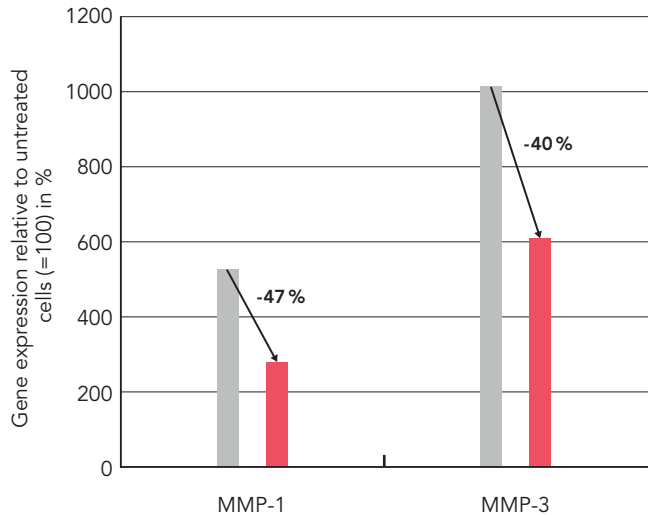
Upregulation of Collagens in Aged Fibroblasts

■ Aged fibroblasts ■ Aged fibroblasts + 0.1% Snow Algae extract



Downregulation of MMP in Aged Fibroblasts

■ After H₂O₂ ■ After H₂O₂ treatment + 0.1% Snow Algae extract



Snow Algae Powder

Study results



Dermal-Epidermal Junction Rejuvenation

A hydrogel containing 2% Snow Algae Powder was applied twice daily for two months to the inner side of the forearm of five Caucasian women aged between 55 and 67 (mean age = 60.6 years old) whilst their other forearm was treated with the corresponding placebo.

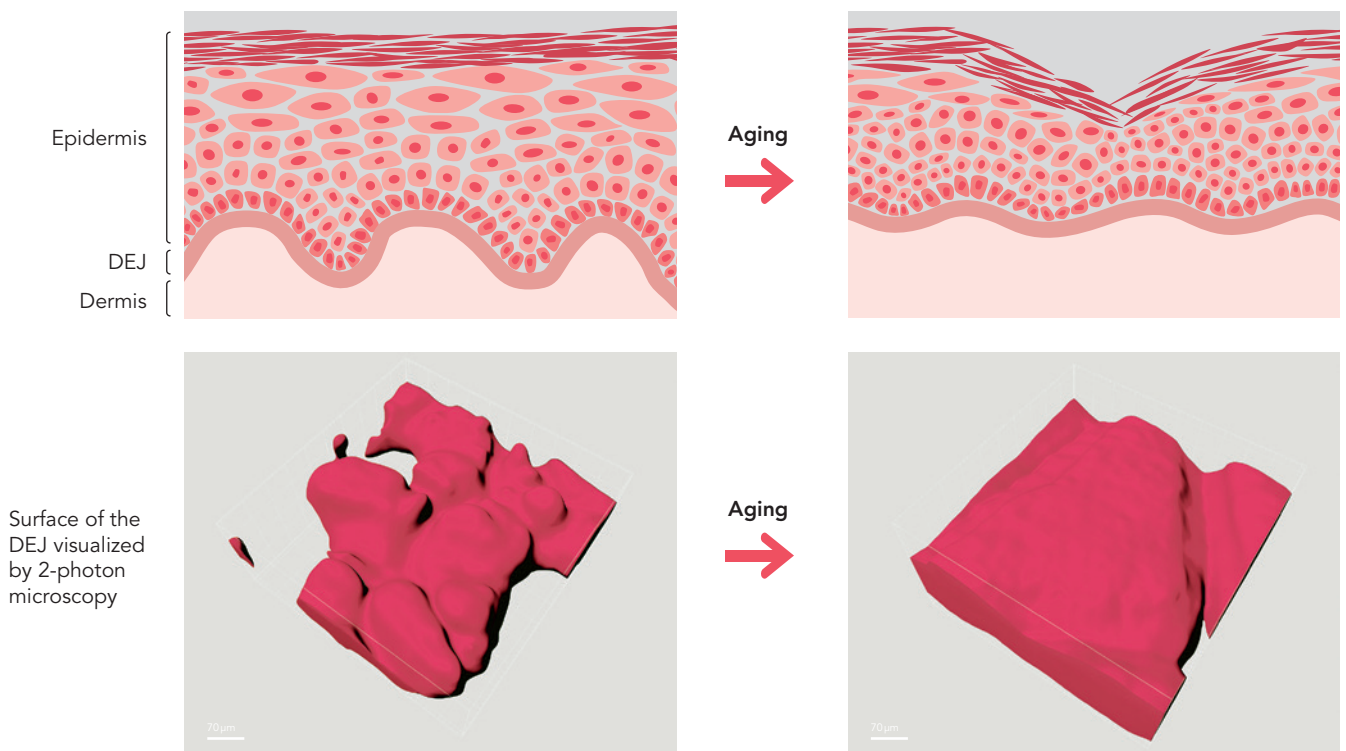
Analysis of the dermal-epidermal junction (DEJ) was performed using a two-photon microscopy. This innovative, non-invasive 3D technique visualizes deeper skin structures. It is based on the principle that infrared laser irradiation can cause autofluorescence of some molecules (e.g. elastin) or second harmonic generation (e.g. collagen). A special software enabled the reconstruction of the papillary surface (DEJ area) from this data.

The DEJ is the area that tightly binds the dermis to the epidermis. Its permeability regulates exchanges between both tissues. In addition, the DEJ contributes to the cohesion of the epidermis and to the wound healing process.

In young subjects, the structure of the DEJ is wavy, maximizing the surface area of connection between the epidermis and the dermis.

As we get older, the architecture of the DEJ changes. This is notably because of the modification in quality and quantity of elastin and collagen. As a consequence, the DEJ becomes more flattened and the connection between the epidermis and the dermis is reduced.

Modification of the Structure of the DEJ with Age



Multi-photon skin tomography images provided by Neurotar LTD (Skinvivo services, www.skinvivo.com)



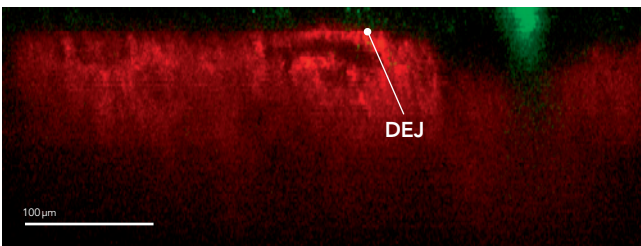
Dermal-Epidermal Junction Rejuvenation

DEJ was analyzed using a two-photon microscopy before and after treatment.

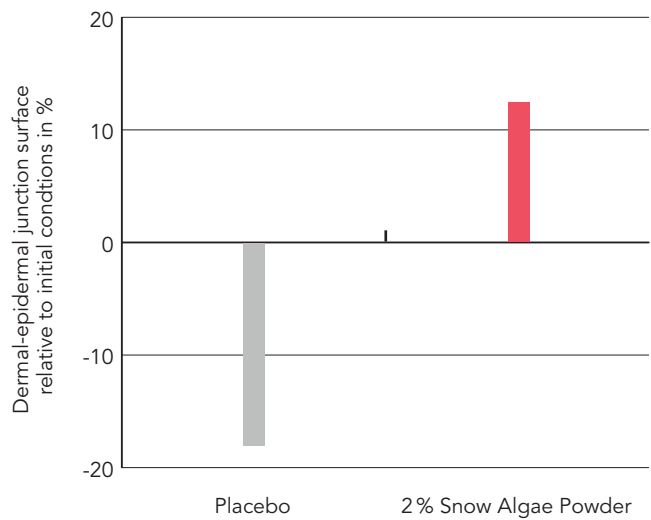
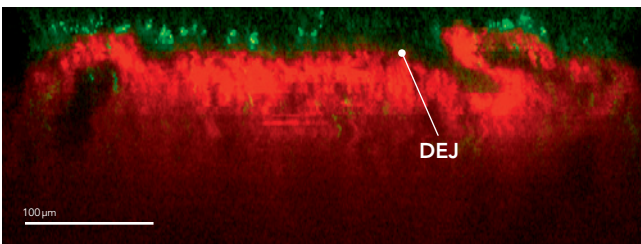
Results showed that Snow Algae Powder improved the undulations of the JDE and increased its surface by 12.5% compared to initial conditions and by 30.5% compared to the placebo. This indicates a rejuvenating effect of this connecting tissue.

Increase of the Dermal-Epidermal Junction's Surface

t0



After two months of treatment with 2% Snow Algae Powder



Snow Algae Powder

Study results



Reinforcement of the Skin Barrier in Challenging Conditions

In a double-blind study performed with 21 Caucasian volunteers (19 women and 2 men) of phototype II and aged between 30 and 57 (mean age = 43.8 years old) during winter time, Snow Algae Powder was shown to reinforce the skin barrier by decreasing water loss.

Volunteers applied twice a day for 21 days:

- an emulsion with 3% Snow Algae Powder on one half of their faces
- the corresponding placebo on the other half of their faces.

TransEpidermal Water Loss (TEWL) was measured before and after treatment on the face (cheeks) using a tewameter.

- Winter weather conditions led to an increased loss of water by the skin as shown with the placebo.
- However, Snow Algae Powder was shown to counteract this effect and to even reduce TEWL.

Therefore, Snow Algae Powder is able to reinforce the skin barrier even under challenging conditions.

Anti-Age Spots Effect

During the previously described study, high definition photographs were taken both before and after treatment using the VisioFace Quick®.

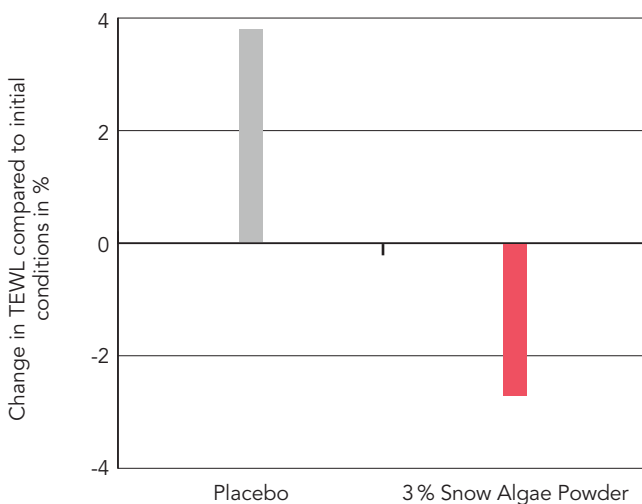
On these photographs, which were taken with white light, the aspect of age spots was evaluated by trained assessors. This analysis was performed on the six volunteers presenting age spots at the beginning of the study.

For the volunteers who applied the placebo, the visibility of their age spots was increased for half of them and was unchanged for the other half.

For the volunteers who were treated with Snow Algae Powder, the visibility of their age spots was reduced in 67% of the cases.

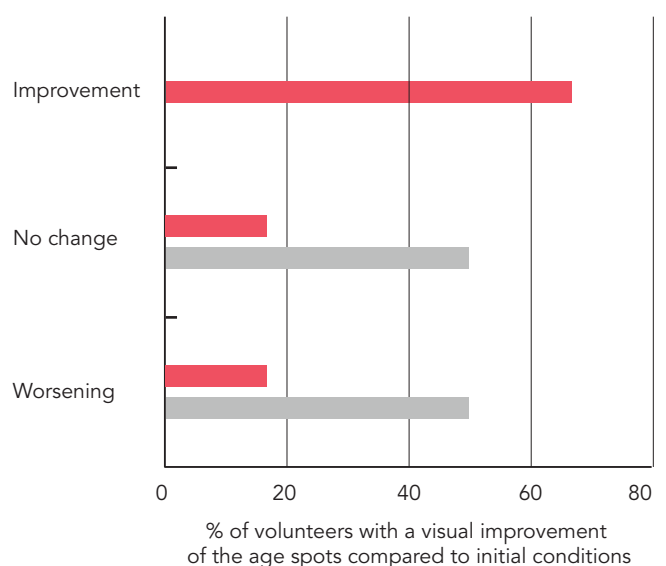
Snow Algae Powder is therefore able to reduce the visibility of age spots.

Reduction of the Loss in Water



Reduction of the Visibility of Age Spots

■ Placebo ■ 3% Snow Algae Powder



Snow Algae Powder

Key to skin's longevity

Claim Ideas for Snow Algae Powder

- Protects and activates longevity factors in skin cells
- Rejuvenates and protects skin at cellular level
- Safeguards skin's youthfulness by activating Klotho
- Strengthens cellular defense mechanisms through calorie restriction mimetic activity

Applications

- Rejuvenating and repair formulas
- Age-defense products
- Youth protecting and promoting skin care
- Formulas to increase skin's longevity – face, body, hand

Marketing Benefits

- Unique extremophile algae
- Sustainable production by biotechnology
- Novel anti-aging concept by Calorie Restriction Mimetic Activity
- Free logo to label your products that contain Snow Algae Powder; as a quality label
- Preservative-free
- Patent (US 8,206,721 B2)

Innovating for your success

Mibelle Biochemistry designs and develops innovative, high-quality actives based on naturally derived compounds and profound scientific know-how. Inspired by nature – Realized by science.

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