mibelle GROUP biochemistry


PLANT STEM CELLS
PhytoCellTec.ch™
SKIN CELL PROTECTION

# PhytoCellTec™ nunatak®

## Secrets of beauty from the ice age

SWISS QUALITY PRODUCT

**Dermal Stem Cell Protection with a Plant that Survived the last Ice Age**

PhytoCellTec™ nunatak® is the first active ingredient that is based on Saponaria pumila, a rare flower that survived the last ice age in the Alps.

In fact, during this period of glaciation the Saponaria pumila plant managed to escape the ice by moving to rare ice-free mountain peaks, which are known as nunataks. As a result of being constantly exposed to low temperatures and high amounts of UV radiation, Saponaria pumila developed protecting and repair mechanisms that enabled it to adapt to its challenging environment. This extremophile plant which features large pink flowers can still be found to this day in the Alps. However it is considered a protected species in some regions.

Thanks to our PhytoCellTec™ technology which makes possible the large scale cultivation of cells from rare and endangered plant species, we were able to develop PhytoCellTec™ nunatak®, an extract of Saponaria pumila stem cells. Consequently, the survival characteristics of Saponaria pumila can be transferred to human skin.

PhytoCellTec™ nunatak® was shown in vitro to efficiently protect dermal stem cells against UV-induced stress and maintain their stemness.
Clinical studies confirmed the positive effect of PhytoCellTec™ nunatak® on the dermis as skin density, firmness and elasticity were greatly improved after just one month.
Furthermore, our results revealed that PhytoCellTec™ nunatak® helped the skin to cope better with aggressions when used in pre-treatment (protective effect).

# Saponaria pumila
## A survivor of the last ice age

**Nunatak, a Mountain Refuge during the Ice Age**
During the last ice age, which ran from 110,000 BC until 10,000 BC, large parts of the northern hemisphere were covered under a thick sheet of ice. This climatic condition not only represented a threat to mankind and animals but also to all plant species. Indeed, during this period, over 90% of all terrestrial life vanished from the northern hemisphere and only those organisms that were truly robust and could quickly adapt to the conditions were able to survive the permanently cold and dry climate.

Whilst most of the plants were unable to survive the last ice age, a small number of plant species managed to escape the ice by moving to rare ice-free mountain peaks called nunataks (Inuit word referring to a mountain top that protrudes above the surface of a glacier). In these unique places, water is often present in liquid form and can be assimilated by plant roots.

**Alpine Nunataks Plants are the Original alpine Plants**
There are several examples of nunataks in the Swiss Alps: Rothorn and Hörnli near Arosa, Zermatt and Simplon region, Pilatus, Säntis and Rigi.

In the Alps, the so called "nunatak plants" are amongst the very few alpine plants that managed to survive the harsh conditions of the last ice age.

In the postglacial era, these extremophile plants progressively re-colonized the alpine region. Therefore, the original alpine plants are actually the nunatak plants and not the so-called alpine plants such as the "Edelweiss", which in fact only migrated from Asia to the alpine region after the ice age.

**Saponaria Pumila, an alpine Extremophile Plant which is now Protected**
- Saponaria pumila (caryophyllaceae family) took refuge on nunataks in the Alps.
- This perennial plant features large pink flowers.
- Within the Alps, it is mainly found in presumptive refugial areas. Furthermore, it is considered a protected species in some regions.
- Saponaria pumila grows on acidic and siliceous bedrock.

# PhytoCellTec™ nunatak®
## Advanced biotechnology to cultivate plant stem cells from rare plants

**PhytoCellTec™ by Mibelle Biochemistry**
Mibelle Biochemistry has developed the PhytoCellTec™ technology which enables the large scale cultivation of plant stem cells. This technology is based on the unique totipotency of plant cells. This specifically means:
- the capacity of every plant cell to regenerate new organs or even the entire plant
- the capacity of every plant cell to dedifferentiate and become a stem cell.

Our PhytoCellTec™ technology relies on the wound healing mechanism of a plant: following an injury, the healing of the cut surface begins with the formation of callus cells. This healing tissue consists of dedifferentiated cells, which are stem cells.

Saponaria pumila germinated sprouts were therefore injured to induce callus formation. The callus cells were then cultivated in an appropriate medium and large-scale production was achieved in a specialized bioreactor system.

To obtain PhytoCellTec™ nunatak®, these stem cells are harvested and homogenized at 1200 bar together with phospholipids to encapsulate and stabilize oil-soluble and water-soluble components into liposomes. The resulting extract is carefully sprayed on a powder based on isomalt.

All stem cells, regardless of their origin (plant, animal or human) contain specific epigenetic factors, the function of which is to maintain the self-renewal capacity of the stem cells. Therefore, PhytoCellTec™ nunatak® is rich in epigenetic factors and metabolites that can help to maintain the regenerative potential of human skin stem cells.

**Advantages of PhytoCellTec™ Technology**
This innovative technology that has been developed by Mibelle Biochemistry delivers the following advantages:
- preservation of the plant (as the technique requires a small quantity of plant material just once)
- availability of plant material regardless of the season and market demand
- plant material that is completely free of environmental pollutants and pesticides
- constant concentrations of metabolites in the stem cells.

# PhytoCellTec™ nunatak®
## Study results

**Maintenance of Dermal Stem Cell Vitality and Protection against Irradiation**

Dermal progenitor/stem cell vitality can be monitored based on the characteristic ability of these cells to grow in colonies and form three-dimensional spheres in vitro.

Dermal progenitor cells, which were isolated from the dermal papilla of plucked hair follicles, were firstly cultured in a specific medium either with or without the Saponaria pumila stem cell extract and then exposed to a broad spectrum light source (300–800 nm) at 6 J/cm$^2$. Following this stress, cells were detached and seeded into non-adherent culture flasks in a second specific medium where dermal progenitor cells spontaneously form spheres instead of monolayers. This enabled the evaluation of their ability to form spheres (stem cell characteristic).

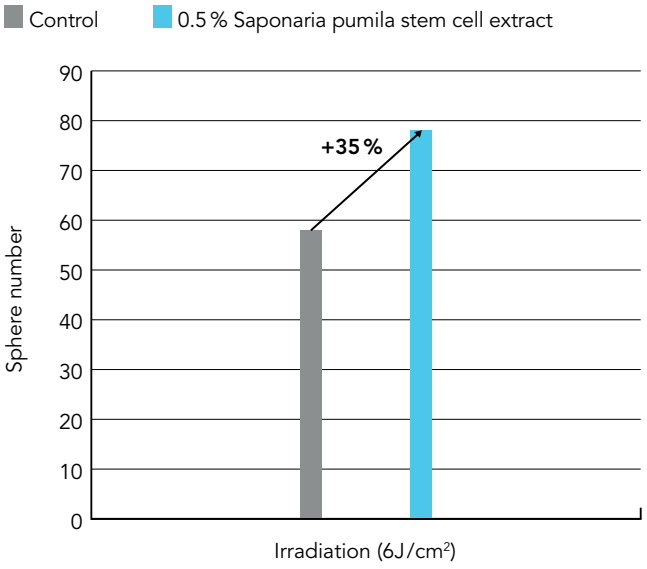
In unexposed cultures, the treatment of dermal progenitor cells with the Saponaria pumila stem cell extract led to an increase of the sphere number (data not shown).

Therefore, PhytoCellTec™ nunatak® helps the dermal stem cells to maintain both their vitality and stem cell characteristics ("stemness").

In cultures exposed to UV and visible light, the sphere number decreased compared to unexposed cultures. However, treatment with the Saponaria pumila stem cell extract was found to protect against irradiation as the sphere number was 35 % higher compared to the unprotected control.

PhytoCellTec™ nunatak® can therefore protect dermal stem cells against the harmful effects of UV and visible light.

**Dermal Stem Cells Vitality after UV Stress**



During the previously described study, a new parameter was also evaluated: the size of the spheres that were formed. This provides an important indication regarding the quality of the dermal stem cells.

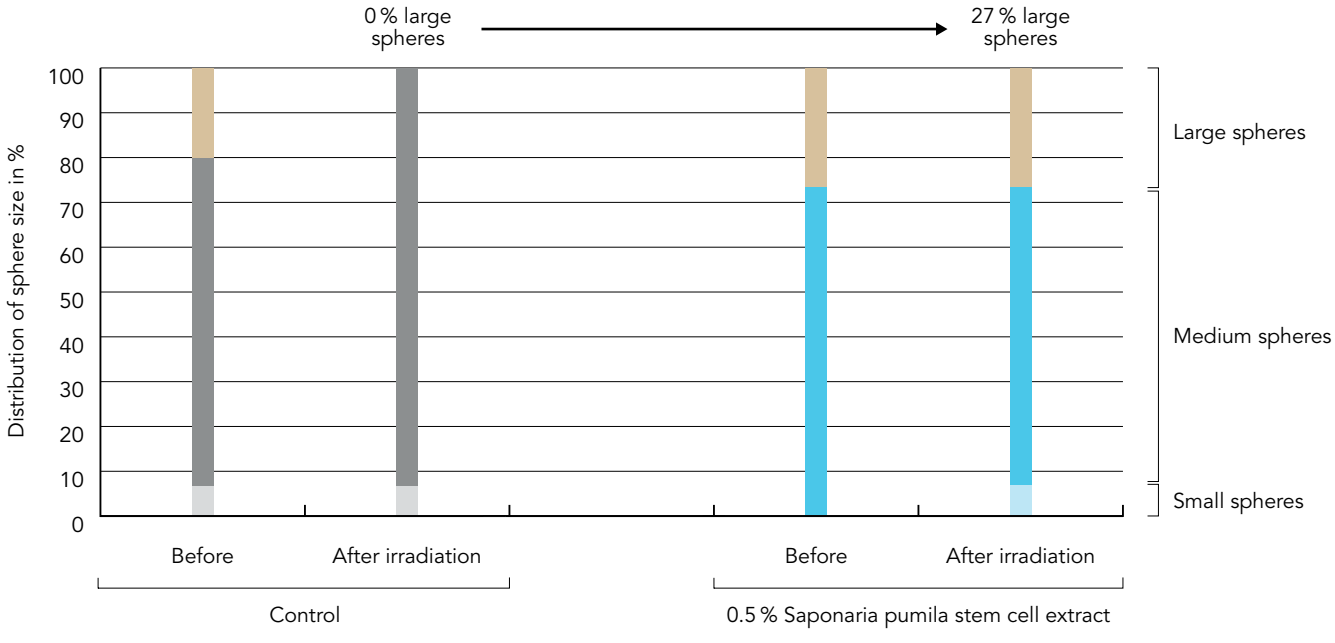
The spheres were classified into three groups:
- small spheres (diameter ≤ 50 µm) regroup cells that have almost lost their capacity to regenerate
- medium spheres (51 µm ≤ diameter ≤ 100 µm) regroup cells with a moderate capacity to renew.
- large spheres (diameter ≥ 100 µm) regroup cells with a real regenerative potential.

This test also involved dermal progenitor cells being exposed to a broad spectrum light source (300–800 nm) at 3 J/cm$^2$.

In the control culture, irradiation led to the disappearance of the largest spheres. This shows that irradiation reduces the proliferation capacity of the most vital cells.

However, with PhytoCellTec™ nunatak®, the repartition of the sphere size was almost entirely unaffected by irradiation as the proportion of large spheres remained at the same level. This demonstrates that PhytoCellTec™ nunatak® can maintain the renewal potential of the dermal stem cells in spite of irradiation.

## Maintenance of the Renewal Potential of Dermal Stem Cells following Irradiation



# PhytoCellTec™ nunatak®
## Study results

**Protecting Effect in spite of UV Stress**

In a study performed before and during holidays in sunny climates involving volunteers with skin that is not sun-adapted, PhytoCellTec™ nunatak® was shown to prevent skin roughness.

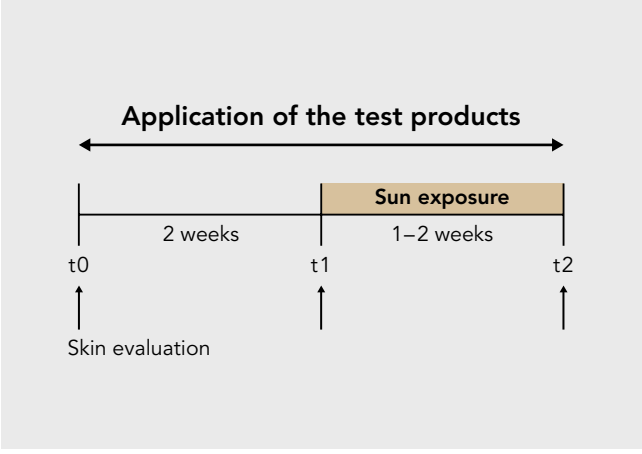
Eleven Caucasian women aged from 44.7 to 68.4 years (mean age: 56.2 years) applied twice daily for four weeks:

- a cream with 1 % PhytoCellTec™ nunatak® on one half of their faces
- the corresponding placebo on the other half of their faces.

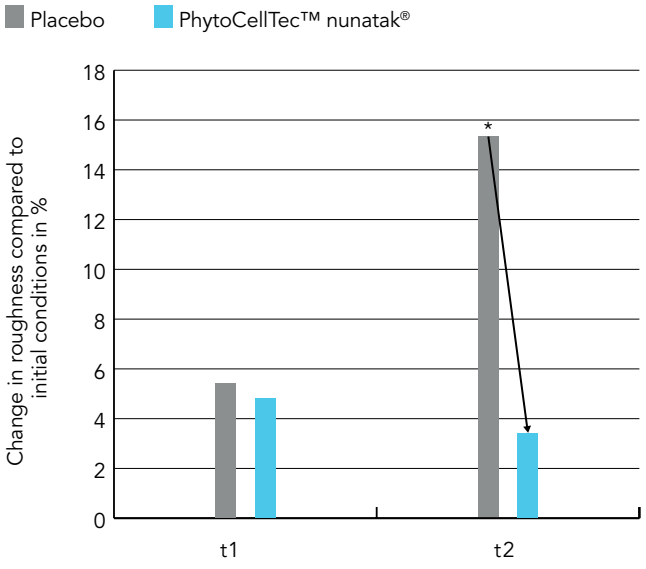
The application of test products started two weeks before a period of one to two weeks of seaside holidays.
The roughness of the skin of the volunteers was measured on the temple area using the PRIMOS system.

Results showed a statistically significant increase in skin roughness on the placebo-treated facial area. This can be explained by the sudden exposure to the sun and seawater stress.
On the contrary, there was almost no change in terms of skin roughness on the half of the face that was treated with PhytoCellTec™ nunatak®. This reveals the capacity of PhytoCellTec™ nunatak® to protect the skin.

### Study Design



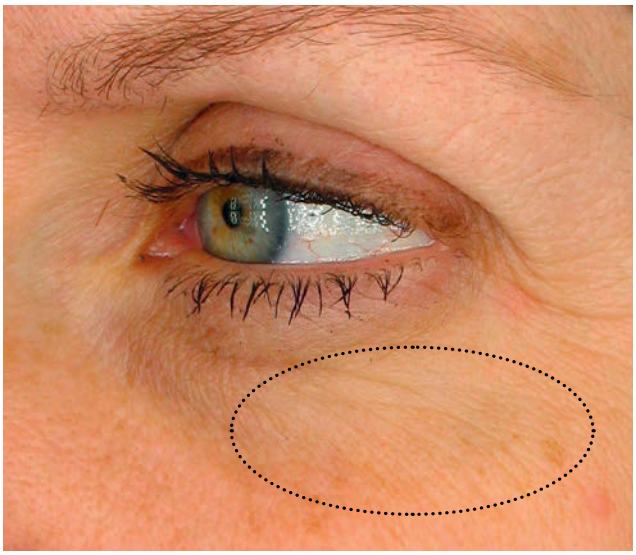
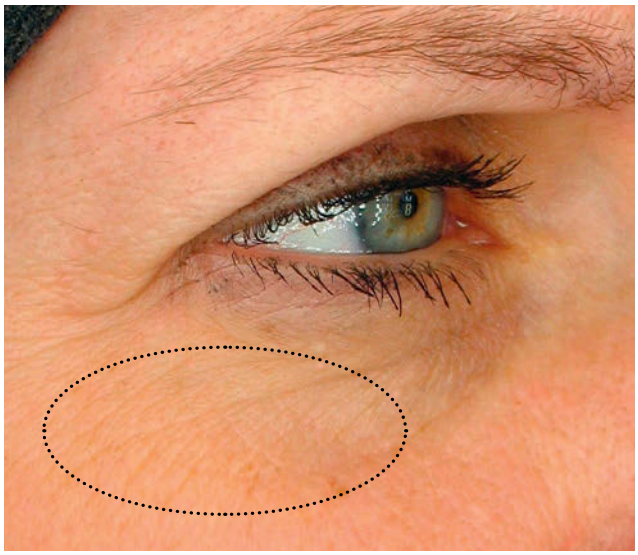
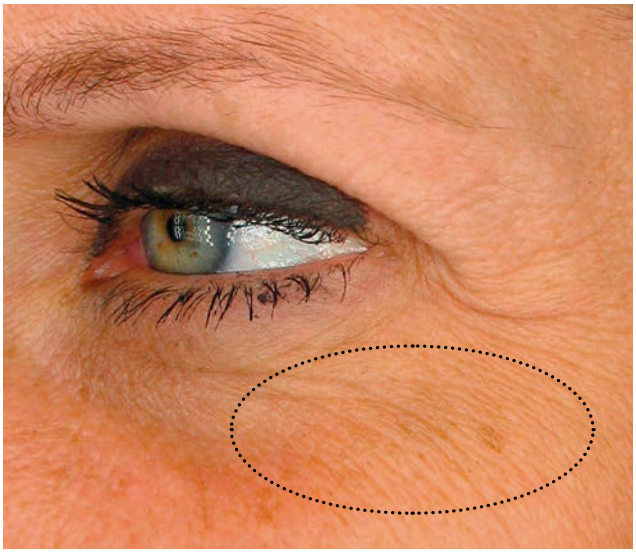
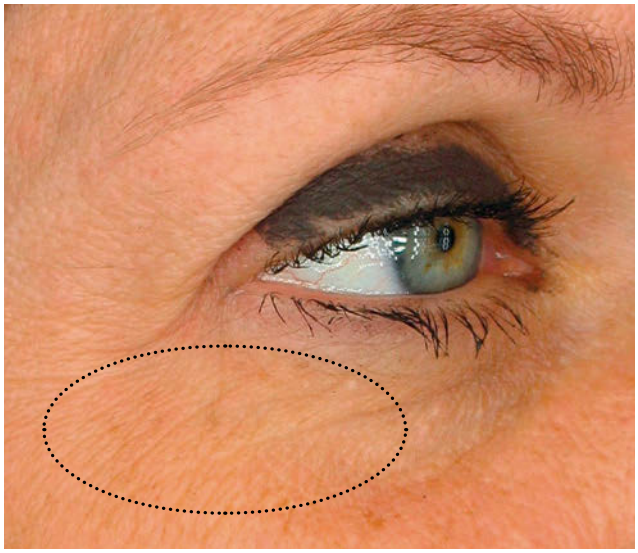

### Protecting Effect




*p=0.048 versus initial conditions

Therefore, PhytoCellTec™ nunatak® may exert a pre-conditioning treatment that would help the skin to better cope with aggressions and result in a smoother texture.

## Improvement of Skin Texture

| | Placebo | PhytoCellTec™ nunatak® |
|---|---|---|
| t1 |  |  |
| t2 |  |  |

# PhytoCellTec™ nunatak®
## Study results

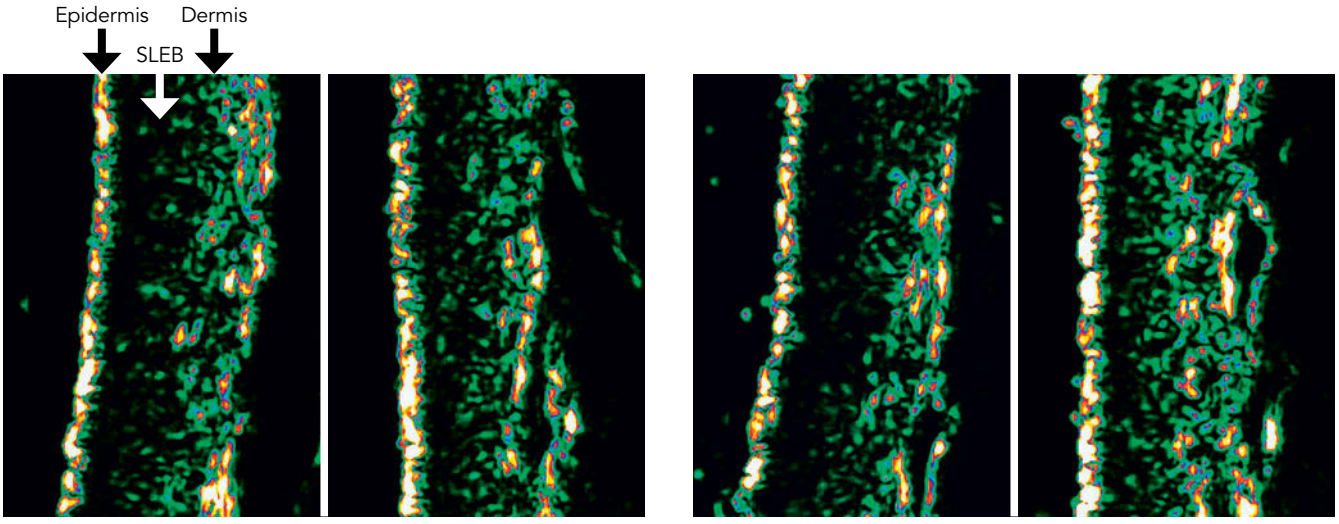
**Improvement of Skin Density**
Twenty women aged between 44.2 and 59.5 years (mean age: 53.5 years) and with sun-damaged skin applied either a cream with 0.4 % PhytoCellTec™ nunatak® or the corresponding placebo twice daily for 28 days to the inner side of their forearms. The density of their skin (epidermis + dermis) was determined by ultrasonic measurements.

The ultrasonic wave generates echoes when it is partially reflected at the boundaries between different tissue structures. The intensity of the reflected echoes can be evaluated and visualized in a color image. The collagen and elastic fiber structure of an intact dermis yields many reflections that are visible as bright colors in the ultrasonographic image.

However, disruption to this regular architecture leads to weaker reflections and dark patches as can be seen in the ultrasonographic images below at Day 0. These so-called subepidermal low-echogenic bands (SLEB) are commonly found in aged and photo-damaged skin.

Ultrasonographic images showed that PhytoCellTec™ nunatak® increased the density of the dermal tissue and this led to a reduction of the SLEB.

**Dermal Skin Density in Sun-Damaged Skin**



Ultrasonic measurements realized during the previously described study enabled the quantification of the skin density before and after treatment.

Results showed that PhytoCellTec™ nunatak® significantly increased the density of the dermis by almost 10% compared to initial conditions after 28 days of treatment.

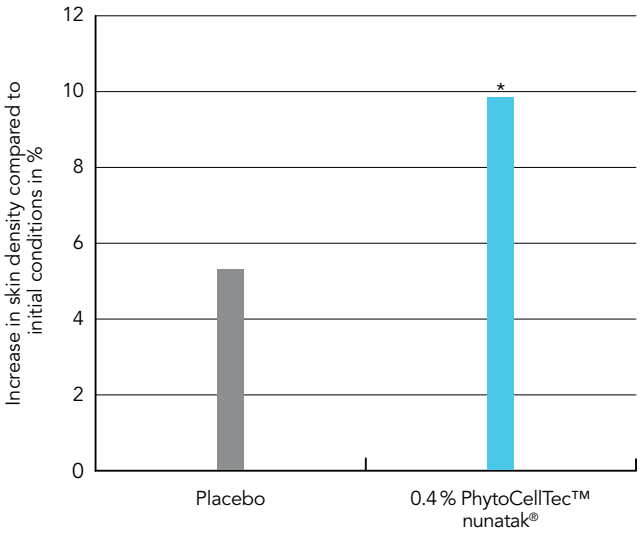
**Improvement of Skin Elasticity and Firmness**

Twenty women aged between 44.2 and 59.5 years (mean age: 53.5 years) and with sun-damaged skin either applied a cream with 0.4% PhytoCellTec™ nunatak® or the corresponding placebo to the inner side of their forearms twice daily for 28 days.

The elasticity and firmness of their skin were determined by using a cutometer and the Reviscometer® system respectively.

Results showed a significant increase of both parameters compared to initial conditions. In particular, the increase in firmness was especially high - more than 14% compared to the placebo after just one month of treatment.

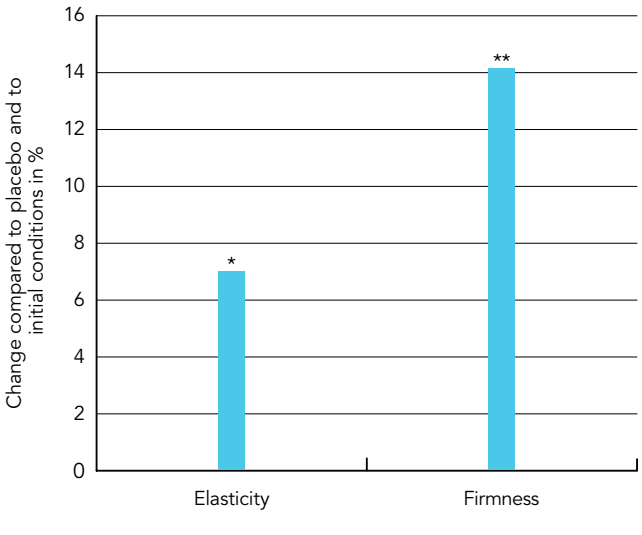
## Increase of Tissue Density




*p=0.0007 versus initial conditions



## Improvement of Skin Elasticity and Firmness




*p=0.0296 versus initial conditions
**p=0.0525 versus initial conditions

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