PhytoCellTec™ Malus Domestica

Plant stem cells for skin stem cell protection

EUROPEAN COSMETICS
INNOVATION PRIZE 2008
CATEGORY: BEST ACTIVE INGREDIENT

SWISS QUALITY PRODUCT

Uttwiler Spätlauber - an Apple with an Excellent Storage Ability

- Storage ability of apples was an important factor to guarantee fresh fruits over winter in the 18th century
- Modern apple cultivars are selected for a sweet flavour and therefore the Uttwiler Spätlauber disappeared
- Today the Uttwiler Spätlauber apple is an endangered apple variety with only a few trees left in the world

The Secret of Storage Ability

- Longevity of cells
- High acid and tannin concentrations
- Undiscovered metabolites?

→ Can we use this apple for skin care?

Young and Old

Plant Cell Culture Technology (PCT)

- PCT makes it possible to cultivate cells of endangered and rare species
- The technology is based on dedifferentiated plant cells
- These cells are plant stem cells and can be cultivated in liquid systems

What are Stem Cells?

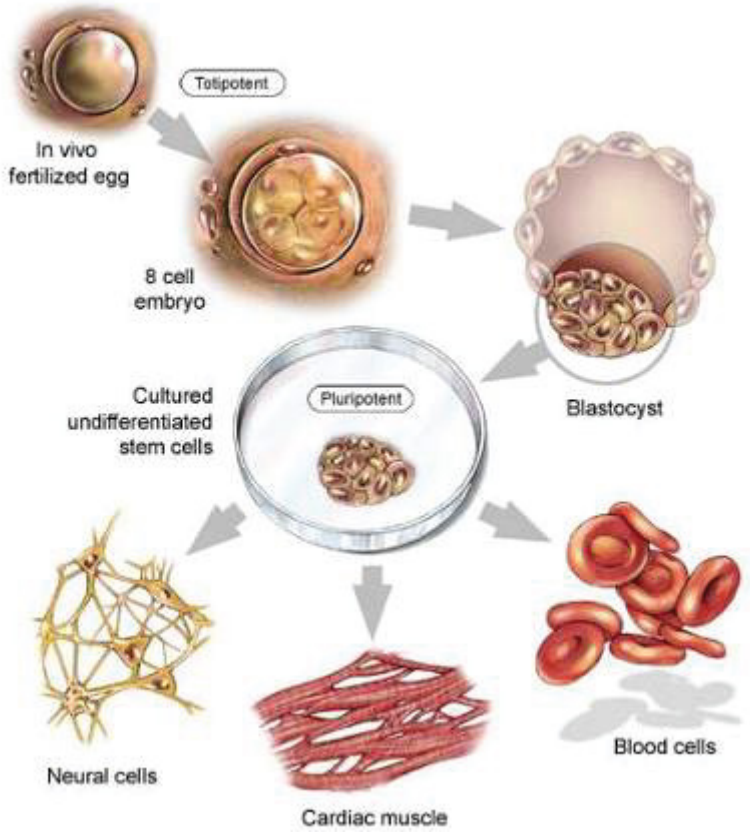
- Stem cells have two properties:

  Self-renewal - the ability to go through numerous cycles of cell division in an undifferentiated state

  Differentiation - the capacity to differentiate into other cell types

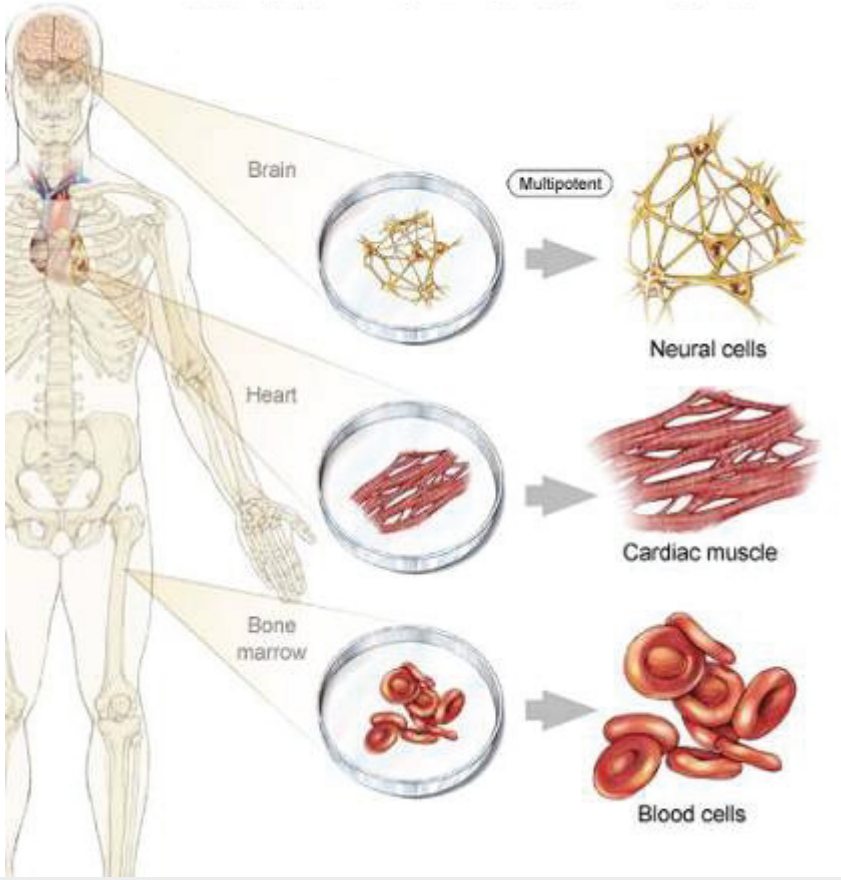
- Toti-, pluri-, multi-, unipotent stem cells

Human: Embryonic Stem Cells (Pluripotent)



- Cells derived from the inner cell mass of an early stage embryo
- Blastocyst, 4-5 days post fertilization, 50-150 cells
- Pluripotent: Differentiate into one of the more than 220 different cell types of an adult body
- The use of embryonic stem cells in medicine is discussed controversially

Human: Adult Stem Cells (Multipotent)



- Undifferentiated cells among differentiated cells in a tissue or organ
- Generate all the cell types of the organ from which they originate — potentially regenerating the entire organ from a few cells
- Primary roles: Replenish dying cells and regenerate damaged tissues

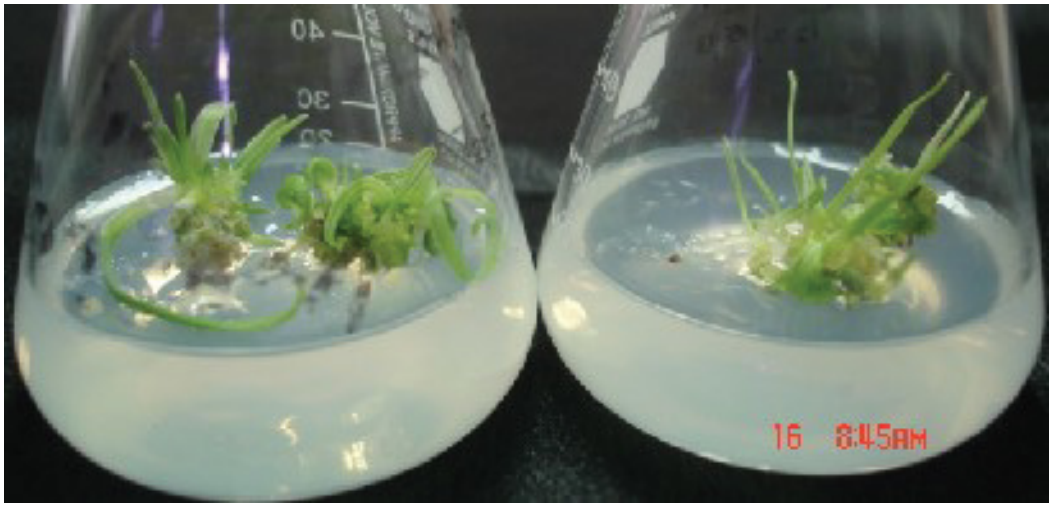
Plant Stem Cells
Formation of Callus Tissue

- If a plant is wounded, a callus tissue is formed (wound healing tissue)
- In the callus tissue the normal differentiated cells go back into a dedifferentiated form and become stem cells (not possible in humans)
- Mechanism is controlled by different plant hormones (auxins)

PhytoCellTec™ by Mibelle Biochemistry

- PhytoCellTec™ utilizes this unique totipotency of plant cells
- Only a small quantity of plant material of endangered species is needed
- Induction of callus tissue by cutting plant parts
- Isolation of stem cells and cultivation on agar plates

From Callus to Plants

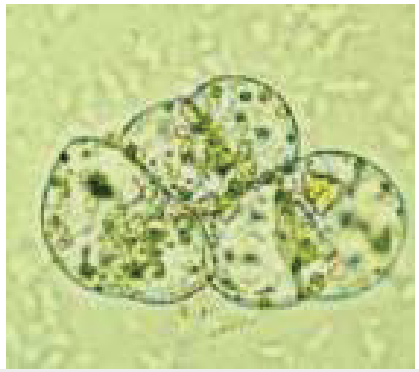


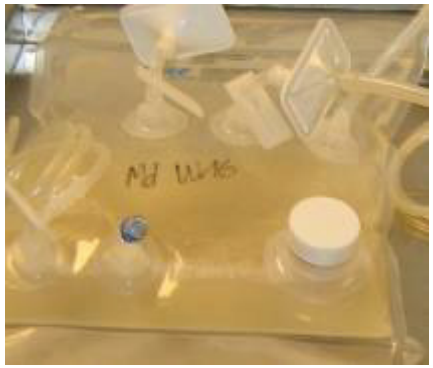
PhytoCellTec™

- Complete dedifferentiation of the cells into a homogenous culture (4 -12 months)
- Transformation of the cells into liquid suspension

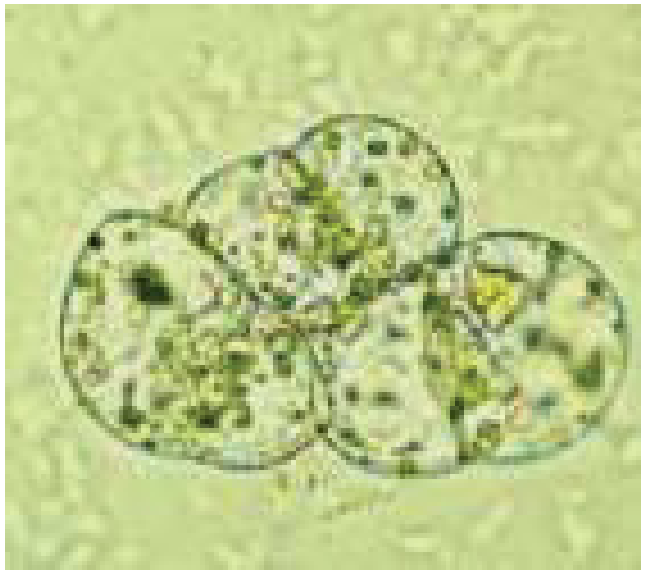
  (1 - 6 months)
- Find optimal conditions for cell cultivation

  (1 - 6 months)





Advantages of PhytoCellTec™



- Preservation of endangered plants
- Use of rare plants for cosmetic applications
- Growth of plant cells in-vitro
- Controllable and reproducible production of metabolites
- No limitation of plant sources due to seasonal restrictions

Production of the Cosmetic Active: PhytoCellTec™ Malus Domestica

- Suspension cultures from apple stem cells (Uttwiler Spätlauber) are grown in bioreactors
- Stem cells grow with oxygen and without light (chlorophyll free)
- Batch is harvested after all sugar is used
- The cells are homogenized to release the secondary metabolites
- Oil- and water-soluble ingredients are stabilized by liposomal structures
- Suspension is fixed in a Xanthan Gum gel

PhytoCellTec™ Malus Domestica

Composition

Malus Domestica Stem Cells	9.0 %
Phospholipids	0.14%
Glycerin	0.4 %
Phenoxyethanol	0.8 %
Keltrol T	1.0 %
Deionized water	ad.100 %

CTFA/INCI

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

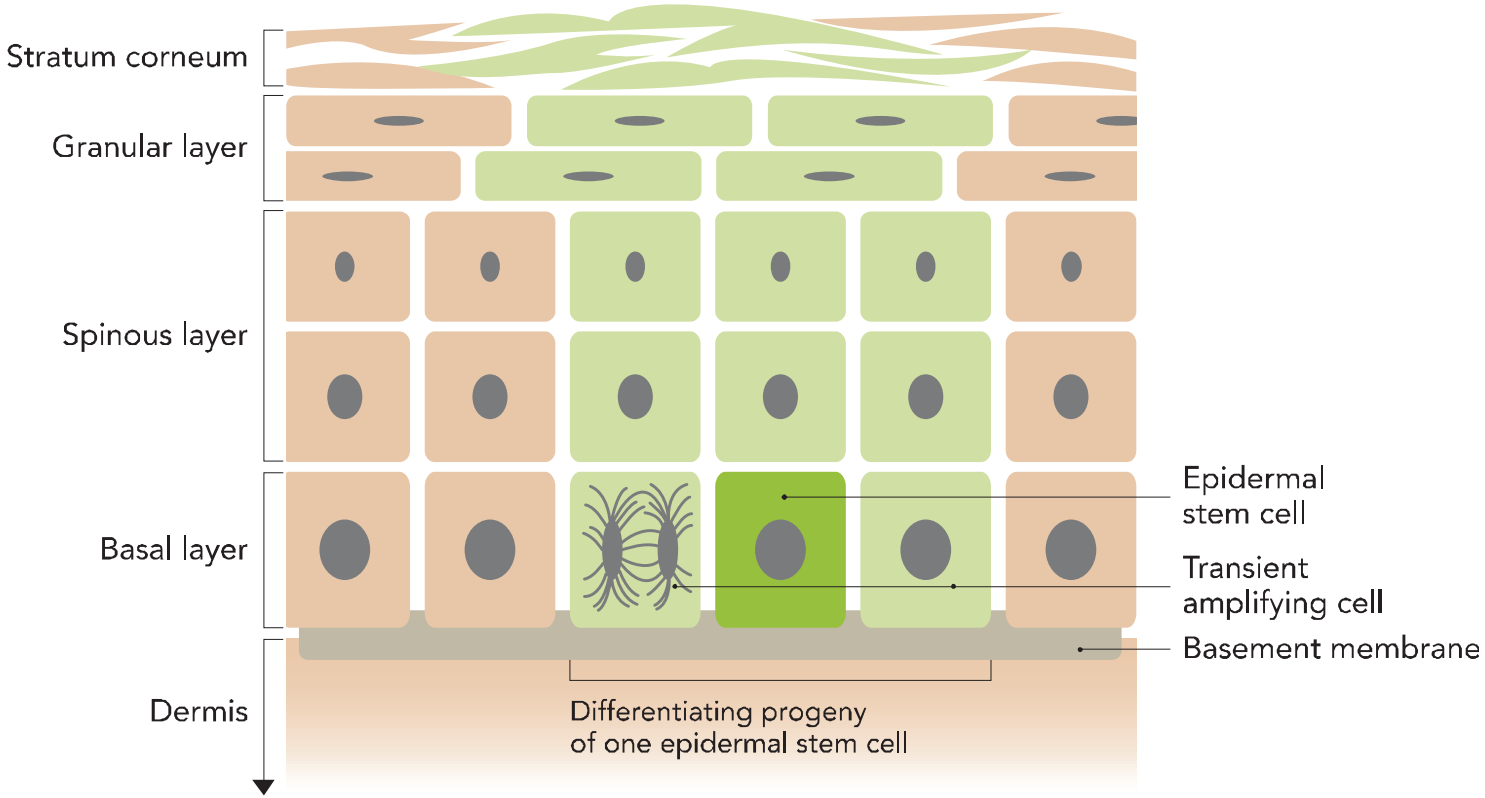
SWISS QUALITY PRODUCT

Stem Cells in Skin

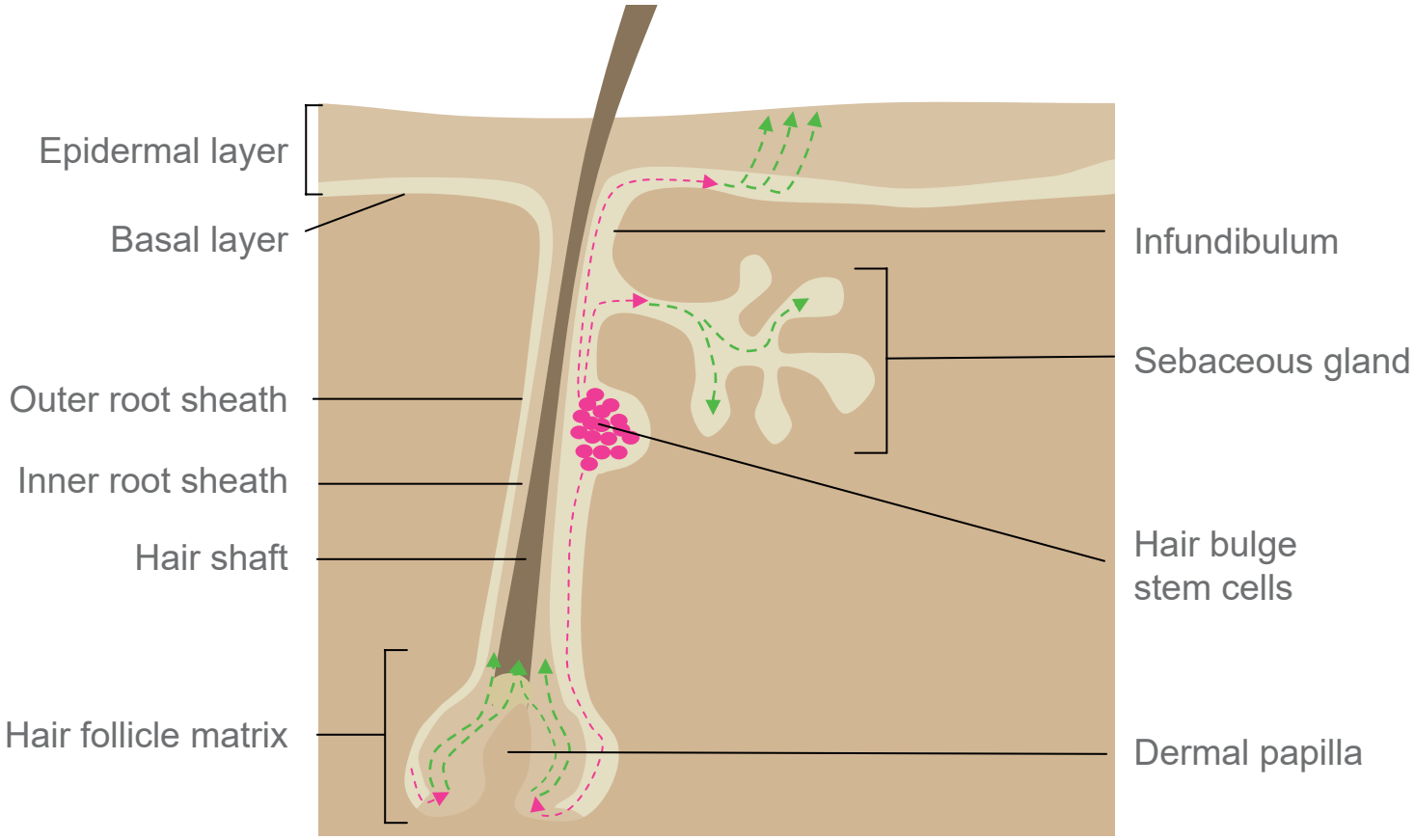
In skin, different types of stem cells have been found:

Type	Place
Epithelial skin stem cells	Basal layer of the epidermis
Hair bulge stem cells	Hair follicle

Epithelial Stem Cells



Hair Bulge Stem Cells



Stem Cells and Aging

- Stem cells are responsible for the rejuvenation of skin
- Stem cells are sensitive to environmental threats
- Chronological aging leads to the depletion of the number and activity of skin stem cells

→ Protection of stem cells and activation of stem cell function is of great importance in cosmetics

Epigenetic Factors in Stem Cells

- Normal differentiated cells express only a limited amount of genes (fibroblast: collagen; blood cells: hemoglobin)
- Stem cells have the possibility to express the whole DNA
- The epigenetic profile regulates the gene expression in stem cells

Activity of PCT Malus Domestica

1. Use of plant stem cells to protect skin stem cells

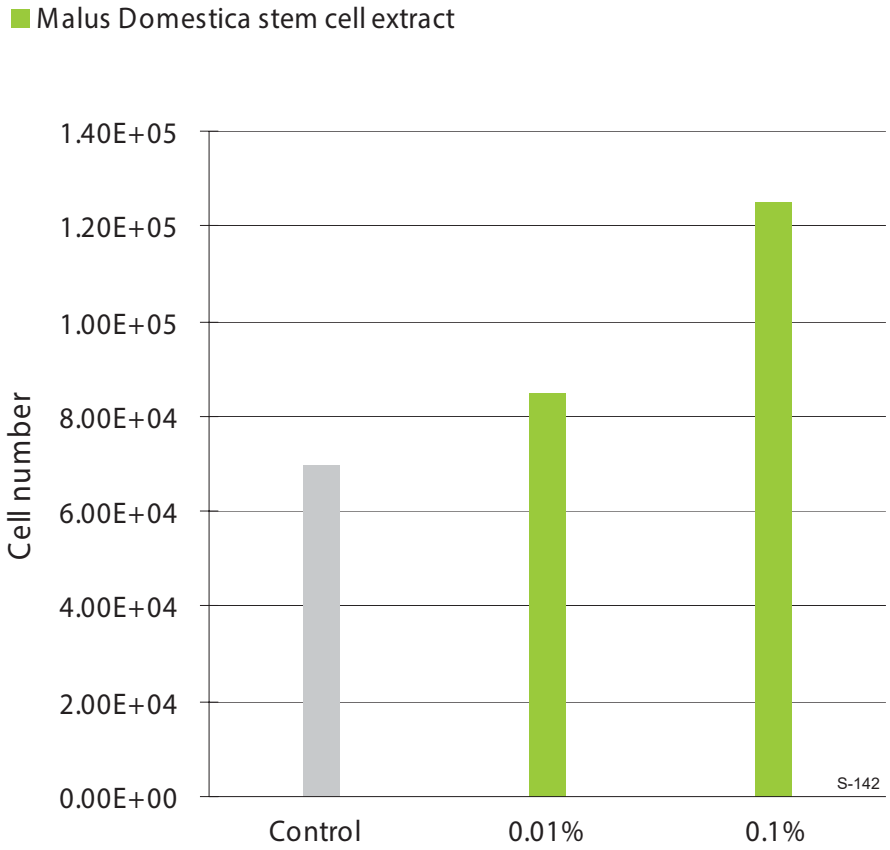
The components of *Malus domestica* stem cells are rich in epigenetic factors relevant to protect and maintain the function of skin stem cells.

2. Use of plant metabolites to delay senescence

The active ingredients of *Malus domestica* var. Uttwiler Spätlauber cells contain metabolites which insure longevity of skin cells.

Study Results

Increase in Cell Number (Human Umbilical Cord Stem Cells)



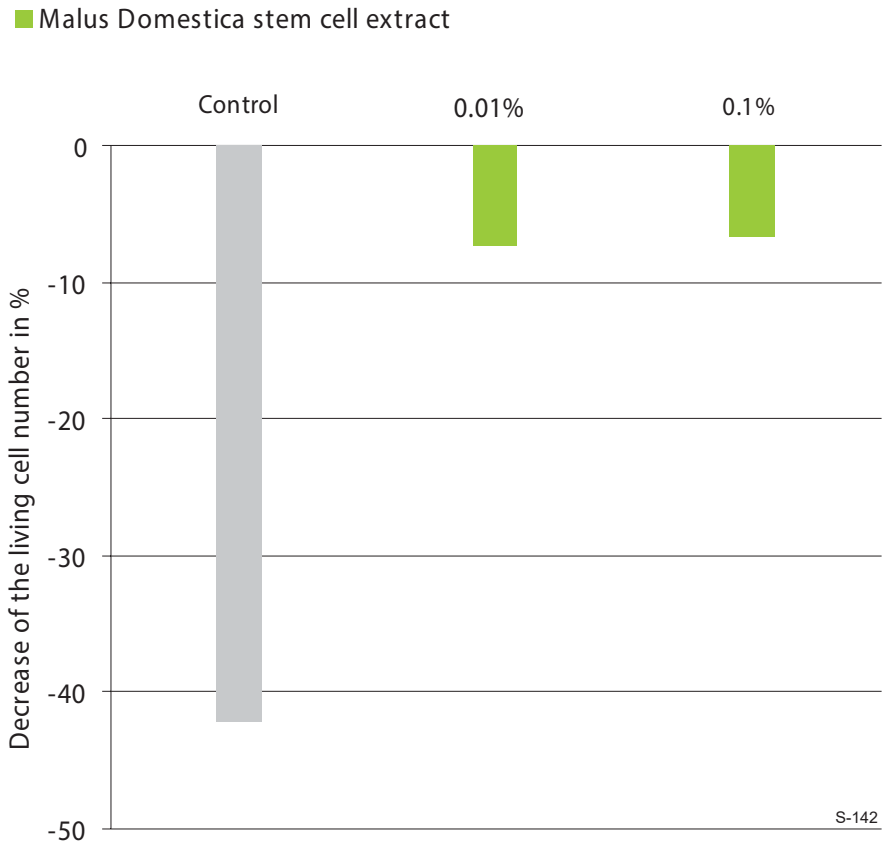
Umbilical Cord Derived MSC

10% FBS

10% FBS

+ 0.1% PhytoCellTec™ Malus Domestica

Protection Against UV (Umbilical Cord Stem Cells)



Growth of Hair Follicle

Hair follicles	From human skin fragments (from micro dissection)
Test substance	PhytoCellTec™ Malus Domestica 0.2%
Design	Length of hair follicle increases until day 14 → after day 14 induction of senescence → length of hair follicle decreases

Day 0

Day 4

Day 7

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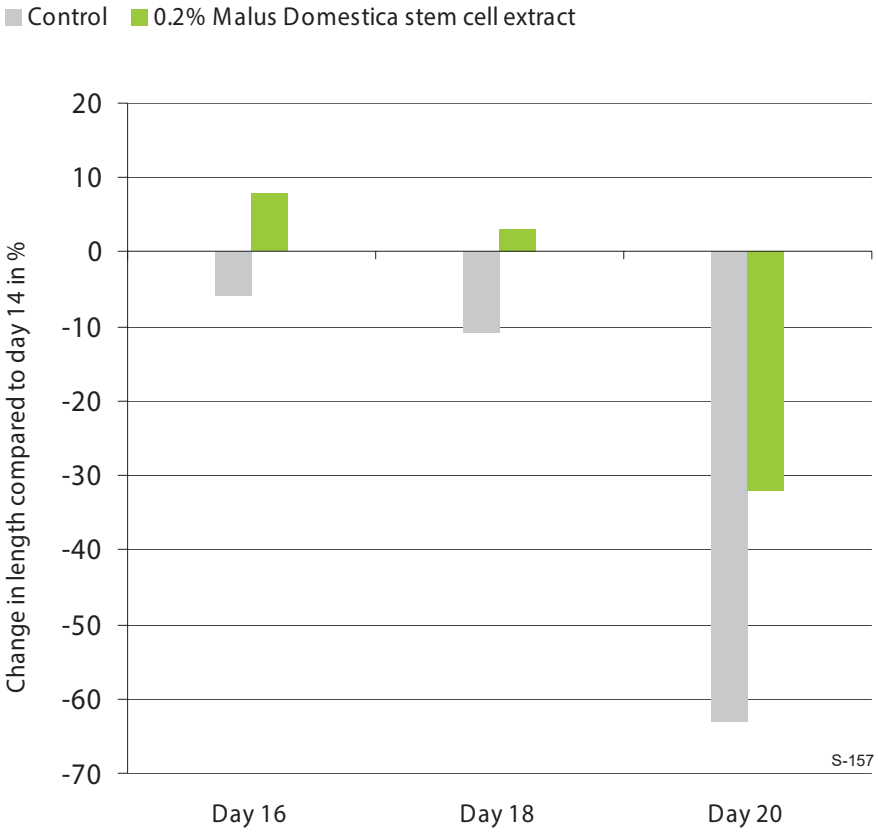
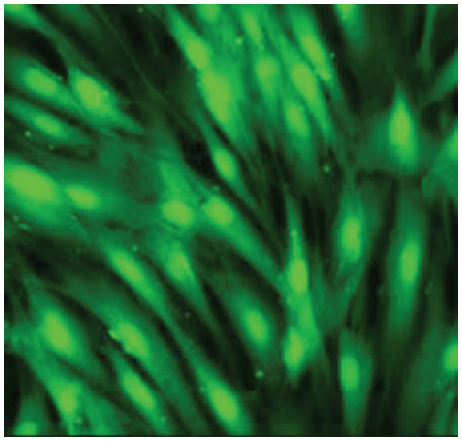
Length of Hair Follicle



Senescence of hair
(stem cells) can be delayed

PhytoCellTec™ Malus Domestica Effect on Gene Expression in Senescent Dermal Fibroblasts

Cell line	Normal Human Dermal Fibroblasts
Design	• H_2O_2 induced senescence (2h) followed by a treatment with 2% PhytoCellTec™ Malus Domestica or not (144 h)
Parameters	• Gene profile (microarray)



PhytoCellTec™ Malus Domestica Gene Profile (Microarray)

↓ Gen, that is down-regulated by senescence induction (see control)

↑ Gen, that is up-regulated or normalized in senescent cells by PhytoCellTec Malus Domestica

Gen	After H_2O_2 Control	After H_2O_2 + 2% Malus Domestica stem cell extract
cyclin B1: induces proliferation	73 ↓	130 ↑
cyclin E1: tumor inducer	78 ↓	135 ↑
p53: tumor suppressor gene	63 ↓	137 ↑
insulin-like growth factor II: cell proliferation enhancer	71 ↓	117 ↑
heme oxigenase 1: antioxidans enzyme	89 ↓	211 ↑

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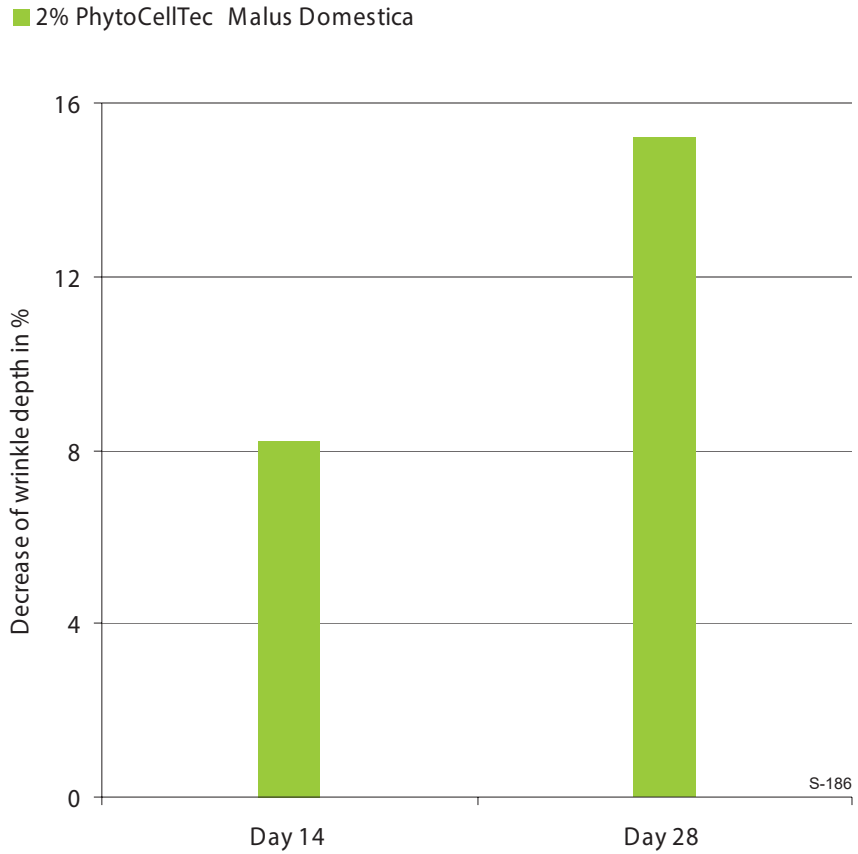
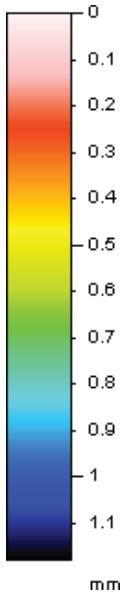
In-Vivo Study: Anti-Wrinkle Effect of PhytoCellTec™ Malus Domestica

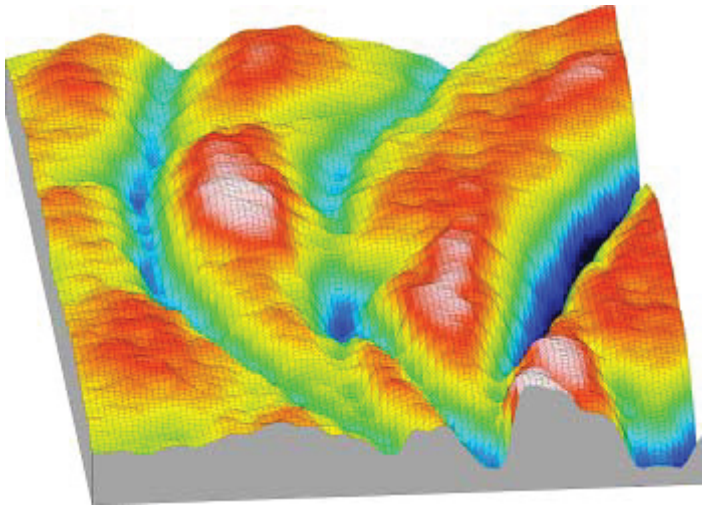
- Analysis of wrinkle depth in the crow‘s feet area
- 20 volunteers
- 28 days
- Application twice daily
- Against initial conditions

In-Vivo Study: Anti-Wrinkle Effect of PhytoCellTec™ Malus Domestica

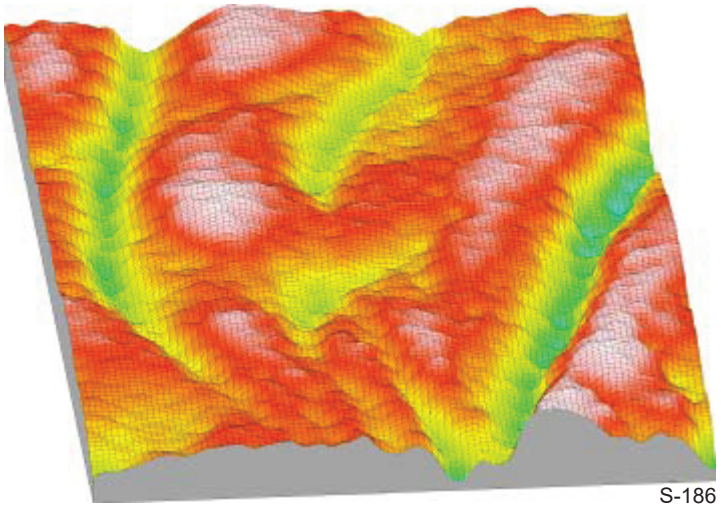


In-Vivo Study: Anti-Wrinkle Effect of PhytoCellTec™ Malus Domestica





Day 0



Day 28
2% PCT Malus D

Summary

Product from rare Swiss *Malus domestica* var. Uttwiler Spätlauber PhytoCellTec™ to produce stem cells from this apple

Product activity

1. Activation and protection of skin stem cells
2. Delay of senescence

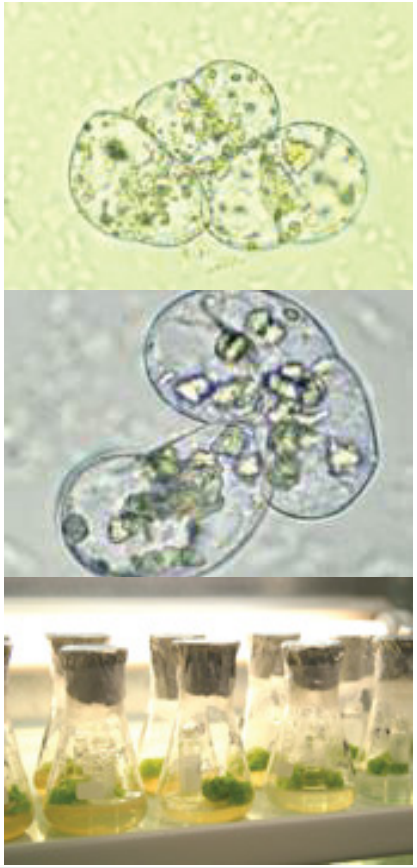
Results show

- Protection of stem cells against UV-radiation
- Increase of activity of stem cells
- Decrease of senescence markers in fibroblasts
- Increase of life span of hair follicles
- Reduction of skin wrinkles

Claims

- Delays senescence of essential cells
- Protects longevity of skin stem cells
- Protection of skin stem cells
- Rejuvenation of skin
- Maintains the power of regeneration
- Decrease in wrinkles
- Combats chronological aging

Properties of PhytoCellTec™



- PhytoCellTec™ is a preparation of plant stem cells
- PhytoCellTec™ is produced in Switzerland by a novel plant cell culture technology
- PhytoCellTec™ contains plant stem cells to protect and maintain skin stem cells
- The protection of skin stem cells is a breakthrough approach in anti-aging cosmetics
- Studies showed that PhytoCellTec™ protects human stem cells and rejuvenates them
- PhytoCellTec™ delays chronological aging

Application of PhytoCellTec™

- Face care of mature skin to rejuvenate the basal layer and extend the life span of basal keratinocytes
- Face care of all age to protect the most important cells in the skin which are the stem cells
- Eye serum to improve firmness and rejuvenate the extracellular matrix
- Pure PhytoCellTec™ product can be used as a fresh cell extract to rejuvenate the skin extensively

Marketing Story for Consumers

- PhytoCellTec™ plant stem cells are a breakthrough in anti-aging therapy
- Skin stem cell treatment is a revolutionary new approach in cosmetics
- Plant stem cells are extremely efficient but also safe to use
- PhytoCellTec™ is based on real plant stem cells to trigger the rejuvenation of the skin
- PhytoCellTec™ delays the natural aging process by maintaining the activity of skin stem cells

Marketing Story for Consumers

- PhytoCellTec™ triggers the skin's stem cell driven potential for infinite regeneration
- Epigenic factors of PhytoCellTec™ maintain the self renewing capacity of skin stem cells; this is the breakthrough in anti-aging
- PhytoCellTec™ is the most potential skin care to stop chronological aging
- PhytoCellTec™ gives you a promise of "for ever young"
- Stem cell technology is believed to be the "holy grail" of skin care
- Plant stem cells are very active but safe and will therefore write a new history in skin care