mibelle GROUP biochemistry

PLANT STEM CELLS
PhytoCellTec.ch™
SKIN CELL PROTECTION

EUROPEAN COSMETICS
INNOVATION PRIZE 2008
CATEGORY: BEST ACTIVE INGREDIENT

Approved by ECOCERT RAW MATERIAL
COSMOS APPROVED

PhytoCellTec™
Malus Domestica *Hair*
Plant stem cells to rejuvenate hair follicles

SWISS QUALITY PRODUCT

PhytoCellTec™ Malus Domestica
Plant stem cells to rejuvenate hair follicles

A Revolutionary Technology to Protect Hair Stem Cells
PhytoCellTec™ Malus Domestica is a patented liposomal preparation that is based on the stem cells of a rare Swiss apple.

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

Mibelle Biochemistry has developed a novel technology that enables the cultivation of such rare and endangered species as Uttwiler Spätlauber. Thanks to this technology, which is called PhytoCellTec™, plant stem cells can be obtained and incorporated into cosmetic products to ensure the longevity of hair cells.

Studies have shown that PhytoCellTec™ Malus Domestica:
- helps keratinocyte and dermal papilla stem cells to maintain their stem cell characteristics
- stimulates hair growth
- prolongs the anagen phase of hair
- delays hair aging
- prevents hair loss.

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

PhytoCellTec™ Malus Domestica
- Vitalizes hair stem cells
- Delays senescence of hair follicles
- For a visibly improved hair density

Applications
- Stem cell cosmetics
- Rejuvenation of hair follicles
- Delay of hair aging
- Anti-hair loss and hair regrowth formulations

** Raw material approved by ECOCERT GREENLIFE in accordance with the COSMOS Standard*

PhytoCellTec™ Malus Domestica
Swiss apple stem cells for the protection of hair stem cells

A Rare Apple Featuring Remarkable Properties
Uttwiler Spätlauber is a variety of a Swiss apple that derives from a seedling first planted in the middle of the 18th century. It was well-renowned for its excellent storability without shriveling. Nowadays, apple cultivars are selected to maximize crop yield and sweet flavor. Consequently, Uttwiler Spätlauber, with its acidic taste, is disappearing.

Uttwiler Spätlauber apples are rich in phytonutrients, proteins, and long-living cells. They appear to have especially long-living tissue stem cells and the particular composition of these cells leads to remarkable storability and longevity properties.

Stem Cells and Longevity
The concept of longevity relates to specific cells, that are known as stem cells and 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 that 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, hair, or intestinal tissues.

The Human Hair Has its own Pools of Stem Cells

The human hair follicle contains stem cells that are located in two main areas, which are also termed stem cell niches:

- in the bulge area that is located within the outer root sheath (ORS) of the hair follicle (the ORS which surrounds the hair fiber is continuous with the epidermal basal layer that also contains stem cells)
- at the base of the hair follicle in the dermal papilla area located in the dermis.

Hair follicle regeneration is dependent on the interaction between these distinct stem cell populations.

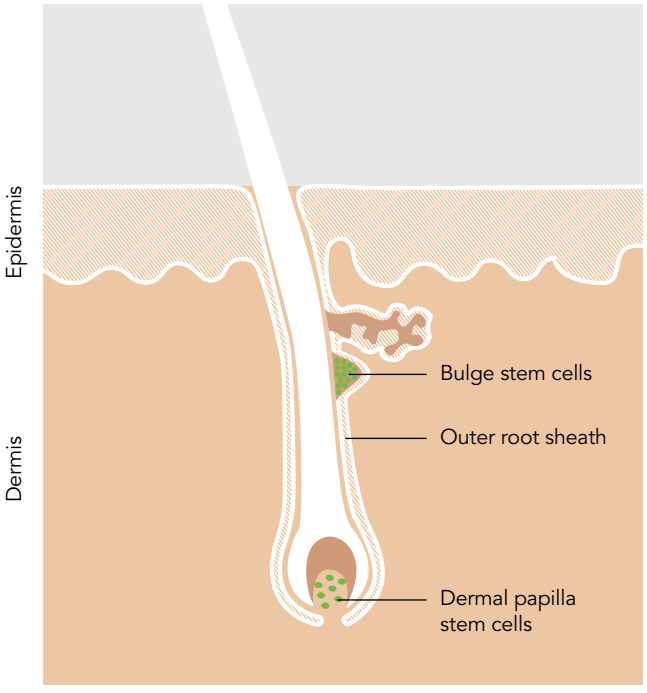
Stem Cells Regulate Hair Growth

The epithelial stem cells located in the hair bulge region of the ORS are required for hair growth and supply cells for the maintenance of the germinative epithelium during the growth phase of the hair. These cells ultimately differentiate into the hair matrix cells.

The mesenchymal stem cells, which are located in the dermal papilla, have distinct functions from epithelial stem cells. These cells are considered to be initiators of the hair cycle by sending instructive signals that induce proliferation of the epithelial stem cells of the bulge and the consequent initiation of hair follicle growth.

In the hair follicle, stem cells notably replenish and maintain the balance of cells within the hair and ensure hair regeneration.

Stem Cells of the Human Hair



PhytoCellTec™ Malus Domestica

Swiss apple stem cells for the protection of hair stem cells

The Hair Undergoes Cyclic Renewal

Hair follicles continuously undergo cyclical growth and regression throughout their life, and this consists of three phases:

- Anagen: during this growth phase, which lasts approximately between three and five years, the stem cells, which are located in the ORS in the bulge area, migrate into the hair bulb to form new cells. This phase is marked by a rapid proliferation of cells.
- Catagen: the involution phase lasts a couple of weeks. During this period, cell proliferation and differentiation ceases and cell apoptosis is initiated.
- Telogen: this is a resting period lasting up to four months, after which the hair is shed when a new anagen hair grows below.

This cycling activity greatly depends on stem cells that supply cells for the hair matrix by cell division and specialization (differentiation).

Hair Stem Cells and Aging

- Hair follicle stem cells have a limited life expectancy and they can undergo only a limited number of cell divisions.
- Intrinsic or extrinsic stress factors can affect their functionality.
- With age, hair stem cells are less active and lower in terms of number.

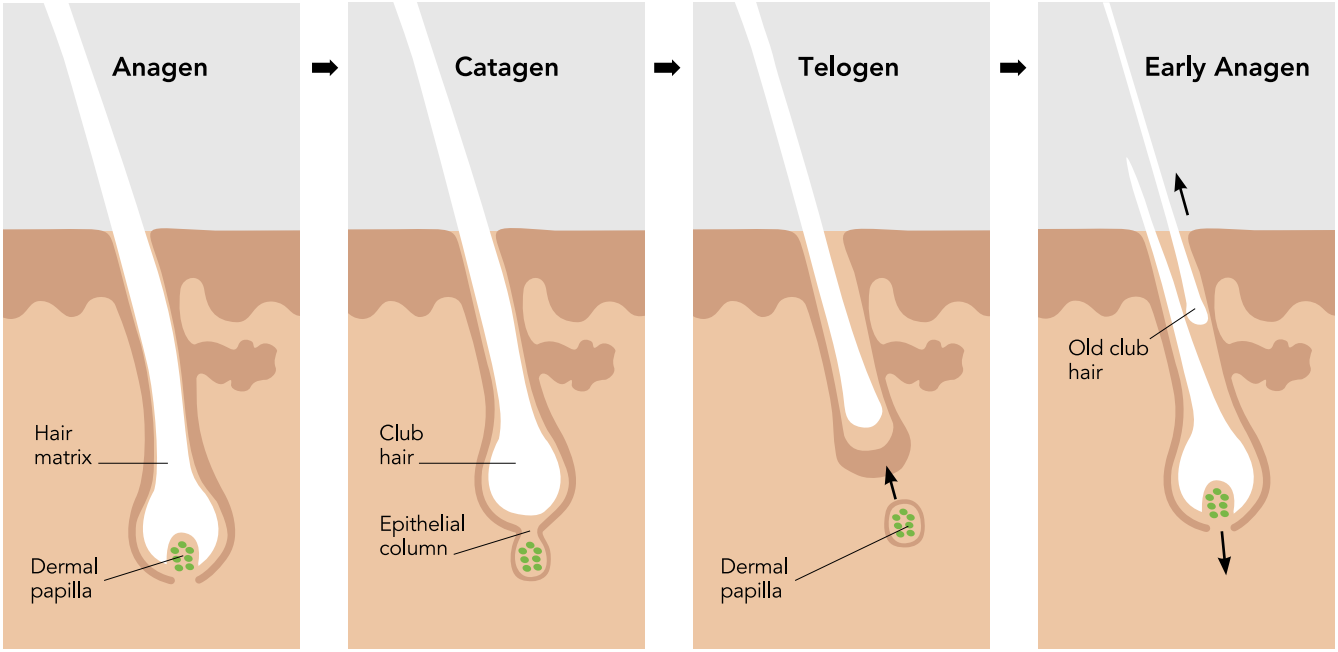
The depletion of hair follicle stem cells leads to a reduced regeneration potential and ultimately to hair aging and hair loss.

Plant Stem Cells to Protect Hair Stem Cells

All stem cells, independently of their origin (i. e. regardless of whether plant, animal, or human) contain specific epigenetic factors, the function of which is to maintain the self-renewal capacity of stem cells. Therefore, the Malus Domestica stem cells are used in order to help preserve the vitality of human hair stem cells.

The topical use of PhytoCellTec™ Malus Domestica is patented in Europe (EP 1 985 280 B1), the USA (US 9,155,916 B2/US 8,580,320 B2) and Korea (10-1470632 B1).

The Hair Growth Cycle



PhytoCellTec™ Malus Domestica
Study results

Preparation of Epidermal Stem Cells
A novel Progenitor Cell Targeting technology was used in order to prepare human epidermal stem cells.

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

This special, fully defined cell culture medium leads to an enrichment of so-called keratinocyte progenitor cells that can be considered as epidermal stem cells. This enrichment was quantitatively controlled through FACS (Fluorescence-activated cell sorting) of cells, which were labelled with CD34 and α6 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/α6 integrin double-labelled cells.

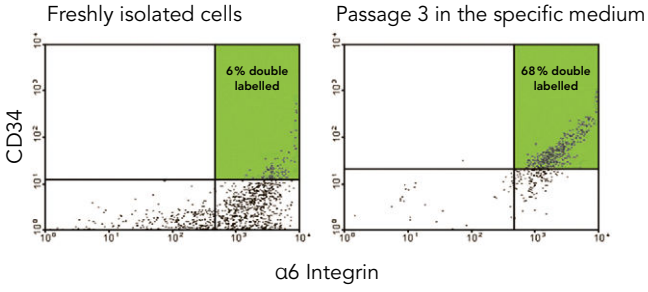
The Progenitor Cell Targeting technology provides a cell culture model that specifically enables the evaluation of the effects of compounds on epidermal stem cells.

The Capacity to Form a Colony is a Characteristic of Stem Cells
Stem cells have the characteristic to form colonies in vitro.

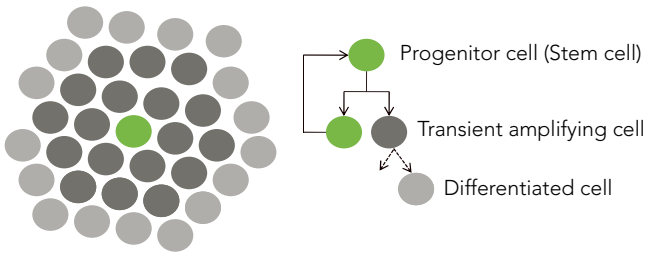
A colony consists of:
- the progenitor cell
- transient amplifying cells (cells in an intermediate state)
- differentiated cells that have lost the capacity to divide.

The number of colonies formed is a value of the vital progenitor/stem cells and it is known as colony forming efficiency (CFE). For the measurement of CFE, cells are seeded at a low density.

Enrichment of Keratinocyte Progenitor Cells



Capacity to Form a Colony (CFE)



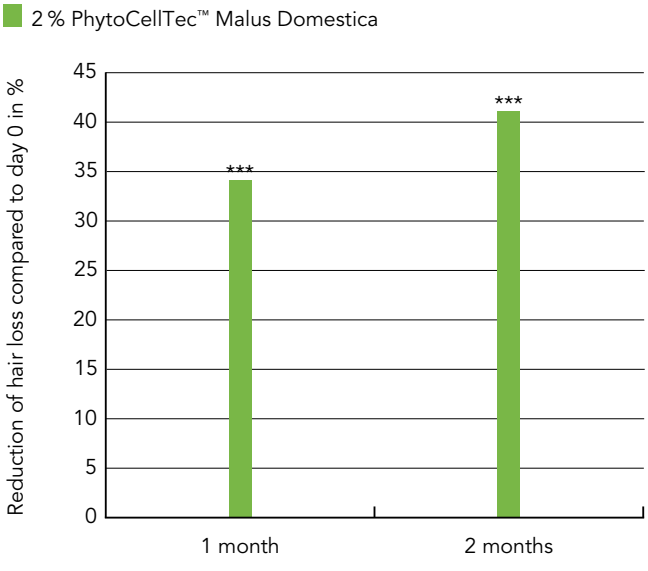
Reduction of Hair Loss

The efficacy of PhytoCellTec™ Malus Domestica to reduce hair loss was assessed in a clinical study. For this study, 18 volunteers (aged from 20 to 63 years) who were losing at least 100 hairs per day were enrolled. The participants applied a hair lotion that contained 2 % PhytoCellTec™ Malus Domestica and 0.1 % caffeine once daily (in the evening) for a period of 2 months. Collections and counting of shed hair were performed at the beginning of the study and after 1 month and 2 months, respectively. Moreover, the visible effects of the treatment were documented by photographs taken of the top of the head of each volunteer.

Results showed that the hair lotion containing 2 % PhytoCellTec™ Malus Domestica significantly reduced hair loss. After one month of treatment, the number of shed hairs decreased by 34 %, and after two months, the number reduced by 41 %. Both reductions were significant compared to the initial conditions.

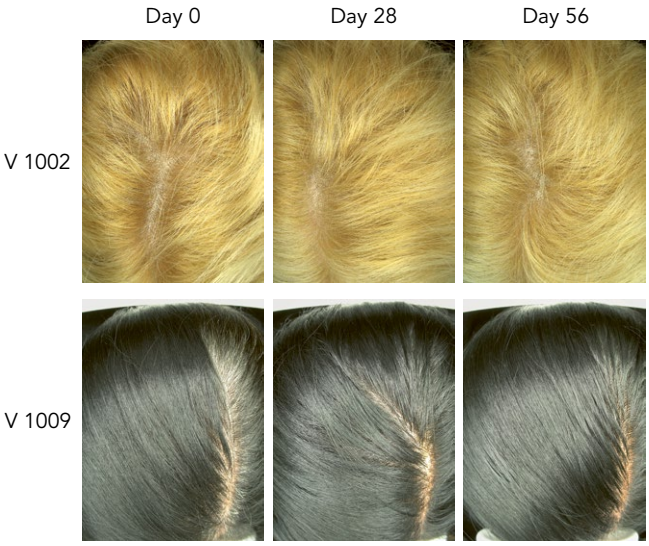
Consequently, the hair density visibly improved compared to initial conditions, which was clear to see in photographs taken from the volunteers after 1 month and 2 months, respectively.

Reduction of Hair Loss after 1 and 2 Months



***p<0.001 versus initial conditions

Visible Improvement of Hair Density



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Maintenance of Epidermal Stem Cell Characteristics – Increase of the Capacity to Form Colonies

The epidermal stem cells obtained using the Progenitor Cell Targeting method were treated with different concentrations of the Malus Domestica stem cell extract. Subsequently, the effect of the Malus Domestica stem cell extract on the CFE was analyzed and compared to a control culture.

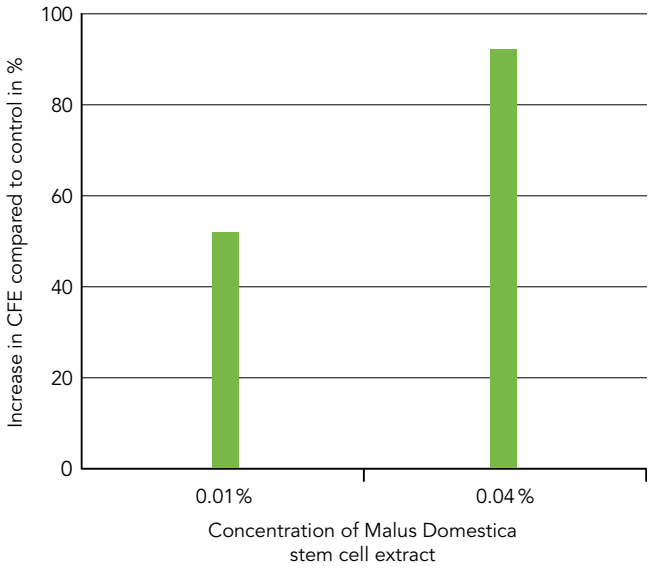
Results showed that the CFE was increased by 92 % in the presence of 0.04 % of the Malus Domestica stem cell extract.

Meanwhile, 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, which indicates that the stimulating effect is not due to the growth medium.

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

Increase of the Colony-Forming Efficiency (CFE)



PhytoCellTec™ Malus Domestica
Study results

Sphere-Formation Assay with Dermal Stem Cells

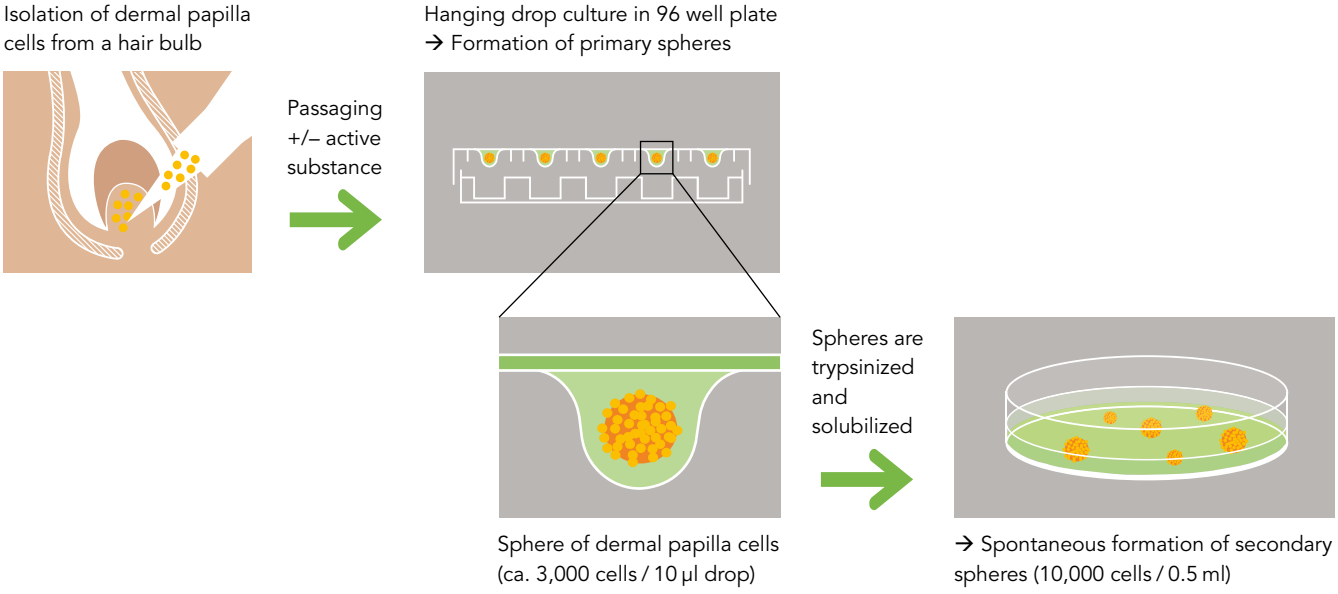
For this experiment, dermal stem cells were isolated from human dermal papilla, a known stem cell niche in the dermis. When cultivated in vitro, these cells grow in colonies to form three dimensional spheres, which is characteristic of dermal stem cells.

Isolated dermal stem cells can subsequently be cultured and passaged in the presence of an active ingredient, while an untreated culture serves as a control. The formation of primary spheres by stem cells in the population is assessed by employing the hanging drop method. This involves ~3,000 cells being seeded in a drop which is then cultivated in a suspended manner. Spheres readily form after a few days.

Primary spheres can be dissociated enzymatically and the resulting single cells are seeded into regular cell culture dishes. The formation of secondary spheres from cells in this suspension is monitored over a period of three weeks and representative pictures are taken thereafter.

The number of secondary spheres is indicative of the portion of stem cells contained in the cell suspension obtained from the dissociated primary spheres.

Isolation of Dermal Papilla Stem Cells and Sphere Formation Assay



Maintenance of Dermal Stem Cell Characteristics

For this experiment, dermal stem cells were isolated from human dermal papilla using the method described on the previous page. These cells form three-dimensional spheres, which is characteristic of dermal stem cells. Moreover, dermal stem cells have been found to specifically express the established stem cell marker Sox2. These two parameters were monitored in order to evaluate the vitalizing effect of PhytoCellTec™ Malus Domestica on human dermal stem cells.

The isolated cells were treated with 0.1 % Malus Domestica stem cell extract, while an untreated culture served as a control. Following the treatment, the effect on the dermal stem cells was analyzed using the above-mentioned parameters.

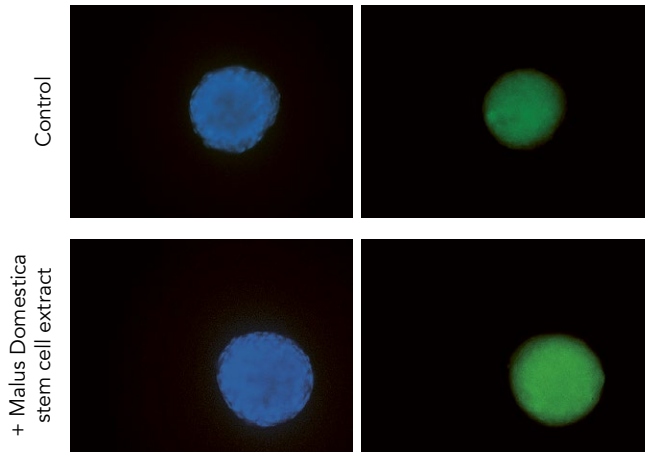
The stem cell marker Sox2 was visualized by immunofluorescent labeling. Compared to the control, the fluorescence signal (green) in spheres formed by dermal stem cells cultured with the Malus Domestica stem cell extract was visibly stronger. This indicates an enhanced production of Sox2.

After several growth passages, cell cultures incubated with the Malus Domestica stem cell extract formed 43 % more spheres when seeded onto culture dishes compared to the control.

These results clearly showed that PhytoCellTec™ Malus Domestica helps the dermal papilla stem cells to maintain their stem cell characteristics.

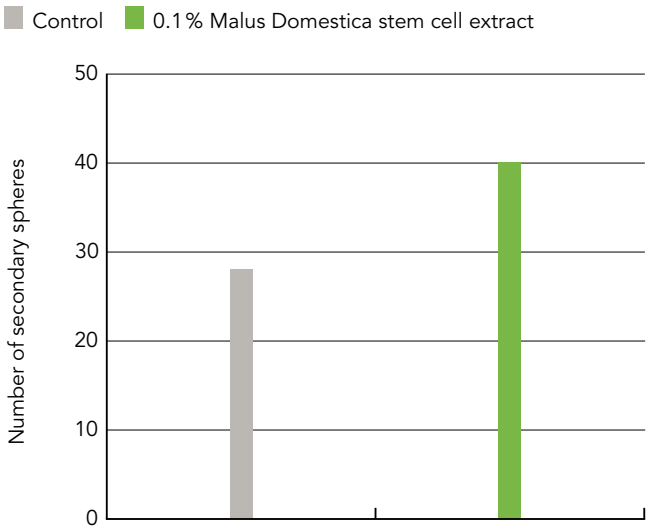
Effect on Sox2 Expression

● Immunofluorescent labeling of stem cell marker Sox2
● Nuclear counter staining



Spheres of dermal stem cells treated with or without Malus Domestica stem cell extract.

Increased Sphere Number



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PhytoCellTec™ Malus Domestica
Study results

Maintenance of Epidermal Stem Cell Characteristics – Increase of Stem Cell Marker

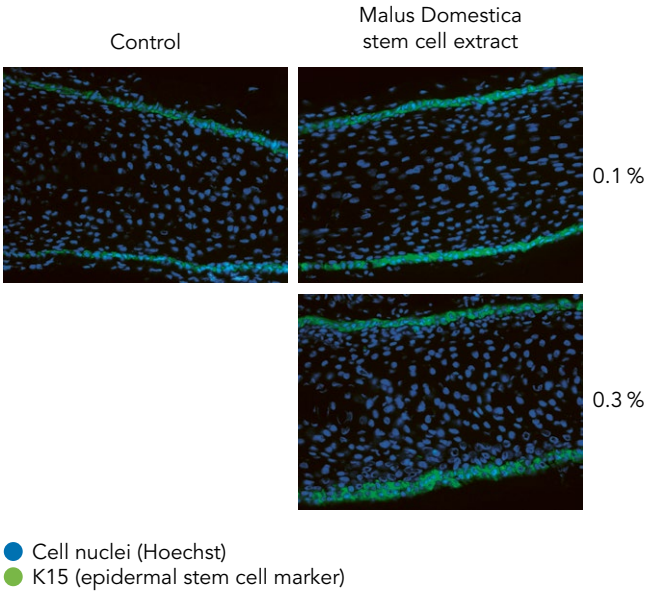
Epithelial stem cells located in the outer root sheath (ORS) in the bulge area are characterized by specific marker proteins, such as Keratin 15 (K15). With the help of this marker, the stem cells can be localized and their stemness can be analyzed.

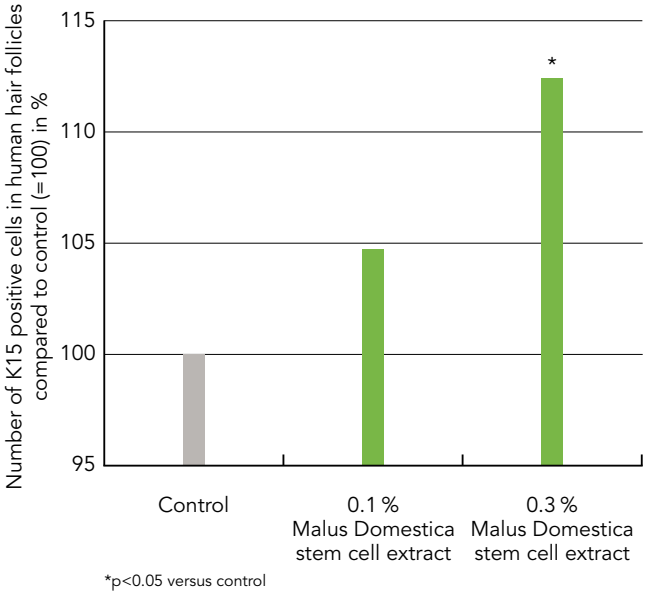
Hair follicles were isolated from a 20-year-old male donor. They were then cultivated with different concentrations of the Malus Domestica stem cell extract. Subsequently, K15 positive cells were visualized and quantified by immunofluorescence in the bulge area. Furthermore, the total quantity of K15 was measured.

Immunofluorescence pictures showed that the Malus Domestica stem cell extract increases the number of K15 positive cells in the basal layer of the ORS in the bulge area. Results of an independent study also demonstrated that the Malus Domestica stem cell extract increased the expression of the stem cell marker K15 in this area (data not shown).

Therefore, the Malus Domestica stem cell extract keeps the stemness of keratinocyte stem cells and consequently maintains their regenerative potential.

Increase of K15 Positive Cells in the ORS of the Hair Follicle – Bulge Area





*p<0.05 versus control

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Age-Delaying Effect on Isolated Human Hair Follicles

Hair follicles are miniature organs that represent a natural co-culture model of dermal, epidermal and melanocyte stem cells, and differentiated cells.

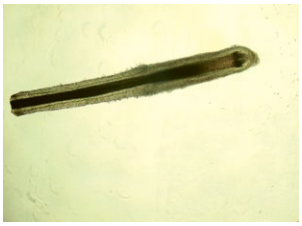
Human hair follicles in the anagen phase were isolated from skin fragments that were obtained following a facelift surgery. The follicles can be maintained in a growth medium where they elongate until approximately day 14. After this, they gradually become senescent or undergo apoptosis due to the lack of blood circulation. As a result, they stop growing and start to shrink in a process that is known as 'necrosis'.

Isolated human hair follicles were incubated with the Malus Domestica stem cell extract or left untreated (control). The addition of 0.2 % of the stem cell extract was found to slightly but clearly postpone senescence and cell death. As a consequence, the follicles cultured, in the presence of the extract continued to elongate until day 18, whereas the control follicles already started to shrink after day 14.

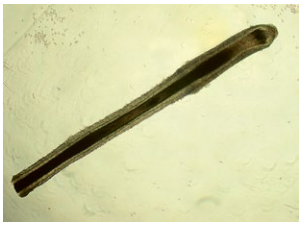
Growth of Hair Follicle in Vitro

Day 0

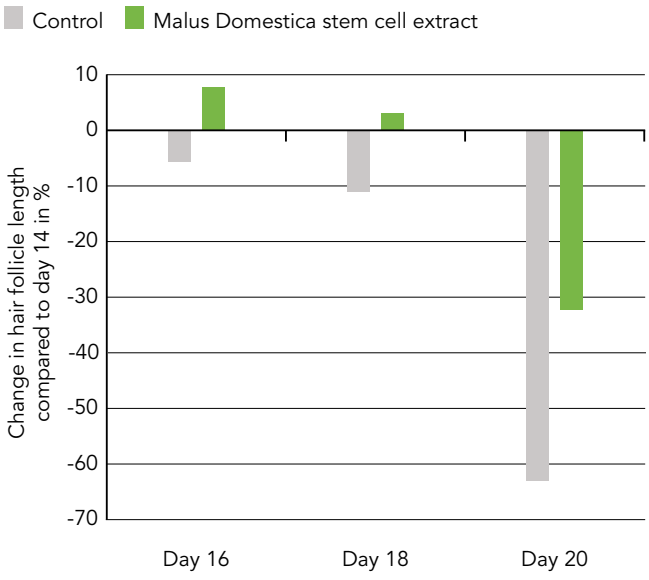
Day 4



Day 7



Increased Lifespan of Isolated Hair Follicles



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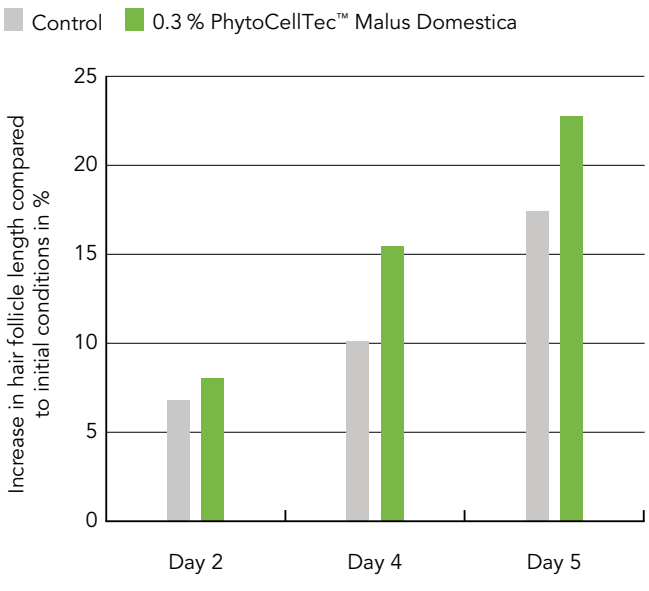
PhytoCellTec™ Malus Domestica
Study results

Growth Stimulation and Extension of the Growth Phase on Isolated Human Hair Follicles

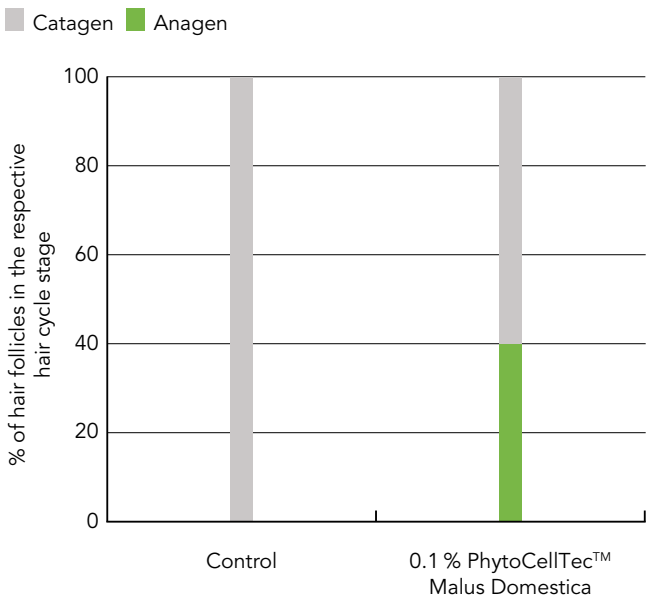
Human hair follicles in the anagen phase were isolated from the occipital scalp skin of a 42-year-old man. The treatment of isolated hair follicles with 0.3 % PhytoCellTec™ Malus Domestica for 5 days clearly increased the elongation of the follicles. Compared to untreated control follicles, the elongation was enhanced by 6 %.

After 5 days of incubation, the growth phase of the hair follicles was analyzed. Following this 5-day period, 100 % of the control follicles were found to be in the catagen phase. In contrast, in the group of follicles treated with 0.1 % PhytoCellTec™ Malus Domestica, 40 % of hair follicles stayed in the anagen phase, while only 60 % of follicles moved to the catagen phase, indicating a delay in hair follicle aging.

Increase in Length



Delay of Anagen-Catagen Transition (Day 5)



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