



PhytoCellTec™

PIONEER
IN STEM CELL
ACTIVES

PhytoCellTec™ Malus Domestica
Plant stem cells
to protect skin stem cells



PhytoCellTec™ Malus Domestica

Plant stem cells to protect skin stem cells

A Revolutionary Technology to Protect Skin Stem Cells

PhytoCellTec™ Malus Domestica is a liposomal preparation based on the stem cells of a rare Swiss apple.

Uttwiler Spätlauber is an endangered apple variety that was well-known for its excellent storability and thus its longevity potential.

Mibelle Biochemistry has developed a novel technology enabling the cultivation of rare and endangered species like Uttwiler Spätlauber. Thanks to this technology called PhytoCellTec™, plant stem cells can be obtained and incorporated into cosmetic products to ensure the longevity of skin cells.

The protection of human stem cells by PhytoCellTec™ Malus Domestica has been shown by various in-vitro experiments. Other studies demonstrated its age-delaying and anti-wrinkle effects. Thus PhytoCellTec™ Malus Domestica is a revolutionary anti-aging active ingredient based on a high-tech plant cell culture technology.

Claim Ideas for PhytoCellTec™ Malus Domestica

- Protects longevity of skin stem cells
- Delays senescence of essential cells
- Combats chronological aging
- Preserves the youthful look and the vitality of your skin

Applications

- Skin care products to protect skin stem cells
- Real rejuvenation for face and body care
- Innovative skin care formulations

Formulating with PhytoCellTec™ Malus Domestica

- Recommended concentration: 2–5%
- Incorporation: For cold processes, mix PhytoCellTec™ Malus Domestica into the aqueous phase. In cold/hot processes, add during the cooling phase below 40°C.
- Thermostability: temperatures of up to 60°C for a short time do not affect the stability of PhytoCellTec™ Malus Domestica.

INCI/CTFA-Declaration

Malus Domestica Fruit Cell Culture Extract (and) Xanthan Gum (and) Glycerin (and) Lecithin (and) Phenoxyethanol (and) Aqua / Water

PhytoCellTec™ Malus Domestica

Swiss apple stem cells to protect skin stem cells

A Swiss Apple Variety with Incredible Properties

Uttwiler Spätlauber is a variety of a Swiss apple that derives from a seedling planted in the middle of the 18th century. It was very famous for its excellent storability without shriveling. Today apple cultivars are selected to maximize crop yield and sweet flavor. Thus Uttwiler Spätlauber with its acid taste is now disappearing.

Uttwiler Spätlauber apples are rich in phytonutrients, proteins and long-living cells. This particular composition leads to incredible storability and longevity properties.

Stem Cells and Longevity

Longevity is related to specific cells called stem cells which have a unique growth characteristic. These unspecialized (undifferentiated) cells can make identical copies of themselves as well as differentiate to become specialized cells. Two basic types of stem cells are present in the human body:

- Embryonic stem cells found in blastocysts can grow and differentiate into one of the more than 220 different cell types which make up the human body.
- Adult stem cells located in some adult tissues can only differentiate into their own or related cell types. These cells act as a repair system for the body but also maintain the normal turnover of regenerative organs such as blood, skin or intestinal tissues.

Research on Stem Cells and Applications

Currently in medicine, adult stem cells are already used particularly in transplant medicine to treat leukemia and severe burns.

In the cosmetic field, scientists are focusing their research on adult stem cells located in the skin. They are studying the potential of this type of cells, their functioning and aging. These researches will help to understand how to protect skin stem cells.

Stem Cells in the Human Skin

In the human skin, two types of adult stem cells have been identified:

- Epithelial skin stem cells which are located in the basal layer of the epidermis.
- Hair bulge stem cells located in the hair follicle.

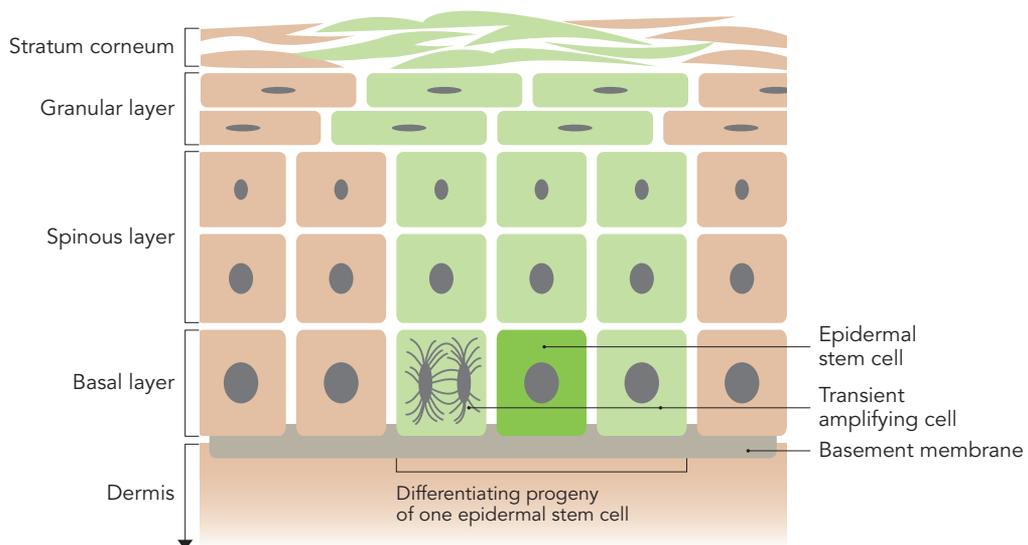
Epidermal stem cells replenish and maintain the balance of cells within the skin tissue and regenerate tissue damages during injury. But with age, the number of skin stem cells decreases and their ability to repair the skin becomes less efficient.

Plant Stem Cells to Protect Skin Stem Cells

In contrast to human, plant cells are totipotent, meaning that every cell has the ability to regenerate new organs (leave, flower, seed...) or even the whole plant. Besides, all plant cells can dedifferentiate and become a stem cell.

All stem cells, independently of their origin (plant, animal or human) contain specific epigenetic factors whose function is to maintain the self-renewal capacity of stem cells.

Epidermal Stem Cells in the Human Skin



PhytoCellTec™

A high-tech biotechnology to cultivate cells from rare plants

PhytoCellTec™ by Mibelle Biochemistry

Mibelle Biochemistry developed a novel technology (PCT) enabling the cultivation of cells from rare and endangered plant species. This PCT technology, based on the unique totipotency of plant cells uses the wound healing mechanism of plants. A part of a plant is wounded to induce the formation of callus cells. This wound healing tissue consists of dedifferentiated cells which are stem cells. Callus cells are harvested and cultivated in a suspension and a novel bioreactor system enables a large scale culture. To obtain the PhytoCellTec™ Malus Domestica cosmetic ingredient, these stem cells are harvested and homogenized at 1200 bar together with liposomes to encapsulate and stabilize oil- and water soluble components.

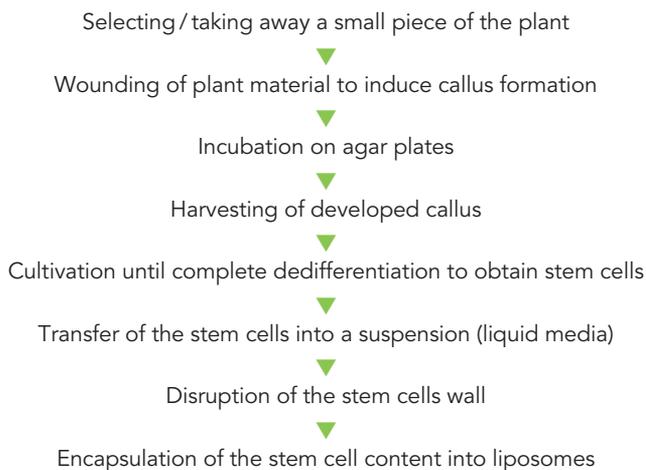
PhytoCellTec™ Malus Domestica is thus rich in epigenetic factors and metabolites which assure the longevity of cells and protect stem cells.

Advantages of PhytoCellTec™ Technology

This innovative technology developed by Mibelle Biochemistry offers the following advantages:

- Possibility to cultivate cells of rare and endangered plants while respecting the environment
- Availability of plant material independent of the season and market's demand
- Plant material completely free of environmental pollutants and pesticides
- Constant concentrations of metabolites in the stem cells

PhytoCellTec™ Process



PhytoCellTec™ Malus Domestica

Study results



Maintenance of Stem Cell Growth

An in vitro test was conducted on umbilical cord blood stem cells with Malus Domestica stem cell extract which is the active component of PhytoCellTec™ Malus Domestica. Umbilical cord blood stem cells (UCBSC) are the “youngest” safely available stem cells for research.

The influence of Malus Domestica stem cell extract on UCBSC artificial growth was evaluated by counting the cell number after incubation.

Results showed that Malus Domestica stem cell extract has a positive effect on UCBSC’s artificial growth thus maintaining the growth and the proliferative activity of UCBSC.



Protection against UV Radiation

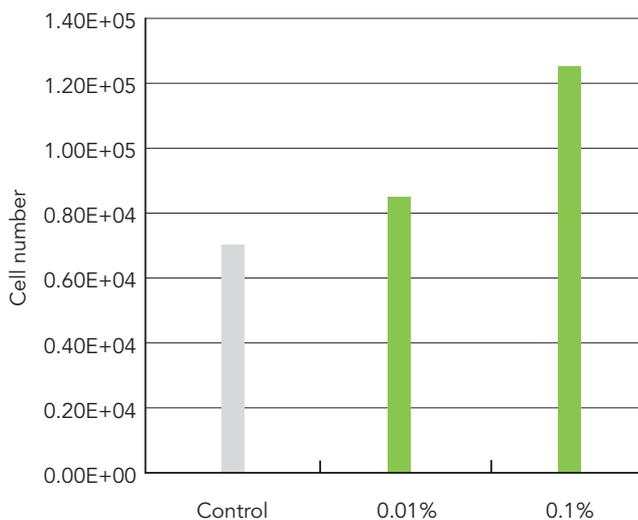
A second in vitro test was conducted on umbilical cord blood stem cells with Malus Domestica stem cell extract which is the active component of PhytoCellTec™ Malus Domestica.

The protective effect against UV damage of Malus Domestica stem cell extract on UCBSC was evaluated by an MTS assay. UCBSC were incubated with different concentrations of Malus Domestica stem cell extract for 24h and were then exposed to UV radiation. The MTS assay, which measures the number of living cells and therefore the damage from UV, was performed 48h after UV radiation.

Results showed the capacity of Malus Domestica stem cell extract to protect UCBSC from UV damage even at low concentrations.

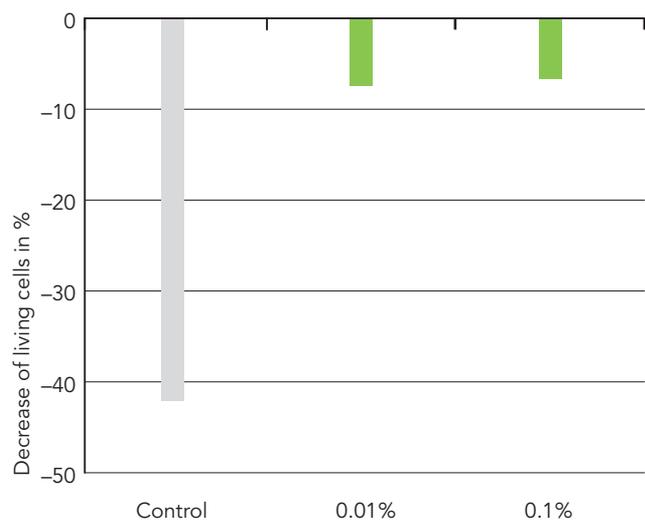
Maintenance of Stem Cell Growth

■ Malus Domestica stem cell extract



Protection of Stem Cells against UV Damage

■ Malus Domestica stem cell extract



PhytoCellTec™ Malus Domestica

Study results



Enhancement of Epidermal Stem Cells Characteristics

A novel Progenitor Cell Targeting technology was used to prepare human epidermal stem cells.

The method consists essentially of culturing primary human keratinocytes in a medium specifically designed to mimic the micro-environment of the *in vivo* stem cell niche. This special, fully defined cell culture medium leads to an enrichment of so called keratinocyte progenitor cells that can be considered as activated stem cells. Compared to freshly isolated cells, the cell population of passage 4 is characterized by a 10-fold increase of CD34/alpha6 integrin double labelled cells. CD34 and alpha6 integrin are known markers of epidermal stem cells.

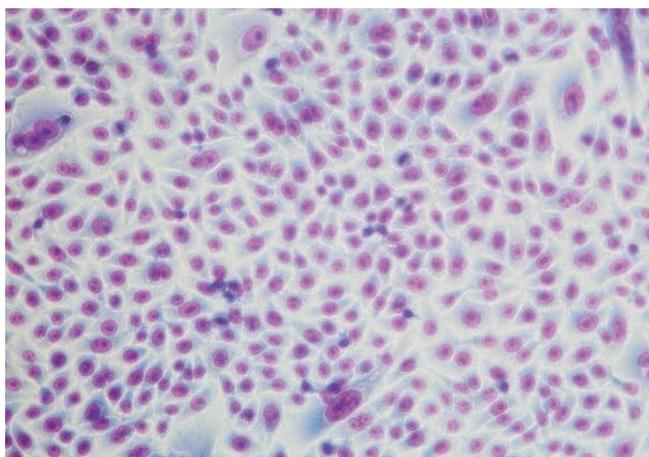
Another characteristic of stem cells is their high colony-forming efficiency (CFE). For analysis of CFE, cells are seeded at low density. The number of colonies formed is a value of the concentration of progenitor/stem cells, because differentiated keratinocytes have lost the capacity to divide.

The epidermal stem cells obtained thanks to the Progenitor Cell Targeting technology were incubated with the Malus Domestica stem cell extract.

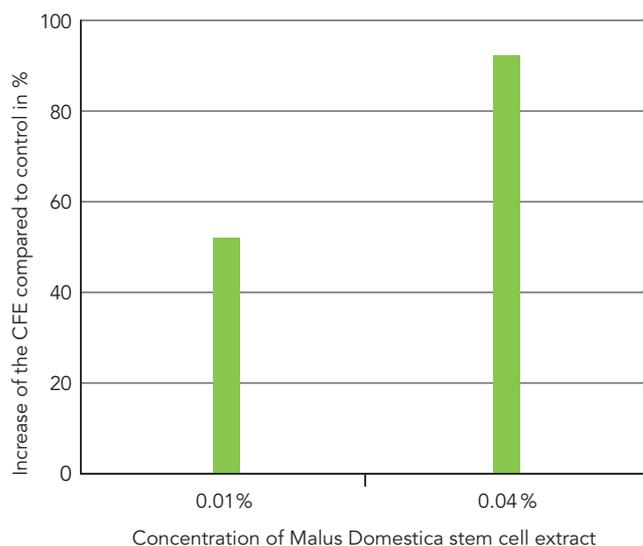
The CFE was determined from cells of different passages and compared to a control culture. Results showed that the CFE was stimulated by 92% in the presence of 0.04% of the Malus Domestica stem cell extract.

This clearly shows an improved maintenance of the stem cell characteristics of epidermal stem cells.

Keratinocyte Progenitor Cells in Culture



Increase of the Colony-Forming Efficiency (CFE)





Effect on Gene Expression in Senescent Dermal Fibroblasts

Recently a cellular model for premature senescence was established based on normal human dermal fibroblasts. After a two hours treatment with H₂O₂ the cells showed the typical signs of senescence. This model was used to prove the anti-senescence effect of the Malus Domestica stem cell extract. After the H₂O₂ treatment, the fibroblast cells were incubated with a 2 % extract for 144 hours. Then gene expression was analyzed with a cDNA array system comprising 150 probes.

Results showed that Malus Domestica stem cell extract can up-regulate specific genes involved in:

- Delay of senescence
- Protection against oxidative stress
- Repair of DNA

Up-Regulation of Specific Genes involved in the Delay of Senescence

↓ Gene, that is down regulated by senescence induction (Control)

↑ Gene, that is up regulated or normalized in senescent cells by PhytoCellTec™ Malus Domestica

Genes	after H ₂ O ₂ Control	after H ₂ O ₂ + 2 % Malus Domestica stem cell extract
cyclin B1: induces proliferation	73 ↓	130 ↑
cyclin E1: cell cycle regulator	78 ↓	135 ↑
p53: tumor suppressor gene	63 ↓	137 ↑
insulin-like growth factor II: cell proliferation enhancer	71 ↓	117 ↑
heme oxygenase 1: antioxidant enzyme	89 ↓	211 ↑

PhytoCellTec™ Malus Domestica

Study results



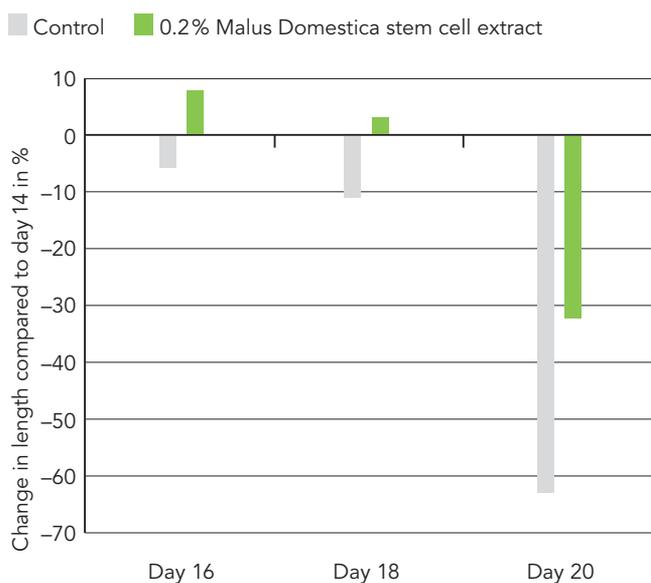
Age-Delaying Effect on Isolated Human Hair Follicles

Human hair follicles can be isolated by microdissection from skin fragments that are left after facelift surgery. Hair follicles are mini organs that represent a natural coculture model of epidermal and melanocyte stem cells and differentiated cells. The follicles can be maintained in a growth medium where they elongate until about day 14. Then the follicle cells gradually become senescent or undergo apoptosis which is a programmed cell death. This necrosis process is caused by the lack of blood circulation. Isolated hair follicles represent a test model to screen for actives that can delay the necrosis process.

Isolated human hair follicles were incubated with Malus Domestica stem cell extract.

Addition of 0.2 % of this extract was found to slightly but clearly postpone senescence- and apoptosis-induced necrosis: follicles kept in presence of the Malus Domestica stem cell extract continued to elongate until day 18, whereas the control follicles started to shrink after day 14.

Age-Delaying Effect





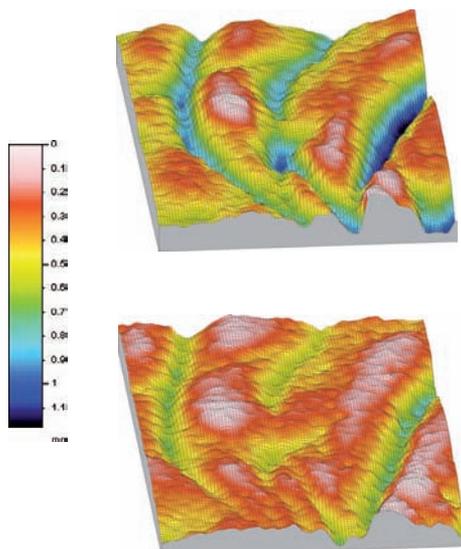
Anti-Wrinkle Effect on "Crow's Feet"

The anti-wrinkle effect of PhytoCellTec™ Malus Domestica was evaluated in a study with 20 volunteers aged from 37 to 64.

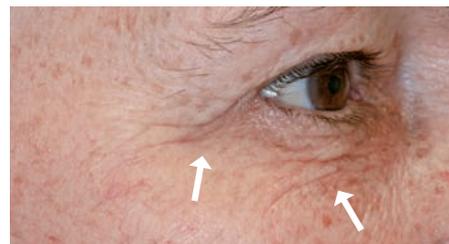
An emulsion containing 2 % of PhytoCellTec™ Malus Domestica was applied twice daily for 28 days to the crow's feet. Wrinkle depth was determined by means of PRIMOS (phase-shifting rapid in vivo measurement of skin).

Results showed a significant and visible decrease in wrinkle depth for 100 % of the subjects.

Anti-Wrinkle Effect on Crow's Feet



Before
(day 0)



After
(day 28)
2 % PhytoCellTec™
Malus Domestica





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Applications

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- Real rejuvenation products for face and body care
- Innovative skin care formulations

Marketing Benefits

- First plant stem cell active ingredient on the market (patent pending)
- Innovation in "stem cells" cosmetics
- Breakthrough in anti-aging

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