PhytoCellTec™ Argan
Vitalizing dermal stem cells to improve skin density
Deep-Seated Rejuvenation of the Skin
In order to maintain the skin in a healthy condition, cutaneous tissue is being continuously regenerated. This regenerative capacity relies on adult stem cells in the skin. While considerable research has been done on epidermal stem cells, dermal stem cells were identified only in 2009. The dermis is the middle layer of the skin and gives it tensile strength and elasticity, therefore it is also the site where wrinkles originate.

PhytoCellTec™ Argan was developed to improve the regenerative capacity of dermal stem cells thereby achieving deep-seated rejuvenation of the skin. PhytoCellTec™ Argan is a powder based on stem cells of the argan tree, one of the oldest tree species in the world.

In order to evaluate which active ingredient effectively promotes dermal stem cell activity, a stable human dermal papilla cell line was used as a new test system: stem cell activity is assessed based on the expression of the Sox2 gene, which is an established stem cell marker. Furthermore, the characteristic property of stem cells to grow in three-dimensional spherical colonies serves as a second observable indicator of stem cell viability in this assay.

Clinical studies performed on healthy volunteers showed that PhytoCellTec™ Argan:
- effectively stimulates the regeneration of dermal connective tissue, thereby increasing skin density
- helps the skin to regain its firmness
- significantly reduces wrinkle depth in crow’s feet area.

PhytoCellTec™ Argan is the very first active ingredient that is capable of both protecting and vitalizing human dermal stem cells. This will not only help to accelerate the skin’s natural repair process but also fights skin aging right at the root.

Claim Ideas for PhytoCellTec™ Argan
- Vitalizes and protects dermal stem cells
- Reduces wrinkles (~26%)
- Tightens and tones skin tissue
- Increases skin firmness and density
- Deep-seated rejuvenation of the skin
- First cosmetic active with proven results for protecting and vitalizing dermal stem cells

Application
- Advanced "stem cell cosmetic" formulas
- Formulas for fundamental rejuvenation
- Anti-wrinkle specialists
- Collagen boosters
- Firming for face and body

Formulating with PhytoCellTec™ Argan
- Recommended use level: 0.4–1%
- Incorporation: Dissolve PhytoCellTec™ Argan into the aqueous phase or add pre-solved, during the cooling phase (<60°C). PhytoCellTec™ Argan is dissolvable at up to 20% in water.
- Thermostability: Temperatures up to 60°C for a short time do not affect the stability of PhytoCellTec™ Argan.

INCI/CTFA-Declaration
Argania Spinosa Callus Culture Extract (and) Isomalt (and) Lecithin (and) Sodium Benzoate (and) Aqua/Water

Additional Information
- PhytoCellTec™ Argan contains 50% of argan stem cell culture
- Phenoxyethanol-free
PhytoCellTec™ Argan
Dermal papillae, a newly discovered niche for dermal stem cells

Dermis – Provides Stabilization and Elasticity to the Skin

While the epidermis acts as a barrier of the skin against the environment, the dermis’s function is to provide mechanical support and elasticity. A healthy tissue is required to maintain these important functions, therefore the skin undergoes a constant cell turnover in order to maintain, renew and repair the skin tissue. New cells are produced to replace damaged or dead ones. Responsible for this regenerative processes are adult stem cells residing in special niches in different layers of the skin:

• epidermal stem cells responsible for the regeneration of the epidermis are located in the basal layer of the epidermis
• hair follicle stem cells ensure renewal of the hair follicle and are located in the hair follicle bulge
• dermal stem cells responsible for maintaining and repairing the dermis were discovered in the dermal papilla.

Adult dermal stem cells were identified only a few years ago, when researchers demonstrated that the dermal papilla serves as a niche for stem/progenitor cells.

PhytoCellTec™ Argan Acts on Dermal Stem Cells

PhytoCellTec™ Malus Domestica
PhytoCellTec™ Solar Vitis
PhytoCellTec™ Alp Rose

Epidermal stem cells

Dermal stem cells

Collagen fibres

Elastin fibres

Dermal stem cells

PhytoCellTec™ Argan

PhytoCellTec™ Argan
**Dermal Stem Cells Play a Decisive Role in Skin Aging**

The recently discovered dermal stem cells were found to self-renew, to induce formation of hair follicles and to differentiate into dermal cell types like fibroblasts. Fibroblast cells are the most prominent cell type in the dermis and responsible for the continuous production of elastin and collagen. These two important structural components build up the extracellular matrix, which gives the skin its properties of elasticity and tensile strength.

However, the regenerative potential of dermal stem cells does not last forever:

**Dermal Stem Cells Do Age**

They have a limited life expectancy. With age, they become less active and decrease in number. As a consequence, senescent fibroblasts are not replaced anymore by new ones, elastin and collagen production drops, and the skin loses its elasticity and firmness – wrinkles appear. Therefore, the protection of dermal stem cells is of great importance.

**Reinforcement of the Regenerative Capacity of Dermal Stem Cells**

The activity of stem cells is regulated by certain epigenetic factors. Such epigenetic factors are present in all stem cells of plants, animals and humans alike. Their function is to maintain the self-renewal capacity of stem cells. Thus epigenetic factors of stem cells from the argan tree are used to help to preserve the vitality of stem cells in the human skin.

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**PhytoCellTec™ Argan**

Argan stem cells to protect dermal stem cells

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**Role of Dermal Stem Cells in Maintenance and Repair of the Dermis**
Endemic to the Southwest of Morocco
The argan tree (Argania spinosa) is indigenous to the arid southwest of Morocco. The tree has perfectly adapted to intense droughts and extremely high temperatures, which are typical in this region. Roots of the argan tree can extend 30m deep into the sandy soil to bring the water from the depth to the surface. This also benefits smaller plants growing in its shade. Thus the tree helps to prevent soil erosion and holds back desertification. Unfortunately, the argan tree population has shrunk because of extensive cultivation of the land, so that nowadays the Moroccan argan tree forests cover an area of only 8000 km². To protect the remaining trees the arganeraie forests in Morocco were designated as a UNESCO International Biosphere Reserve in 1998.

Advanced Biotechnology to Cultivate Plant Stem Cells from the Rare Argan Tree
Mibelle Biochemistry has developed a novel plant cell culture technology enabling the large-scale cultivation of callus cells from rare and protected plant species such as Argania spinosa.

PhytoCellTec™ by Mibelle Biochemistry
This technology is based on the unique totipotency of plant cells:
• the capacity of every plant cell to regenerate any part of or even the whole 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: after 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. For this purpose argan shoots were injured to induce callus formation. Argan callus cells were then cultivated in an appropriate medium and large-scale production was achieved in a specialized WAVE bioreactor system.

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

PhytoCellTec™ Argan is an extract of argan stem cells and is therefore rich in epigenetic factors and metabolites, which maintain the regenerative potential of the skin’s dermal stem cells.
PhytoCellTec™ Argan is Encapsulated into Liposomes

Hair follicles are a promising target for the delivery of active ingredients into the skin and liposomal formulations have been shown to be useful delivery systems.

Therefore PhytoCellTec™ Argan was enveloped into liposomes for improved skin penetration. The dermal stem cells located at the dermal papilla can easily be reached by this method.

The Follicular Delivery System

- Basal membrane
- Sebaceous gland
- Outer root sheath
- Inner root sheath
- Dermal cells in a hair bulb
A New Assay to Measure Dermal Stem Cell Activity
The recently discovered human dermal stem cells have been found to specifically express Sox2, an established stem cell marker. Moreover, they grow in colonies forming three-dimensional spheres, which is characteristic for dermal stem cells.

The new cell line shows the aforementioned two typical stem cell characteristics, which are only expressed by vital dermal stem cells. These two parameters were monitored in order to evaluate the vitalizing effect of PhytoCellTec™ Argan on human dermal stem cells.

Maintenance of Human Dermal Stem Cell Characteristics
The newly established human dermal papilla cell culture was treated with 0.1% of the argan stem cell extract. An untreated culture served as a control. Then, the effect on the dermal stem/progenitor cells was analyzed using the aforementioned parameters.

Compared to the control, the immunofluorescence images clearly show an intensified fluorescence signal (green) in spheres formed by dermal stem cells cultured with the argan stem cell extract, indicating an enhanced production of the fluorescent-labeled Sox2 marker.

After several growth passages, cell cultures incubated with the argan stem cell extract form almost 90% more spheres when seeded onto culture dishes compared to the control.

These results clearly demonstrate that PhytoCellTec™ Argan helps the dermal stem cells to maintain their stem cell characteristics.

### Effect on Sox2 Expression

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<tr>
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<th>Control</th>
<th>+ Argan stem cell extract</th>
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<tbody>
<tr>
<td>Spheres</td>
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**Spheres of dermal stem cells treated with or without argan stem cell extract.**
- Green: Immunofluorescent labeling of stem cell marker Sox2
- Blue: Nuclear counter staining

### Increased Sphere Number

<table>
<thead>
<tr>
<th>Total number of spheres/24 well plate</th>
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<tr>
<td>0.1% Argan stem cell extract</td>
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<td>Control</td>
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**PhytoCellTec™ Argan Study results**
PhytoCellTec™ Argan Improves Skin Density

The capacity of PhytoCellTec™ Argan to improve dermal tissue density was evaluated by visualizing the structure of the dermis using ultrasonography. 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 visible as bright colors in the ultrasonographic image. However, disruption of this regular architecture leads to weaker reflections and dark patches as seen in the left image below. These so-called subepidermal low-echogenic bands (SLEB) are commonly found in aged and photo-damaged skin.

Changes in SLEB of 21 volunteers of average age 49 were monitored by analyzing ultrasonographic images taken from their forearms before and after treatment with either a 0.4% PhytoCellTec™ Argan cream or a corresponding placebo.

Results clearly show that PhytoCellTec™ Argan stimulates the regenerative capacity of the upper dermis tissue compared to the placebo. The SLEB is reduced due to an improved dermal tissue density:

- by 5.5% after 28 days of treatment.
- by 13% after 56 days of treatment.

Improvement of Tissue Density in the Upper Dermis

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<th>Day 0</th>
<th>Day 56</th>
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![Image of ultrasonographic images with SLEB](image)

*Changes in skin density relative to placebo and D0 (%)*

*p=0.004 versus initial conditions*
Anti-Wrinkle Effect on Crow's Feet

The anti-wrinkle effect was demonstrated in a half-face study with 21 healthy volunteers, average age 49. A cream containing 0.4% PhytoCellTec™ Argan extract or a placebo cream were applied twice daily, one to each side of the face including the crow's feet. After 56 days, wrinkle depth was determined by the PRIMOS® technique.

PhytoCellTec™ Argan successfully reduced the wrinkle depth in crow's feet area:
- by 19% after 28 days of treatment.
- by 26% after 56 days of treatment.

* p=0.001 versus initial conditions
** p=0.0005 versus initial conditions
Improvement of Skin Firmness at Women’s Thighs

The skin of the thighs in women is especially susceptible to the appearance of dimples. This is due to the special structure of the subcutaneous tissue of a woman’s thigh. The fat chambers extend vertically to store fat cells, which serve as energy storage. However, if the overlying connective tissue is weak and thin the adipose tissue protrudes into the reticular dermis, leading to a dimpled skin.

A successful method to combat skin dimpling is to tighten the connective tissue.

The efficacy of PhytoCellTec™ Argan in strengthening the connective tissue was evaluated in a double-blind placebo-controlled study with 22 healthy volunteers aged from 29 to 53. A cream containing 0.4% PhytoCellTec™ Argan was applied twice daily for 56 days on one thigh of the volunteers, the corresponding placebo on the other.

In the ultrasonographic images the heterogeneous dermis appears bright, whereas the more homogeneous hypodermis shows up dark. Adipose tissue, which protrudes into the lower dermis, is recognized by the irregular, broad dermis-hypodermis junction area (image day 0). A 56-day treatment with PhytoCellTec™ Argan shows a tightening of the dermal connective tissue compared to initial conditions, leading to a much smoother dermis-hypodermis junction. PhytoCellTec™ Argan reduces the dermis-hypodermis junction area:
• by 14% after 56 days of treatment.

**Tightening of the Connective Tissue of Woman’s Thighs**

**Reduction of the Dermis-Hypodermis Junction Area**

* *p<0.05 versus initial conditions*
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Applications
• Advanced "stem cell cosmetic" formulas
• Formulas for fundamental rejuvenation
• Anti-wrinkle specialists
• Collagen boosters
• Firming for face and body

Marketing Benefits
• Next generation of stem cell cosmetics
• First active with proven efficacy on dermal stem cells
• Sustainable sourcing of raw material
• www.phytocelltec.ch

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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