Clinical Studies from:

- Eyeless
- Lipotec
- Matrixyl
- PhytoCell
- BV-OSC
- SYN-EYE
Clinical Studies

Eyeless
FIGHT AGAINST BAGS UNDER THE EYES

Function and Characteristics:
EYELISS is a combination of 3 active molecules in solution:
- **Hesperidin methyl chalcone**: decreases the capillary permeability.
- **Dipeptide Valyl-Tryptophane (VW)**: increases lymphatic circulation.
- **Lipopeptide Pal-GQPR**: improves firmness and elasticity, decreases inflammatory phenomena.

Cosmetic interest (properties):
Tiredness, hypertension or the intake of certain medicines, in addition to the natural effects of ageing, contribute to the formation of bags under the eyes. EYELISS helps to prevent and fight bags under eyes as well as smoothing the appearance of fine lines by firming and toning the skin.

Applications:
All products (creams, gels, lotions...) intended for the treatment of puffy eyes.

Recommended use level: 3%

CTFA / INCI name:
Water (Aqua) - Glycerin - Hesperidin Methyl Chalcone - Steareth-20 - Dipeptide-2 - Palmitoyl Tetrapeptide-3

Specifications:
- **Appearance**: clear liquid
- **Colour**: brown
- **Odour**: characteristic
- **pH**: 6.0 – 7.0
- **Density**: 1.050 – 1.070
- **Refractive index (at 25°C)**: 1.360 – 1.380
- **Water content (K. Fischer)**: 72 – 82%
- **Val-Trp-OH content**: 0.08 – 0.12%
- **Hesperidine content**: 4 – 6%
- **Pal-GQPR content**: 0.025 – 0.035%
- **Bacteria**: < 100 germs/g
- **Yeast and moulds**: < 10 germs/g


CLAIM SUBSTANTIATION

**IN VITRO**

Anti-inflammatory effect of lipopeptide Pal-GQPR with 3% EYELISS:
- Regulation of UV induced IL6 level produced by keratinocytes: 33%

Drainage stimulation of dipeptide VW with 1% EYELISS:
- Inhibition of Angiotensin Converting Enzyme (ACE): 85%

**EX VIVO**

Effect of hesperidin methyl chalone (HMC) on capillary permeability:
- Decrease of capillaries' permeability: 25%

Clinical study:
20 female volunteers, aged between 40 and 60 presenting chronics bags under the eyes / Twice daily application for 56 days of a gel containing 3% EYELISS.

Morphometric study:

<table>
<thead>
<tr>
<th>Significant results</th>
<th>T28</th>
<th>T56</th>
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</thead>
<tbody>
<tr>
<td>Mean decrease of bag thickness (in mm)</td>
<td>-0.08</td>
<td>-0.20</td>
</tr>
<tr>
<td>Maximum value (in mm)</td>
<td>-0.40</td>
<td>-0.69</td>
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<tr>
<td>Number of volunteers with a reduction of bag volume</td>
<td>65%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Self-evaluation:
- Eye contour smoothing: 62%
- Reduction of the bags: 52%
- Decongestant effect: 52%

Principle of a 3D morphometric study:

Before: Initial data analysis determines the surface of the bag

After: New data analysis

The software measures the distance between the surface of the bag before treatment and the surface of the bag after treatment

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Clinical Studies

Lipotec
GENERAL DESCRIPTION

As skin loses its elasticity and muscles weaken through age, loose skin can accumulate around the eyes, forming folds in the eyelids. Fat, which cushions the eyes in their sockets moves forward out of the ocular cavities and accumulates in bulging bags around the eyelids.

Baggy eyelids are known medically as dermatochalasis and are commonly improved by performing a blepharoplasty, a surgical procedure which involves an inner or outer incision of the eyelid to extract the fat and excess skin. Blepharoplasty is the most common aesthetic procedure performed by plastic surgeons in America [Castro, E, Foster, JA (1999) Upper lid blepharoplasty. Facial Plast. Surg.15 (3): 173 ].

The other major reason for puffy eyes is water accumulation, known as eyelid oedema. Fluid may build up for several reasons, two of the major reasons being poor lymphatic circulation and increased capillary permeability.

EYESERYL® is a tetrapeptide with anti-oedema properties with a proven efficacy in reducing puffy eyebags.
PROPERTIES AND APPLICATIONS

- The draining effect of EYESERYL® achieves a reduction of puffy eyebags in only 15 days – fast anti-eyebag action

EYESERYL® can be incorporated in cosmetic formulations such as emulsions, gels, sera, etc., where a reduction of puffiness under the eyes is desired.

TECHNICAL INFORMATION

PRODUCT SPECIFICATIONS

<table>
<thead>
<tr>
<th>Code</th>
<th>PD070</th>
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<tbody>
<tr>
<td>Appearance</td>
<td>Transparent Solution</td>
</tr>
<tr>
<td>INCI name</td>
<td>Water, Acetyl Tetrapeptide-5</td>
</tr>
<tr>
<td>Active ingredient content</td>
<td>0.1% Acetyl Tetrapeptide-5</td>
</tr>
<tr>
<td>Solvent</td>
<td>Demineralised water</td>
</tr>
<tr>
<td>Preservative</td>
<td>0.5 % Phenonip</td>
</tr>
</tbody>
</table>

PROCESSING AND DOSAGE

EYESERYL® is presented as EYESERYL® Solution, an aqueous solution containing 1 g/L of the powder. It can be incorporated at the final stage of the manufacturing product, provided the temperature is below 40 °C. Taking into consideration the concentration of peptide in EYESERYL® Solution, it is recommended that 3 to 10% of the solution is present in the final formulation in order to obtain significant eyebag-reducing activity.

STORAGE AND SHELF LIFE

Keep in a clean, cool and dark place. If the product is stored as recommended it will remain stable for at least 12 months.

SAFETY

EYESERYL® has been tested in vitro for ocular irritation and is non irritant at the concentration of use. All the raw materials involved in the formulation are regarded as safe at the concentrations of use.
EFFICACY

IN VITRO

Determination of the ACE inhibitory activity of EYESERYL®

It has been suggested that one of the mechanisms involved in the formation of bags under the eyes is the bad circulation of the blood due to hypertension. For this reason, a valid mechanism for an anti-puffiness compound would be the improvement of blood circulation by an anti-hypertensive effect.

Angiotensin I converting enzyme (ACE) is a dipeptide liberating exopeptidase, which has been classically associated with the rennin-angiotensin system regulating peripheral blood pressure. ACE removes a dipeptide from the C terminus of angiotensin I to form angiotensin II, a very hypertensive compound.

The protease renin cleaves angiotensinogen into the inactive decameric peptide angiotensin-I (Ang-I). Angiotensin-converting enzyme (ACE) then cleaves a C-terminal dipeptide from Ang-I to form an active octamer angiotensin-II (Ang-II), which can contribute to hypertension by promoting vascular smooth muscle vasoconstriction and renal tubule sodium reabsorption. ACE can also cleave many other small peptides including the vasodilating peptide bradykinin into an inactive fragment. It can also cleave the Alzheimer amyloid beta-peptide (Abeta), and retard its aggregation, deposition and fibril formation.

The ACE inhibitory activity was determined according to the method of Wang: [Antioxidative and Angiotensin I-Converting Enzyme Inhibitory Activities of Sufu (Fermented Tofu) Extracts” Wang Lijun, Masayoshi Saito, Eizo Tatsumi and Li Lite, JARQ 37, 129-132 (2003)].

In this test, cleavage by ACE of a substrate yields a fluorescent dipeptide which can be measured and its intensity correlated to ACE activity.

The controls used were a standard solution (where EYESERYL® was replaced by distilled water) and a blank (where ACE was replaced by distilled water). The ACE activity was calculated from the following equation and expressed as ACE%:

\[
ACE\% = \frac{(b - c)}{a} \times 100
\]

where \(a\) is the fluorescence intensity of the standard solution (ACE), \(b\) is the fluorescence intensity of the reaction mixture with EYESERYL®, and \(c\) is the fluorescence intensity of the blank solution (without ACE). The ACE inhibitory activity was calculated based on the following equation:

\[
ACE \text{ inhibitory activity (\%)} = 100 - ACE\%
\]
Results

The ACE inhibitory activity of EYESERYL® is shown in Fig. 1. EYESERYL® inhibited ACE activity at all tested concentrations, and the effects were dose dependent.

![Figure 1. ACE inhibitory activity of EYESERYL®](image-url)
IN VIVO

EYESERYL® has been tested in vivo on a group of 20 female volunteers. A cream containing 0.01% EYESERYL was applied twice a day during 60 days. Pictures were taken at 0, 15, 30, 45 and 60 days.

A dermatologist assigned a score to the decrease in eyebag puffiness:
1 – no reduction,
2 – slight reduction,
3 – fairly good reduction,
4 – good reduction

Volunteer A

Day 0

Day 15

Day 30

Day 45
Voluntaria B

Day 0

Day 15

Day 30

Day 45

Voluntaria C

Day 0

Day 15

Day 30

Day 45
Conclusions

Puffiness under the eyes is greatly reduced, even after only 15 days! - 70% of the volunteers had improved at Day 15.

At the end of the test, 95% of the volunteers had improved:
✓ 30% showed a slight improvement (grade 2)
✓ 30% showed a fairly good improvement (grade 3)
✓ 35% showed a good improvement (grade 4)
### GENERAL PRODUCT INFORMATION

<table>
<thead>
<tr>
<th>Trade name</th>
<th>EYESERYL®</th>
</tr>
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<tbody>
<tr>
<td>Product code</td>
<td>PD070</td>
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### INGREDIENTS

<table>
<thead>
<tr>
<th>INCI name</th>
<th>CAS No</th>
<th>EINECS No</th>
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<td>7732-18-5</td>
<td>231-791-2</td>
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<td>ACETYL TETRAPEPTIDE-5</td>
<td>N.L.</td>
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<tr>
<td>PHENOXYETHANOL</td>
<td>122-99-6</td>
<td>204-589-7</td>
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<tr>
<td>ISOBUTYLPARABEN</td>
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<td>224-208-8</td>
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</table>

* a Not Listed
Clinical Studies

Matrixyl
Function:
Wrinkle filler.

Definition:
A dioxygenated lipopeptide
Palmitoyl-Lysyl-Dioxymethionyl-Lysine.

Properties:
Evens out skin relief and smooths wrinkles from the inside by rebuilding the skin where it is needed, particularly on the forehead and crow’s feet.

Characteristics:
Matrikine-like effect that stimulates the synthesis of 6 major constituents of the skin matrix and dermal-epidermal junction (collagen I, III, IV, fibronectin, hyaluronic acid and laminin 5).

Points of interest:
MATRIXYL® synthe’6™ is derived from the tripeptide KMK naturally found in collagen VI and laminins.

INCI Name:
(Check PCPC on-line dictionary for latest INCI name)
Glycerin – Water (Aqua) – Hydroxypropyl Cyclodextrin – Palmitoyl Tripeptide-38

Applications:
Skincare and make-up designed to fight wrinkles.

Formulation:
Water soluble.
Add to the emulsion between 25°C and 80°C.

Recommended use level:
2%

A powerful peptide that boosts skin-rebuilding essentials

3-Dimensional decrease in wrinkles by 31% up to 100%

www.sederma.fr
E-mail: sederma@sederma.fr
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SYNTHESIS OF 6 MAJOR CONSTITUENTS OF THE SKIN

COLLAGEN I, III, IV & HSP47

- COLLAGEN I

Control

MATRIXYL® synthe’6™

+105%, p<0.01

(Immunolabelling on human fibroblasts)

- COLLAGEN III

Control

MATRIXYL® synthe’6™

+104%, p<0.01

(Immunolabelling on human fibroblasts)

Tests performed with 2% MATRIXYL® synthe’6™.

HYALURONIC ACID

- Hyaluronic acid

+174%, p<0.01

(ELISA method on human keratinocytes)

FIBRONECTIN

- Fibronectin

+59%, p<0.01

(ELISA method on human fibroblasts)

MATRIXYL® synthe’6™ significantly increases the synthesis of 6 major constituents of the matrix and dermal-epidermal junction.

ANTI-WRINKLE EFFICACY

25 female volunteers aged from 42 to 70 years old with forehead lines and crow’s feet. Twice daily application of a cream containing 2% MATRIXYL® synthe’6™ for 2 months vs placebo.

ON THE FOREHEAD - FOITS

- Lifting effect

+28%/T0, p<0.05, up to +77%

- Wrinkle volume

-31%/T0, p=0.055, up to -100%

- Maximum wrinkle depth

-16.3%/T0, p<0.05 up to -62%

On the forehead, the following parameters have been measured:

- Wrinkle volume
- Lifting effect
- Maximum wrinkle depth

Tests performed with 2% MATRIXYL® synthe’6™.

LAMININ-5

- Laminin-5

+15%, p<0.05

(Immunolabelling on human fibroblasts)

ON THE CROW’S FEET

- Lifting effect

+12.6%/placebo, p<0.05

- Wrinkle volume

-21.1%/placebo, p<0.05

- Surface occupied by deep wrinkles (>100 μm)

-28.5%/placebo, p<0.05

- Main wrinkle average depth

-15%/placebo, p<0.05

- Wrinkle spread (angle)

+8%/placebo, p<0.05

Tests performed with 2% MATRIXYL® synthe’6™.

MATRIXYL® synthe’6™ rebuilds the skin network that keeps the skin smooth. After 2 months, the forehead and crow's feet wrinkles are evened out just like a facelift.

Formulation

Wrinkle Smoothing Intervention gel-cream

formula Ref.: SED1003840 C

Part A................................................................................................................... %
- Water deionized qsp 100
- Optasense G83 (Carbomer, Croda) 0.40
- Crillet 1 (glycolate 50, Croda) 1.00
- Crodamol GTCC (Copolyol, Croda) 3.00
- Cromomelt DPG (Dimethyl Ether Adipate, Croda) 0.50
- Butylene Glycol 5.00
- Preservatives

Part B................................................................................................................... %
- Perfume

Part D................................................................................................................... %
- Optasense G82 (Acrylamic Acid / Methacrylate Copolymer) 0.20
- DC 200-5 Cips (Dimethicone, Dow Corning) 3.00
- Potassium Sorbate 0.10
- Sodium Deconized 5.00
- Sodium Chloride 30%
- Parfum

Part G................................................................................................................... %
- SEDERMA (Sederma)
- Perfumella (Azalea, Expressions Parfumées) 0.20

Protocol:


Sederma Inc
E-mail: sederma-usa@croda.com
www.crodausa.com
Tel 732-692-1652
Fax 732-417-0804

Non-warranty: This formulation has been subject to limited stability tests and has been shown to perform well. However formulators adopting this approach should ensure that their own satisfaction long term stability and functionality. It is good practice to conduct safety tests on all final formulations prior to marketing. Formulations should not be taken as an endorsement to claiming any existing patents.

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Prepared by: Clare Phelan
Sedermtech 2010

101102
Clinical Studies

BV-OSC
BV-OSC

**Description**

BV-OSC (Tetrahexyldecyl Ascorbate) is a stable, oil soluble form of Vitamin C.

**Properties**

- BV-OSC at 0.1% reduces melanin synthesis by 80%.
- BV-OSC at 3% in vivo reduces Delta-L value by 15% vs. placebo (22 people), a way to measure whitening effect.
- BV-OSC at 0.1% in vitro increases collagen by 50%.
- BV-OSC at 10% in vivo treats acne with 80% of patients (12 people).
- BV-OSC increases collagen synthesis at least twice as much as ascorbic acid.
- BV-OSC inhibits MMP-2 and MMP-9 over 3 times better than ascorbic acid.
- BV-OSC penetrates the skin 4 times better than Magnesium Ascorbyl Phosphate.
- BV-OSC delivers pure Vitamin C 50 times better than ascorbic acid.
- BV-OSC decreases 8-OHdG induced by UV-A.
- BV-OSC decreases p53 expression induced by UV-B.
- BV-OSC protects the cells against UV-B better than other esters of Vitamin C.

**Formulation**

BV-OSC is a slight to pale yellow liquid with a faint characteristic odor. It is very soluble in ethanol, hydrocarbons, esters and vegetable oils. It is insoluble in glycerin and butylene glycol. BV-OSC should be added into the oil phase at temperatures below 80°C. It can be used in formulas with a pH range of 3 to 6. BV-OSC can also be used at pH 7 in combination with chelating agents or antioxidants (guidelines are offered). Use level is 0.5% - 3%. BV-OSC is approved as a quasi-drug in Korea at 2%, and in Japan at 3%.

**Legislation**

INCI Name: Tetrahexyldecyl Ascorbate  
JSCI: 532297  (Ascorbyl Tetra 2-Hexyldecanoate)  
CAS: 183476-82-6  
EINECS:

KOREA: Approved as a functional ingredient for whitening at 2%.

2007-3
The information contained in this technical bulletin is, to the best of our knowledge, true and accurate. No warranty, expressed or implied is made or intended. The use should be based upon the customer’s own investigations and appraisal. No recommendation should be construed as an inducement to use a material in infringement of patents or applicable government regulations.
The following information should be considered when formulating with BV-OSC.

1. pH must be below 6.0 (for the water phase): Although BV-OSC is oil soluble, its stability can be affected by the pH. The factory suggests that the pH be adjusted to below 6.0 before BV-OSC is added to the formulation. (Adjusting it after it is added may be too late).

2. Use of chelating agents and antioxidants (tocopherol) is suggested.

3. Avoid long exposure of the formulation to strong heat.

4. BV-OSC should not be added alone into water. It should be mixed with at least an equal amount (3.5%) of non-polar oils (squalane or esters).

5. Quasi Drug whitening formulation must contain 3% BV-OSC. The specified range is + 10%, which calculates to 2.7 - 3.3%.

6. BV-OSC in the formulation. The factory therefore suggests that 3.3% BV-OSC should be added to the finished formulation to insure compliance with the required range of 2.7 - 3.3%.

7. The use of Hectorites with BV-OSC may cause instability. Customer should evaluate the stability upon the use of BV-OSC and Hectorites.
Clinical Studies

PhytoCell
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.

Claims with 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
• Dermatological tolerance: the dermatological tolerance of PhytoCellTec™ Malus Domestica has been carefully proven in healthy volunteers with an occlusive photo-patch test.
• Recommended concentrations: 2 – 5 %
• Manufacturing of products: PhytoCellTec™ Malus Domestica can be formulated in emulsions (O/W, W/O) and gels. For cold processes, mix PhytoCellTec™ Malus Domestica with the aqueous phase. In cold/hot processes, add during the cooling phase. Homogenization and temperatures of up to 60°C over a short time do not affect the stability of PhytoCellTec™ Malus Domestica.

INCI/CTFA-Declaration
Malus Domestica Fruit Cell Culture (and) Xanthan Gum (and) Glycerin (and) Lecithin (and) Phenoxyethanol (and) Aqua / Water
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.
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

Selecting/taking away a small piece of the plant
↓
Wounding of plant material to induce callus formation
↓
Incubation on agar plates
↓
Harvesting of developed callus
↓
Cultivation until complete dedifferentiation to obtain stem cells
↓
Transfer of the stem cells into a suspension (liquid media)
↓
Disruption of the stem cells wall
↓
Encapsulation of the stem cell content into liposomes
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.
PhytoCellTec™ Malus Domestica
Study results

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.
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

<table>
<thead>
<tr>
<th>Genes</th>
<th>after H₂O₂ Control</th>
<th>after H₂O₂ + 2 % Malus Domestica stem cell extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>cyclin B1: induces proliferation</td>
<td>73 ↓</td>
<td>130 ↑</td>
</tr>
<tr>
<td>cyclin E1: cell cycle regulator</td>
<td>78 ↓</td>
<td>135 ↑</td>
</tr>
<tr>
<td>p53: tumor suppressor gene</td>
<td>63 ↓</td>
<td>137 ↑</td>
</tr>
<tr>
<td>insulin-like growth factor II: cell proliferation enhancer</td>
<td>71 ↓</td>
<td>117 ↑</td>
</tr>
<tr>
<td>heme oxigenase 1: antioxidant enzyme</td>
<td>89 ↓</td>
<td>211 ↑</td>
</tr>
</tbody>
</table>

Gene that is down regulated by senescence induction (Control)
Gene that is up regulated or normalized in senescent cells by PhytoCellTec™ Malus Domestica
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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.2% Malus Domestica stem cell extract</th>
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<tbody>
<tr>
<td>Day 16</td>
<td>-70</td>
<td>-10</td>
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<tr>
<td>Day 18</td>
<td>-60</td>
<td>-20</td>
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<tr>
<td>Day 20</td>
<td>-60</td>
<td>-40</td>
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Change in length compared to day 14 in %
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.
PhytoCellTec™ Malus Domestica
Plant stem cells for skin stem cell protection

Claims with 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 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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Clinical Studies

SYN-EYE
SYN®-EYE
Lineless looking eyes within days
The windows to the world

It's been said the eye is the window to the outside world. This is why it attracts a lot of attention and it requires a lot of care. With long-standing experience in skin care, DSM has created a multi-functional, patented ingredient complex that fulfills consumers' expectations for high- and fast-performing eye care products.

Key facts

**Unique product features:**
- SYN®-EYE combines SYN®-Peptide know-how with superior ingredients to create a multi-functional eye care formula
- It offers multi-target science-based approach to fight against the most common concerns in eye care — the appearance of lines & wrinkles and dark circles
- It shows significant effects in only 7 days
- SYN®-EYE is a patented complex and fully China compliant

**Benefits:**
- Reduces visibly the appearance of lines and wrinkles around the eye within 7 days
- Reduces the appearance of dark circles
- Improves the skin's texture and smooths the delicate skin around the eyes
- Moisturizes, protects, and energizes the skin around the eyes

**Cosmetic applications:**
- Fast-acting and multi-functional eye care formulas
- Anti-aging eye care products to minimize the visual aspect of lines and wrinkles
- Product that focuses on youthful, fresh, and smooth facial skin
- Product that reduces the appearance of dark circles

**Suggested concentration:**
1% SYN®-EYE

**INCI name (active):**
Palmityl Tripeptide-5, Panthenol, Sodium Hyaluronate, Algae (Dunaliella Salina) extract

**Need for multi-functional products**

The eye area ages faster as it is the thinnest and most fragile area of skin. Over the years, the production of collagen slows down and an extensive muscle contraction contributes to the formation of lines and wrinkles.

The delicate area around the eyes needs special care and focus on different parameters, such as collagen reduction, microcirculation, and skin thinning, all of which needs to be taken care of.

Most of the products in the market only treat one aspect and disregard others. However, the multi-functionality of SYN®-EYE offers more opportunity to maintain this sensitive area in an effective and gentle way. This multi-functionality is highly accredited in consumer feedback.

48% of global skin care shoppers found it important or very important to minimize the number of products used in their health and beauty regime.

11% said multi-functional products are convenient and time-saving.
68% said multi-functional products save me money instead of buying multiple products.
60% said multi-functional products allow me to reduce or replace the number of skincare products I use.
SYN®-EYE efficacy was evaluated in a 4-week, split-face study amongst European female volunteers in the age range of 35-55. It compared 2 samples (SYN®-EYE and a competitive benchmark) against a placebo in an eye care cream. Measurement of different parameters on wrinkle and dark circles has been conducted by Primos or DermaTop; image analysis has been performed by Northwest.

Visible difference in skin color in the dark circle area

**Result:** SYN®-EYE shows a visibly brighter skin color in dark circle area after only 14 days.
Continuous visible improvement on fine lines and wrinkles

Significant reduction in the appearance of wrinkles compared to benchmark and placebo

Smoothing – noticeable reduction in roughness compared to day 0

Result: SYNP-EYE shows a significant anti-wrinkle appearance in 7 days.

Result: SYNP-EYE shows a significant effect on the appearance of smoother skin in only 7 days.

Let's ask the consumer!
Consumer questionnaire: products efficacy on wrinkles

Consumers confirmed outstanding activity of SYNP-EYE

Result: Higher consumer acceptance in all key aspects compared to benchmark.
**SYN®-EYE: 4-in-1 blend composition**

**Palm-Lys-Val-Lys-OH**

Palm-Lys-Val-Lys-OH is a synthetic tripeptide with a unique sequence that mimics the skin's natural OHX mechanism to reduce the appearance of fine lines and wrinkles.

**Key benefits:**
- Protects from collagen network from the appearance of degradation
- Serves as an anti-wrinkle and anti-aging ingredient

**Dunaliella salina extract**

Dunaliella salina is a very small, unicellular microalgae discovered in 1939 by Dunaliella species. It grows in salt water with up to 10% salinity which is more than three times saltier than sea water. Living in such harsh conditions, D. salina has developed an important source of nutrients, including fatty acids, vitamin D, astaxanthin, carotenoids, and antioxidants. It is known to increase cell metabolism.

**Key benefits:**
- Helps to regenerate and protect the skin
- Stimulates in vivo antioxidant systems

**Hyaluronic acid**

Hyaluronic acid (HA) is a naturally occurring polysaccharide in all human tissue. It is mainly found in the dermal layer of skin, HaA helps to retain moisture produced by degeneration followed by an extended duration. During the hyaluronic acid injection, it is an active ingredient that improves the domain properties and still radiating benefits.

**Key benefits:**
- Excellent moisturizing benefit
- Anti-bacterial and soothing effect

**Panthenol**

Panthenol is a derivative of vitamin B5 or D. Panthenol has been used for years in various cosmetic products. It functions as a humectant, increasing the water content in hair and improves elasticity. When applied to the skin, Panthenol readily converts to pantetheine and pantothenic acid, also known as vitamin B5. It is a part of the B group vitamins and is classified as a water-soluble vitamin. Panthenol is also about twice as thio-panthenol or phosphatidylcholine and can enhance the antioxidative appearance of skin.

**Key benefits:**
- Helps to keep the skin moist and supple
- Minimize the appearance of irritation and redness