



PhytoCellTec™ Symphytum
Speed up your skin renewal
through stem cell activation





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Improving Skin Renewal during the Aging Process with Plant Stem Cells

PhytoCellTec™ Symphytum is a powder based plant stem cell extract of comfrey (symphytum) roots that is prepared by our PhytoCellTec™ technology.

The skin protects our body from the environment. In order to perform this barrier function efficiently, the epidermis is constantly renewed and repaired by the epidermal stem cells. This constant renewal, which is important for the quality of the barrier, also ensures that the skin remains smooth and even. However, as we age, this renewal process slows down considerably due to the reduced regenerative capacity of the epidermal stem cells. This leads to a thinner, dry and rough skin that in turn results in a dull complexion.

PhytoCellTec™ Symphytum has been shown to clearly restore the regenerative capacity of epidermal stem cells cultivated in an aging environment. To this end, a pro-aging medium was developed by Mibelle Biochemistry in collaboration with stem cell technology experts in order to mimic the tissue environment of elderly people. In these pro-aging conditions, PhytoCellTec™ Symphytum was also shown to help the epidermal stem cells in building a thicker 3D epidermis with a higher content of hyaluronic acid.

Clinical studies performed with twenty women demonstrated a significant increase of the skin renewal rate as well as an 11% improvement in skin smoothness.

PhytoCellTec™ Symphytum can thus boost and improve the skin renewal from the deepest layers of the epidermis during aging. Consequently, PhytoCellTec™ Symphytum prevents the age-related thinning of the epidermis and ensures that the skin remains smooth.

Claim Ideas for PhytoCellTec™ Symphytum

- Recharges youthfulness of skin stem cells
- Boosts cell turnover to rejuvenate the skin from the inside out
- Maintains the regenerative capacity, even as the years pass by
- Reveals a fresher and smoother skin

Applications

- Rejuvenating formulas
- Age-defense products for both face and body
- Skin care products to vitalize the most valuable skin cells – the stem cells

Formulating with PhytoCellTec™ Symphytum

- Recommended use level: 0.4–1%
- Incorporation: Dissolve PhytoCellTec™ Symphytum into the aqueous phase or add pre-solved, during the cooling phase (<60°C). PhytoCellTec™ Symphytum 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 PhytoCellTec™ Symphytum.

INCI/CTFA-Declaration

Symphytum Officinale Callus Culture Extract (and) Isomalt (and) Lecithin (and) Sodium Benzoate (and) Aqua/Water

Additional Information

- PhytoCellTec™ Symphytum contains 50% of Symphytum stem cell extract
- Phenoxyethanol-free

Symphytum Officinale

An old medicinal plant with powerful healing properties

A Healing Plant Used Since Ancient Times

PhytoCellTec™ Symphytum is based on the stem cells of Symphytum roots.

Symphytum officinale, which is also called comfrey is one of the oldest known medicinal plants. Its healing and analgesic effects have been experienced since ancient times. Indeed, the name Symphytum derives from the Greek word *symphyo* which means "to grow together" and *phyton* which means "plant".

Comfrey, the more common name derives from the Latin word *confervere* which means "to grow together" or "to heal". The other common names given to this plant also refer to its healing powers: boneset, bruisewort, con-sound and knitbone.

Comfrey grows in rich, moist, well-drained soil and has been used since ancient times for its healing properties:

- ancient sources recorded that the legion warriors of ancient Rome used comfrey for its healing properties.
- the ancient Greek physician and philosopher Dioscorides prescribed it for healing wounds, broken bones as well as respiratory and gastrointestinal problems. Meanwhile, other well-known Greek physicians including Herodotus, Nicander and Galen also used this plant.
- the famous 12th century German nun Hildegard of Bingen, who was also a herbalist, recommended comfrey for wound healing.
- in the Middle Ages, comfrey was widely cultivated in monastery gardens and vegetable gardens and its roots were used both internally and externally as a universal remedy for many diseases.
- during the Renaissance, comfrey was one of the most popular medicinal herbs. The German alchemist and pharmacologist Paracelsus used its roots to treat wounds, ulcers and fractures.

A Versatile Remedy Used Both Internally and Externally

Comfrey roots have been used internally (to heal inflammation of the stomach and digestive tract) and externally (to heal burned and frost-bitten skin areas and to treat eczema, rashes as well as bruises, sprains, fractures and rheumatism).

Nowadays, comfrey is still notably used externally for wound healing and inflammatory and traumatic lesions of muscles and joints. It is also employed in phytotherapy, homeopathy and anthroposophic medicine as well as in TCM and Ayurveda.

Healing by Speeding Cell Proliferation

Comfrey grows in rich soil where it develops a deep root network in search of nutrients. Therefore, comfrey can easily concentrate and accumulate nutrients from the soil. This explains both its highly complex composition as well as its use as a fertilizer in organic agriculture.

Furthermore, comfrey is very rich in mucilage polysaccharides which help the plant to store water efficiently.

Comfrey speeds up the healing of injuries thanks to its capacity to stimulate cell proliferation and thus to promote tissue formation.

It also has anti-inflammatory, analgesic and decongestant effects.

PhytoCellTec™ Symphytum

Preventing the age-related slowdown of skin renewal at the source

Skin Barrier is Controlled by Epidermal Stem Cells

The epidermis and particularly the stratum corneum which is its upper layer, protect our bodies from the environment and external stress while simultaneously preventing water loss from the inside.

To perform this barrier function, the epidermis undergoes constant maintenance, renewal and repair. This process occurs thanks to the epidermal stem cells that are located in the basal layer which is the deepest layer of the epidermis. From there, the epidermal stem cells replenish the epidermis with new cells. When keratinocytes migrate from the basal layer, they no longer divide but instead undergo differentiation to form the outermost horny layer (stratum corneum). This constant renewal, which is important for the quality of the barrier, also ensures that the skin remains smooth and even.

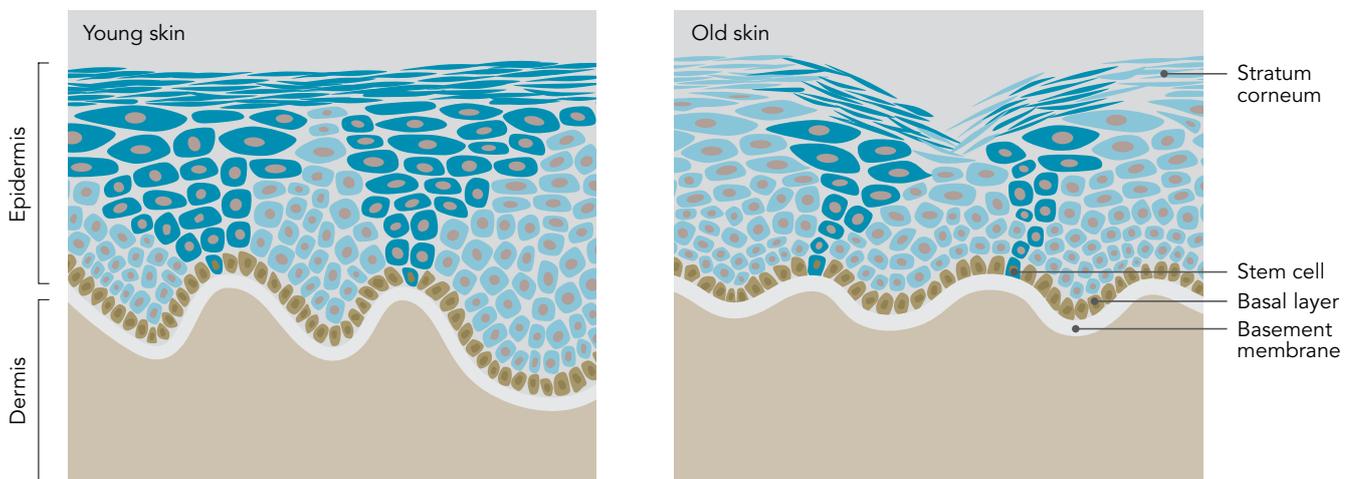
Skin Renewal Depends on the Regenerative Capacity of Epidermal Stem Cells

The regenerative capacity of epidermal stem cells – that is to say their ability to give birth to new cells – decreases with age. Consequently, the turnover of the epidermis slows down: between the ages of 30 and 80, the turnover rate decreases by 30% to 50%. This leads to a reduced production of key barrier lipids, an impaired skin barrier and an increased transepidermal water loss (TEWL). As a result, the skin becomes dry, rough, uneven and dull.

PhytoCellTec™ Symphytum Prevents the Age-Related Slowdown of Skin Renewal

PhytoCellTec™ Symphytum can clearly restore the regenerative capacity of epidermal stem cells in an aging environment. In this way, it is able to boost the skin turnover from the source, i.e. from the deepest layers of the epidermis. This results in a smoother skin with an improved barrier function.

Regeneration of the Epidermis in Young and Old Skin



Young skin with active, highly proliferating stem cells (left) in contrast to old skin with senescent stem cells (right). Senescence of stem cells results in a smaller progeny of differentiated keratinocytes leading to a thinner epidermis with a lower turnover rate.

PhytoCellTec™ Symphytum

Advanced biotechnology to cultivate plant stem cells

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 is specifically:

- 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.

Symphytum officinale roots were thus 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.

The PhytoCellTec™ technology is especially well adapted to plant roots as it provides access to this vital part of the plant without destroying them.

To obtain PhytoCellTec™ Symphytum, 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 stem cells. Therefore, PhytoCellTec™ Symphytum 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 developed by Mibelle Biochemistry offers the following advantages:

- preservation of the plant (as the technique requires only once a small quantity of plant material)
- availability of plant material regardless of the season and market demand
- plant material completely free of environmental pollutants and pesticides
- constant concentrations of metabolites in the stem cells.

PhytoCellTec™ Actives Targeting the EPIDERMIS

Active ingredients	Chronological aging		Photo-aging	Result on the skin
	Prevention	Treatment	Prevention	
PhytoCellTec™ Malus Domestica	✓			Extended capacity to regenerate
PhytoCellTec™ Symphytum		✓		Extended capacity to regenerate in an aging environment
PhytoCellTec™ Solar Vitis			✓	Stronger resistance to UV stress
PhytoCellTec™ Alp Rose			✓	Stronger resistance to environmental stress

PhytoCellTec™ Symphytum

Study results



Preparation of Epidermal Stem Cells

The Progenitor Cell Targeting technology which was developed by Mibelle Biochemistry in collaboration with stem cell technology experts was used to prepare human epidermal stem cells.

This technology consists of culturing a skin sample in a medium that is specifically designed to mimic the micro-environment of the stem cell in the epidermis. This leads to an enrichment of so-called keratinocyte progenitor cells that can be considered as stem cells. The enrichment was quantitatively controlled through FACS (Fluorescence-activated cell sorting) of cells which were labeled with CD34 and $\alpha 6$ integrin, two well-known markers of epidermal stem cells. Compared to freshly isolated cells, the cell population of passage 3 was characterized by a tenfold increase of CD34/ $\alpha 6$ integrin double labeled cells.

The Progenitor Cell Targeting technology provides a cell culture model that specifically enables the evaluation of the effects of compounds on epidermal stem cells.



Development of a Pro-Aging Environment in Cell Cultures for Epidermal Stem Cells

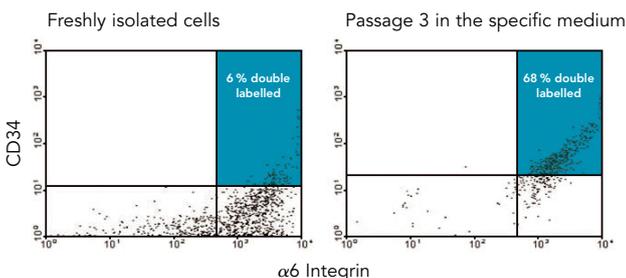
Cell cultures require essential elements (amino-acids, trace elements) and are supplemented with protective compounds to enhance the survival of the cells and to stimulate their proliferation. However, these nutritive and protective substances prevent the study of the effects of chronological aging on the cells. Indeed, they need to be "neutralized" and therefore require the use of unnaturally high doses of stressors such as H_2O_2 in order to mimic aging.

In collaboration with stem cell technology experts, Mibelle Biochemistry has developed a novel in vitro pro-aging model which allows epidermal stem cells to age in conditions that more closely reflect reality. To this end, a completely new specific culture medium has been established that contains the essential elements for growth but in which:

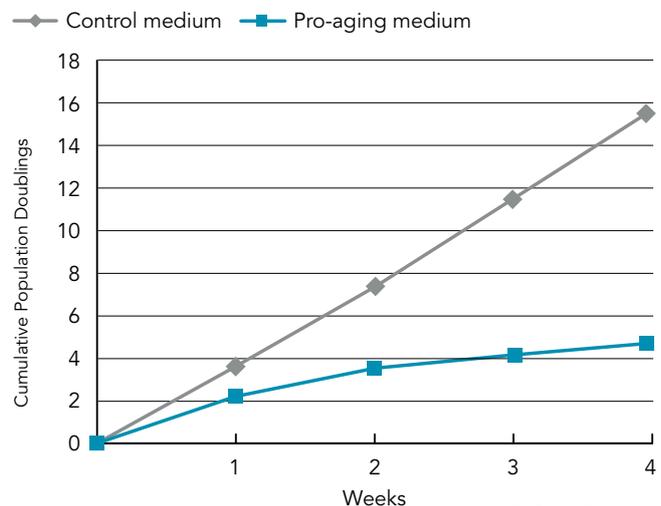
- no protective compounds are added
- such proliferation-accelerating substances as growth factors are missing.

Epidermal stem cells (that are obtained with the Progenitor Cell Targeting technology) grown in this specific pro-aging environment exhibited a reduced proliferation and a shorter lifespan whilst remaining vital and maintaining a normal morphology. Compared to a classic medium (control medium), cells cultured in the pro-aging medium reached much lower population doublings (number of generations produced in a week).

Enrichment of Keratinocyte Progenitor Cells



The Pro-Aging Medium Mimics Chronological Aging



PhytoCellTec™ Symphytum

Study results



Inhibition of the Age-Related Decrease of the Regenerative Capacity of Skin Stem Cells

Thanks to this pro-aging medium, we can now screen substances for their ability to activate the functions of epidermal stem cells in an aging environment.

A preliminary cytotoxicity test was conducted to define the sub-toxic concentration of the test substances.

Epidermal stem cells obtained with the Progenitor Cell Targeting technology were cultured for 3 passages in the pro-aging medium either with or without the Symphytum stem cell extract (0.06 % or 0.125 %).

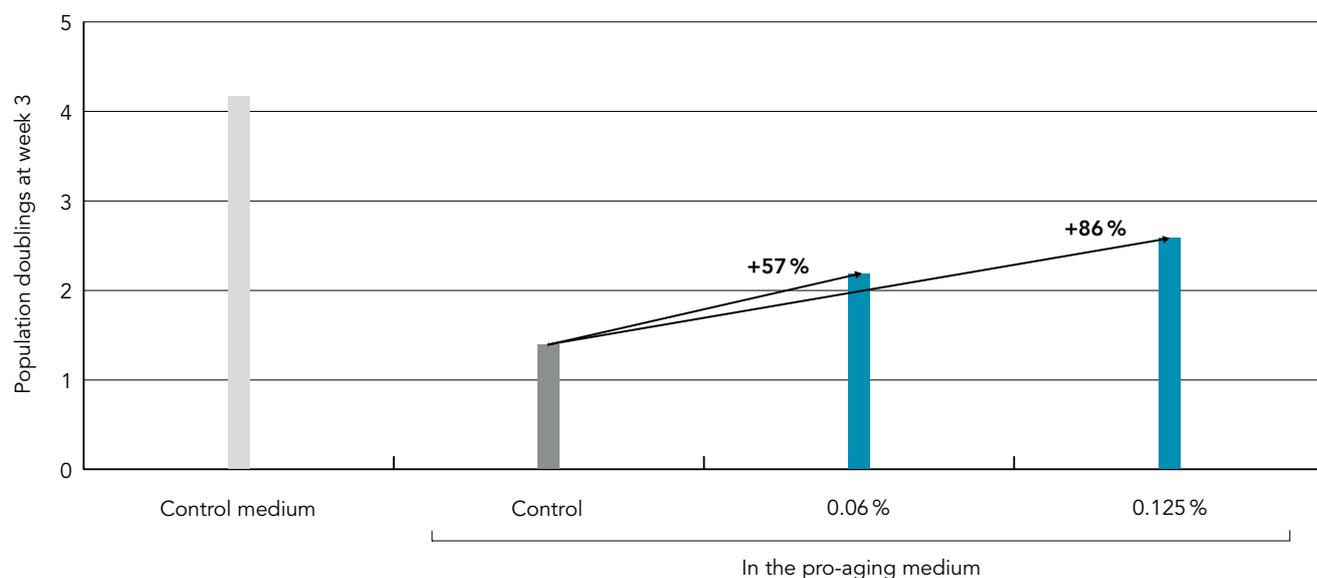
Each week, cells were detached and counted before being re-seeded in order to calculate the population doubling rate that corresponds to the number of generations produced during that week.

Following 3 passages in the pro-aging medium, the epidermal stem cells showed a reduced proliferation compared to cells cultured in the classic medium (control medium). Treatment with the Symphytum stem cell extract could clearly restore the proliferation capacity of epidermal stem cells.

The number of generations produced in week 3 could almost be doubled with 0.125 % Symphytum stem cell extract.

Activation of the Regenerative Capacity of Epidermal Stem Cells in a Pro-Aging Environment

■ Symphytum stem cell extract





Maintenance of the Capacity to Build New Tissues in a Pro-Aging Environment

Epidermal stem cells obtained with the Progenitor Cell Targeting technology were cultured for 10 days in the pro-aging medium either with or without 0.125% Symphytum stem cell extract.

The cells were then transferred into a pro-aging differentiation medium to induce the formation of a stratified epidermis (3D epidermis).

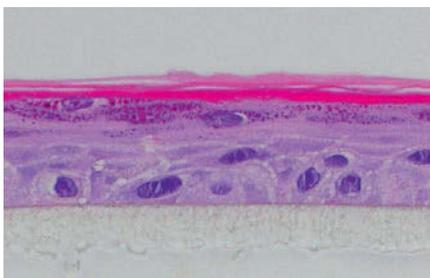
Cells incubated in the pro-aging medium formed a thinner epidermis with lower stratification / differentiation and a reduced hyaluronic acid (HA) content.

However, cells treated with the Symphytum stem cell extract formed a thicker and more differentiated epidermis with higher HA content.

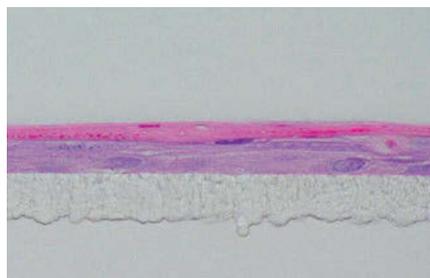
Consequently, through the activation of the regenerative capacity of epidermal stem cells, the Symphytum stem cell extract helps these cells to maintain their ability to build new tissues during aging.

Improvement of the Epidermal Thickness and Hyaluronic Acid Content

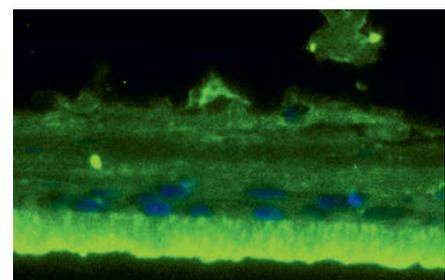
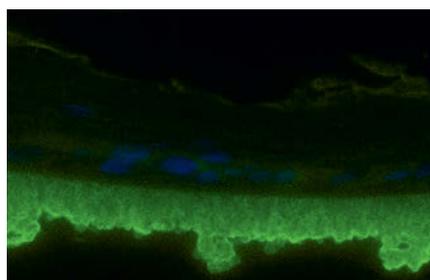
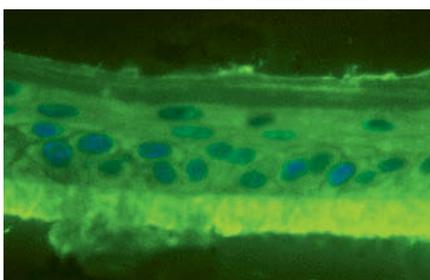
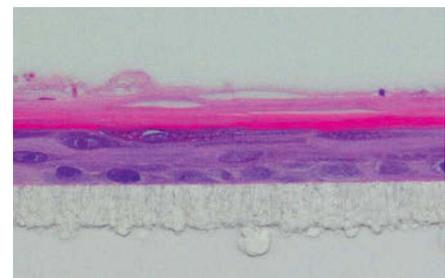
Control medium



Pro-aging medium



Pro-aging medium + Symphytum stem cell extract



Immunofluorescent staining of hyaluronic acid in green – Cell nuclei in blue (DAPI)

PhytoCellTec™ Symphytum

Study results



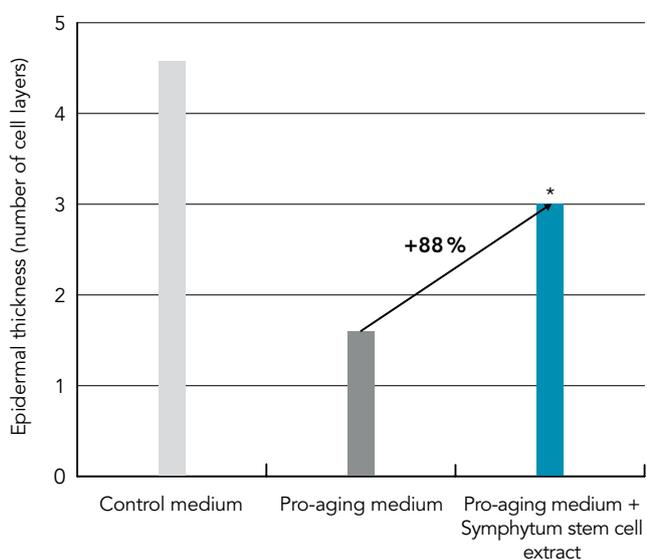
Maintenance of the Capacity to Build New Tissues in an Aging Environment

From there, the thickness of the epidermis formed was measured.

Epidermal stem cells cultivated in the pro-aging medium generated a thinner epidermis compared to the classic medium (control medium): Its thickness was reduced by approximately 65% whereas it was reduced by only 35% in the presence of the Symphytum stem cell extract. This means that in an aging environment, the Symphytum stem cell extract increases the thickness of the epidermis by 88% compared to the control in the pro-aging medium.

Therefore, the Symphytum stem cell extract can prevent the age-related thinning of the skin.

Inhibition of the Age-Related Thinning of the Epidermis



*p=0.016 versus the pro-aging medium



Increase of the Skin Renewal

The effect of PhytoCellTec™ Symphytum on the renewal time of the epidermis was measured using a fluorescent dye (dansyl chloride) that irreversibly stains the stratum corneum cells. And it is only after a complete renewal of the stratum corneum that the dansyl chloride is entirely removed. Twenty women aged between 40 and 60 (average age: 49.3 years) applied the test products (0.4% PhytoCellTec™ Symphytum cream and the corresponding placebo) twice daily for 56 days to the inner side of their forearms. At day 28, a 5% dansyl chloride suspension was applied to the test areas under occlusive patches for 24 hours. Following this, the level of fluorescence was assessed visually under UV light from day 42 onwards until the skin was no longer fluorescent. Results showed that PhytoCellTec™ Symphytum increased the turnover of the epidermis as it led to a reduction of the skin renewal time of more than 11% compared to untreated and almost 8% compared to placebo.



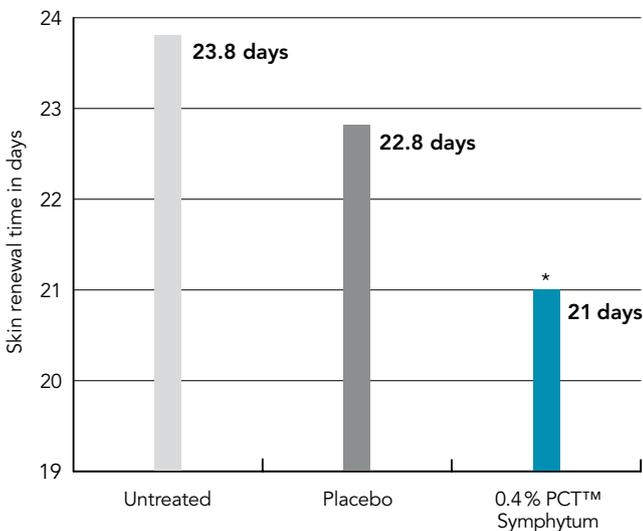
Smoothing Effect

Twenty women aged between 40 and 60 (average age: 49.3 years) applied either a cream with 0.4% PhytoCellTec™ Symphytum or the corresponding placebo twice daily for 28 days to the inner side of their forearms. The smoothness of their skin was determined using the PRIMOS system.

Results showed that PhytoCellTec™ Symphytum significantly increased skin smoothness.

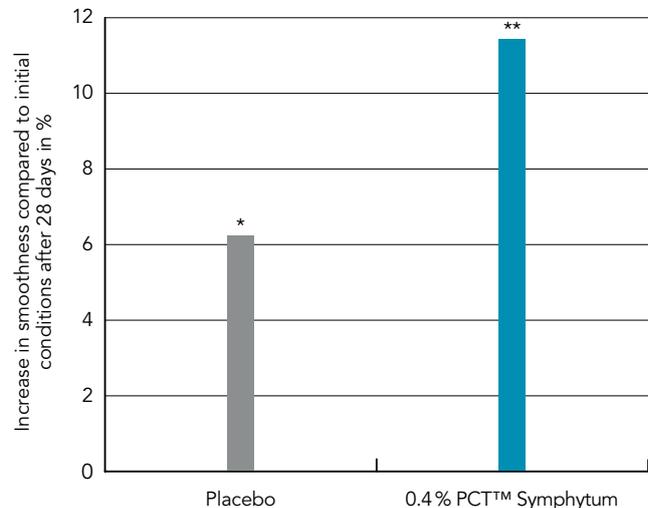
In another placebo-controlled clinical study, the effects of PhytoCellTec™ Symphytum on the water content in different depths of the stratum corneum were evaluated using Raman spectroscopy. Following eight weeks of application, PhytoCellTec™ Symphytum was found to increase the water content in the stratum granulosum (deep moisture).

Increase in the Epidermal Turnover



*p<0.05 versus untreated and placebo

Increase in Skin Smoothness



*p<0.05 versus untreated, **p<0.05 versus untreated and placebo

PhytoCellTec™ Symphytum

Speed up your skin renewal through stem cell activation

Claim Ideas for PhytoCellTec™ Symphytum

- Recharges youthfulness of skin stem cells
- Boosts cell turnover to rejuvenate the skin from the inside out
- Maintains the regenerative capacity, even as the years pass by
- Reveals a fresher and smoother skin

Applications

- Rejuvenating formulas
- Age-defense products for both face and body
- Skin care products to vitalize the most valuable skin cells – the stem cells

Marketing Benefits

- Cosmetics Business Awards Winner
- Plant stem cells to protect skin stem cells (patent pending)
- Proven efficacy on skin stem cells
- Based on stem cells from a plant that is well known for its medicinal benefits
- Sustainable sourcing of raw material
- Free logo to label your products that contain PhytoCellTec™ actives; as a quality label



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.