



**PIONEER**  
IN STEM CELL  
ACTIVES

PhytoCellTec™ Malus Domestica  
Plant stem cells  
for skin stem cell protection



# PhytoCellTec™ Malus Domestica

## Plant stem cells for skin stem cell protection

### 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.

Studies showed the double activity of PhytoCellTec™ Malus Domestica:

- it helps skin stem cells to maintain their characteristics and their capacity to build new tissues
- it delays aging and has an anti-wrinkle effect.

PhytoCellTec™ Malus Domestica is the first plant stem cell active ingredient on the market whose effect was evaluated on human skin stem cells. This unique and revolutionary ingredient is able to protect the most precious skin cells, the skin stem cells, against premature aging.

### Claim Ideas for PhytoCellTec™ Malus Domestica

- Protects longevity of skin stem cells
- Delays senescence of essential cells
- Increases the vitality of skin stem cells
- Combats chronological aging

### Applications

- Advanced "stem cell cosmetic" formulas
- Real rejuvenation for face and body care
- Innovative skin care formulations

### Formulating with PhytoCellTec™ Malus Domestica

- Recommended use level: 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

### Additional Information

- PhytoCellTec™ Malus Domestica contains 9 % of Malus Domestica stem cell extract
- Available in a phenoxyethanol-free version
- Available in powder form

# PhytoCellTec™ Malus Domestica

## Swiss apple stem cells to protect skin stem cells

### **A Rare Apple with Incredible Properties**

Uttwiler Spätlauber is a variety of a Swiss apple that derives from a seedling planted in the middle of the 18<sup>th</sup> 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. They must have especially long-living tissue stem cells. Their 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.



### 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.

Fast regenerating tissues such as the epidermis are particularly dependent on their stem cells because only stem cells retain the capacity to divide and thus to provide new tissues.

Epidermal stem cells have 2 main functions:

- replenish and maintain the balance of cells within the skin
- regenerate damaged tissues.

### Skin Stem Cells and Aging

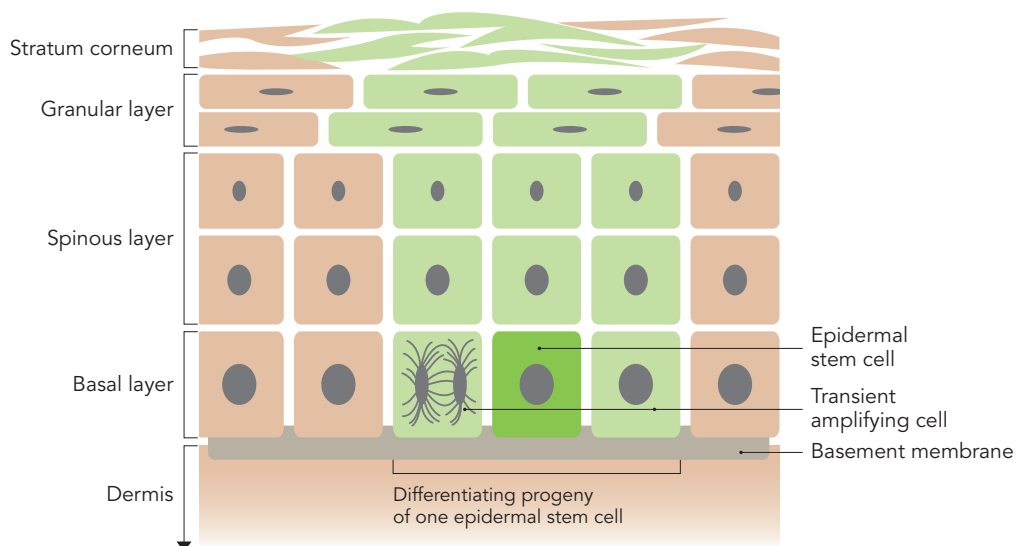
- Skin stem cells have a limited life expectancy.
- Intrinsic or extrinsic stress factors can affect their functionality.
- With age, skin stem cells are less active and lower in number.

This explains why the depletion of skin stem cells is regarded as the primary cause of skin aging.

### Plant Stem Cells to Protect Skin Stem Cells

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. Therefore the *Malus Domestica* stem cells are used to help to preserve the vitality of human skin stem cells.

## Epidermal Stem Cells in the Human Skin



# PhytoCellTec™ Malus Domestica

## Advanced biotechnology to cultivate cells from rare plants

### PhytoCellTec™ by Mibelle Biochemistry

Mibelle Biochemistry has developed a novel technology called PhytoCellTec™, enabling the large scale cultivation of cells from rare and endangered plant species.

This technology is based on the unique totipotency of plant cells that is to say:

- the capacity of every plant cell to regenerate new organs 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: part of a plant is wounded to induce the formation of callus cells. This healing tissue consists of dedifferentiated cells which are stem cells. Callus cells are harvested and cultivated in a suspension. A novel bioreactor system enables the large scale production.

To obtain the PhytoCellTec™ Malus Domestica cosmetic ingredient, these stem cells are harvested and homogenized at 1200 bar together with phospholipids to encapsulate and stabilize oil- and water-soluble components into liposomes.

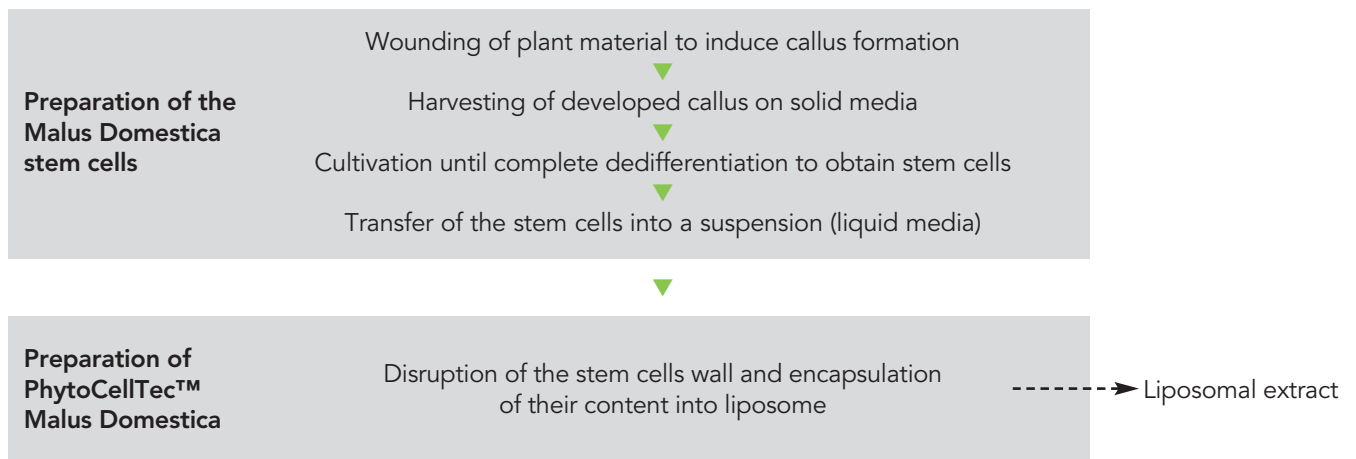
PhytoCellTec™ Malus Domestica is thus rich in epigenetic factors and metabolites which assure the longevity of cells and protect stem cells.

### Advantages of PhytoCellTec™ Technology

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

- possibility to cultivate cells of rare and endangered plants based on small amounts of origin plant material
- availability of plant material independent of the season and market demand
- plant material completely free of environmental pollutants and pesticides
- constant concentrations of metabolites in the stem cells

### PhytoCellTec™ Malus Domestica Process



# PhytoCellTec™ Malus Domestica

## Study results



### Preparation of Epidermal Stem Cells

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

This technology consists of culturing a skin sample in a medium specifically designed to mimic the micro-environment of the stem cell in the epidermis.

This special, fully defined cell culture medium leads to an enrichment of so called keratinocyte progenitor cells that can be considered as stem cells. This enrichment was quantitatively controlled through FACS (Fluorescence-activated cell sorting) of cells which were labelled 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 10-fold increase of CD34/ $\alpha 6$  integrin double labelled cells.

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



### The Capacity to Form a Colony is a Characteristic of Stem Cells

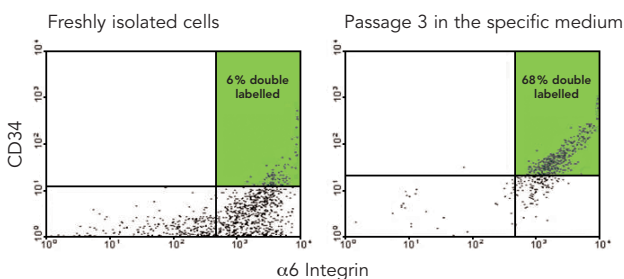
Stem cells have the characteristic to form colonies in vitro.

A colony consists of:

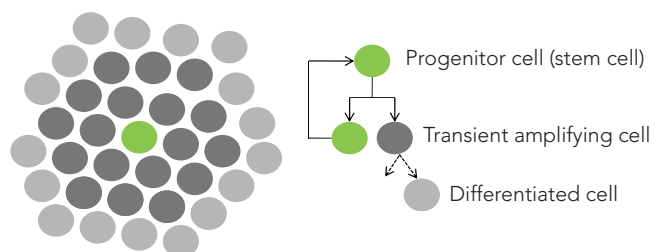
- the progenitor cell
- transient amplifying cells (cells in an intermediate state)
- differentiated cells that have lost the capacity to divide.

The number of colonies formed is a value of the vital progenitor /stem cells and is called colony forming efficiency (CFE). To measure CFE, cells are seeded at a low density.

### Enrichment of Keratinocyte Progenitor Cells



### Capacity to Form a Colony (CFE)



# PhytoCellTec™ Malus Domestica

## Study results



### Maintenance of Epidermal Stem Cell Characteristics

The epidermal stem cells obtained using the previously described method, were treated with different concentrations of the Malus Domestica stem cell extract. Then, the effect of the Malus Domestica stem cell extract on the CFE was analysed and compared to a control culture.

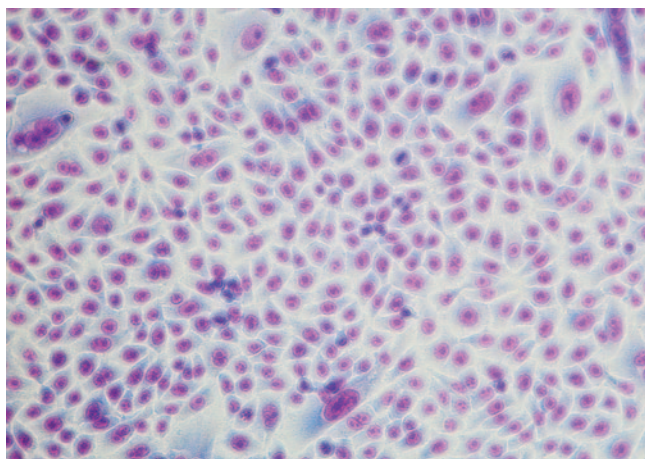
Results showed that the CFE was increased by 92% in the presence of 0.04% of the Malus Domestica stem cell extract.

Two other studies using this novel Progenitor Cell Targeting technology also showed that:

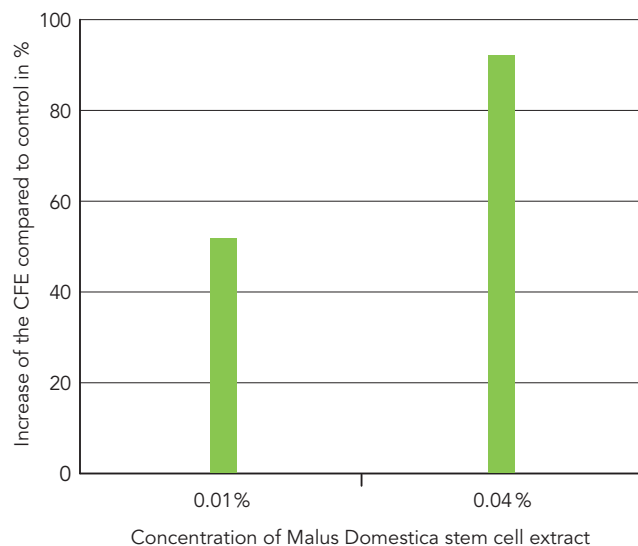
- this improvement of the CFE is not based on a general growth stimulation of the progenitor cells
- CFE is still improved when the Malus Domestica stem cells are washed indicating that the stimulating effect is not due to the growth medium.

This clearly shows that Malus Domestica stem cell extract helps the epidermal stem cells to maintain their stem cell characteristics ("stemness").

### Keratinocyte Progenitor Cells in Culture



### Increase of the Colony-Forming Efficiency (CFE)







### Maintenance of the Capacity to Build New Tissues

Epidermal stem cells were obtained using the novel Progenitor Cell Targeting technology previously described. They were then cultured for different numbers of passages with and without 0.01% Malus Domestica stem cell extract. Afterwards, their capacity to form a stratified epidermis was evaluated.

From the fresh epidermal stem cells, two kinds of cells were tested:

- “young” epidermal stem cells obtained from early passage (passage 5)
- “old” epidermal stem cells obtained from late passage (passage 14).

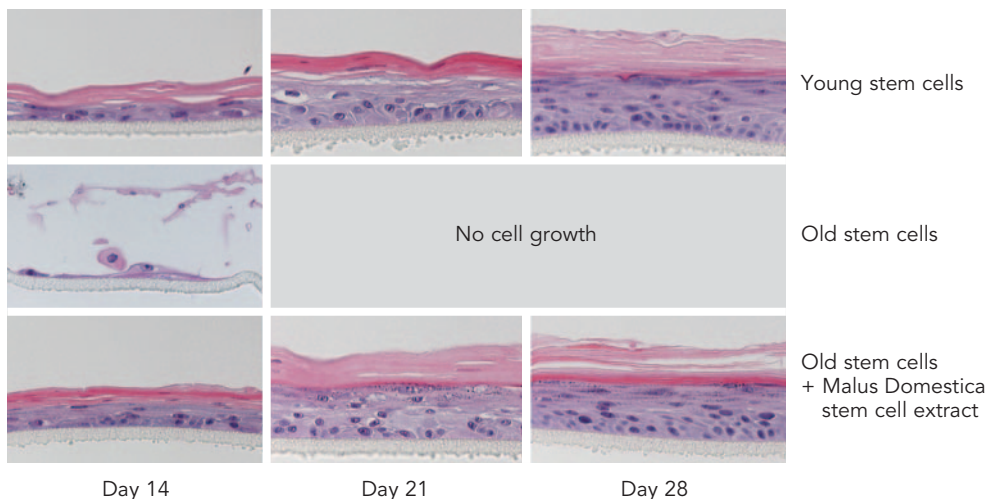
The Malus Domestica stem cell extract was added to the culture from early passage.

Results showed that:

- “old” epidermal stem cells lost their capacity to build a 3D epidermis
- but, if treated with Malus Domestica stem cell extract from their early passage, “old” epidermal stem cells retain their capacity to form a stratified epidermis. This 3D epidermis was made of all 4 epidermal layers (stratum basale, spinosum, granulosum, corneum) and was indistinguishable from the one obtained with the “young” epidermal stem cells.

In presence of Malus Domestica stem cell extract, skin stem cells keep longer their ability to proliferate and to undergo the complex process of stratification and differentiation. Thus, the Malus Domestica stem cell extract helps the epidermal stem cells to retain their capacity to build new tissues.

### Maintenance of the Capacity to Form a 3D Epidermis



# PhytoCellTec™ Malus Domestica

## Study results



### Effect on Gene Expression in Senescent Dermal Fibroblasts

Recently a cellular model for premature senescence was established based on normal human dermal fibroblasts.

The fibroblasts were stressed with H<sub>2</sub>O<sub>2</sub> in order to induce premature senescence. The fibroblasts were then incubated with 2 % Malus Domestica stem cell extract. Then gene expression was analyzed with a cDNA array system comprising 150 probes.

Results showed that, in the control culture, several genes important for cellular proliferation and stimulation of cellular growth were down-regulated. But the Malus Domestica stem cell extract was found to neutralize this down-regulation and even induce an up-regulation of specific genes involved in the:

- delay of senescence
- protection against oxidative stress.



### Age-Delaying Effect on Isolated Human Hair Follicles

Human hair follicles in anagen phase were isolated 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, they gradually become senescent or undergo apoptosis due to the lack of blood circulation: they stop growing and start shrinking, this is 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 the presence of the extract continued to elongate until day 18, whereas the control follicles started to shrink after day 14.

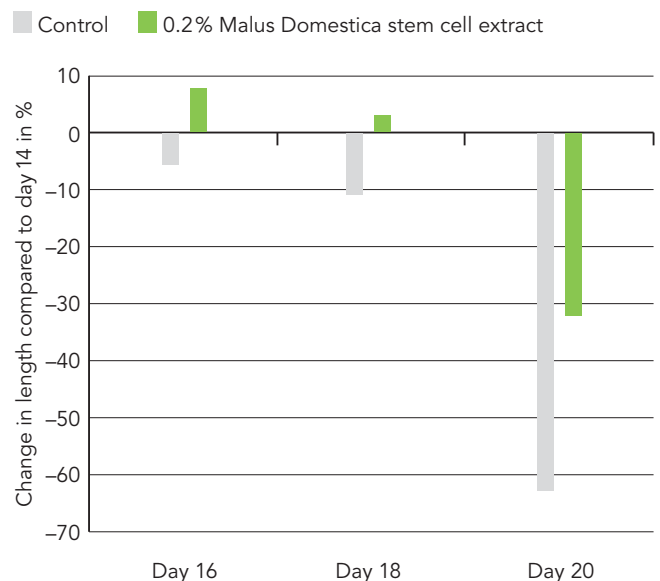
#### Reversion of Senescence Signs in Fibroblasts

Genes	after H <sub>2</sub> O <sub>2</sub> Control	after H <sub>2</sub> O <sub>2</sub> + 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 ↑

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

↑ Gene, that is up-regulated or normalized in senescent cells by Malus Domestica stem cell extract

#### Increased Lifespan of Isolated Hair Follicles





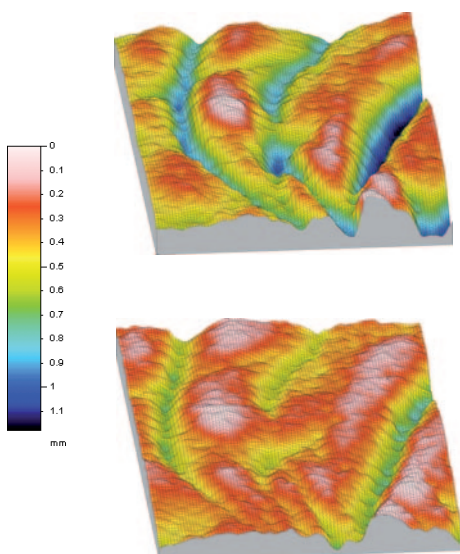
### Anti-Wrinkle Effect on "Crow's Feet"

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

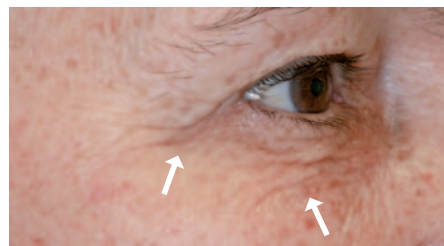
An emulsion containing 2% of PhytoCellTec™ Malus Domestica was applied twice daily for 28 days to the crow's feet. Wrinkle depth was determined by means of PRIMOS (phase-shifting rapid in vivo measurement of skin).

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

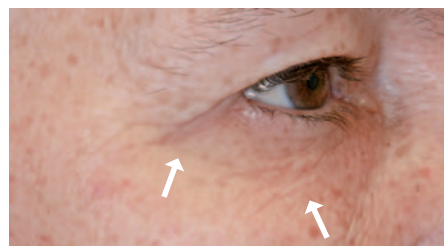
### Anti-Wrinkle Effect on Crow's Feet



Before  
(day 0)



After  
(day 28)  
2% PhytoCellTec™  
Malus Domestica





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### Applications

- Advanced "stem cell cosmetic" formulas
- Real rejuvenation for face and body care
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### Marketing Benefits

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

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