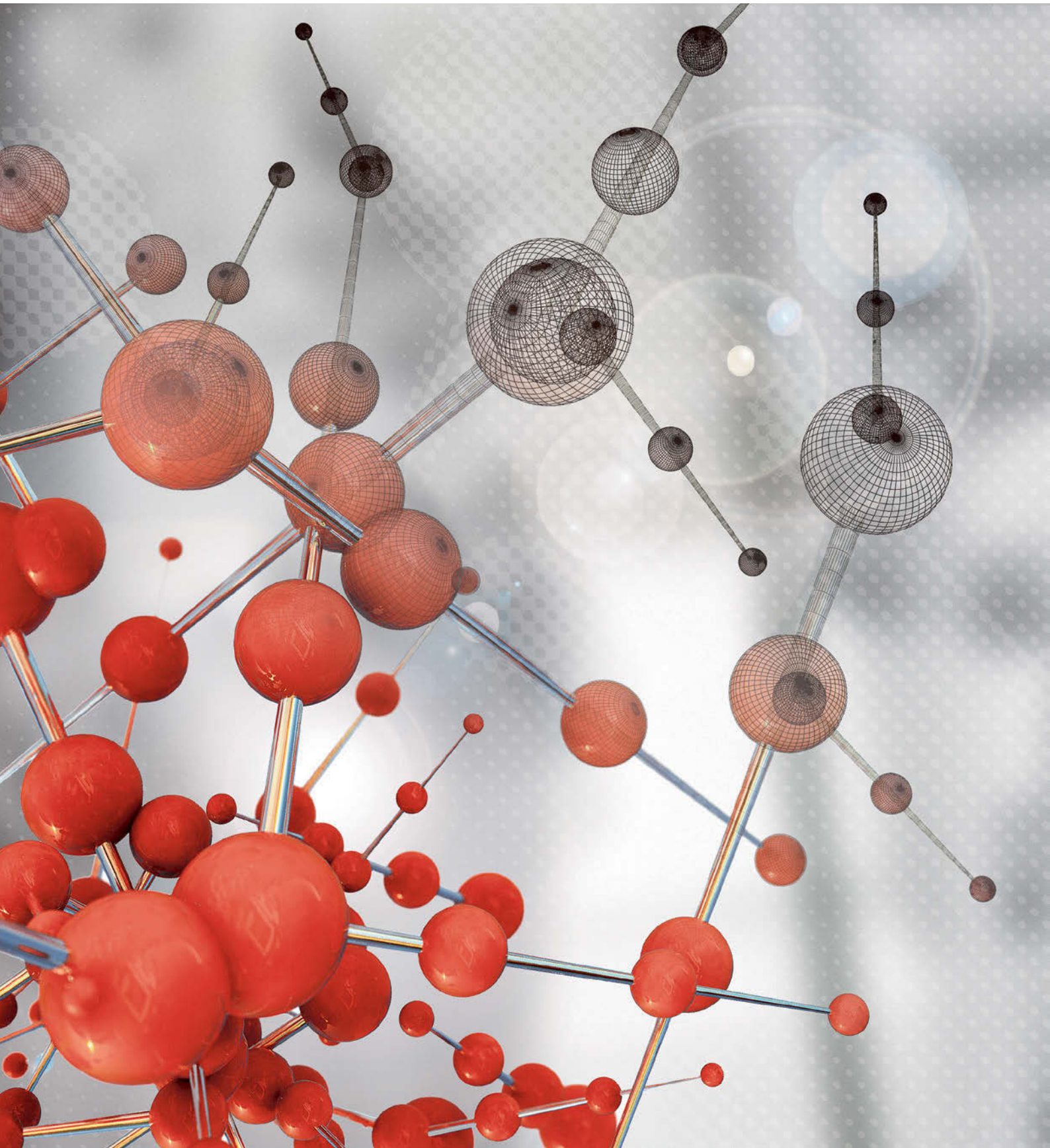
mibelle GROUP biochemistry

PerfectionPeptide P7
Cellular defense regenerator

SWISS QUALITY PRODUCT



PerfectionPeptide P7
Cellular defense regenerator

Introducing a New Anti-Aging Peptide Designed to Actively Protect the Skin from the Inside
PerfectionPeptide P7 is a new biologically active heptapeptide that stimulates the skin's own self-defense mechanisms against oxidative stress.

Our cells respond to oxidative stress generated either internally or externally by activating the transcription factor Nrf2, which is a master switch in the cellular self-protection system. However, aging down regulates this natural process.

In unstressed cells, Nrf2 activity is repressed by the Keap1 protein via a specific binding sequence. However, Mibelle Biochemistry has designed a heptapeptide that mimics this binding sequence and in doing so stimulates Nrf2 activity to enhance protection against reactive chemical species. For improved skin uptake, the peptide was incorporated into a soft sphere carrier system based on shea butter.

Cell culture assays and clinical studies have confirmed the capacity of PerfectionPeptide P7 to both boost Nrf2 activity and protect the skin from oxidative stress at the cellular level:
- DNA damage was significantly reduced.
- UV-induced formation of sunburn cells and the depletion of Langerhans cells were also significantly and greatly reduced.

Therefore, PerfectionPeptide P7 offers a unique strategy of ensuring protection against oxidative stress by activating Nrf2. In this way, the new anti-aging peptide actively prevents both extrinsic and chronological aging.

Claim Ideas for PerfectionPeptide P7
- Activates the skin's self-protection capacity
- Fights both environmental and chronological aging
- Protects DNA against oxidation
- Neutralizes free radicals and empowers the skin's own antioxidant capacity

Applications
- DD creams (daily defense)
- Protecting and regenerating skin care
- Age prevention products
- Sun protection

Formulating with PerfectionPeptide P7
- Recommended use level: 2 %
- Incorporation: For cold processes, dissolve PerfectionPeptide P7 into the aqueous phase. In hot/cold processes, add during the cooling phase below 45 °C.
- Thermostability: Temperatures of up to 45 °C for a short time will not affect the stability of PerfectionPeptide P7.

INCI/CTFA-Declaration
Acetyl sh-Heptapeptide-1 (and) Hydrogenated Lecithin (and) Glycerin (and) Butyrospermum Parkii (Shea) Butter (and) Phenethyl Alcohol (and) Ethylhexylglycerin (and) Aqua/Water

Additional Information
Preservative-free

Nrf2

The antioxidant master switch in extrinsic and chronological aging

Nrf2 Manages the Cellular Self-Protection System

Free radicals generated by oxidative stress and electrophilic toxic compounds represent the main threat at the cellular level:

- they either directly or indirectly damage DNA, lipids and proteins.
- these stressors are produced by environmental factors such as UV radiation. However, they are also endogenously produced as byproducts of the energy metabolism in mitochondria. Therefore, they are linked to both extrinsic and chronological aging.

Our cells respond to these toxic chemicals by activating the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2). This is a master switch in the cellular self-protection system that leads to:

- an increased synthesis of cell protecting enzymes that fight oxidants, free radicals and toxins.
- the replenishment of used cellular antioxidants such as glutathione.

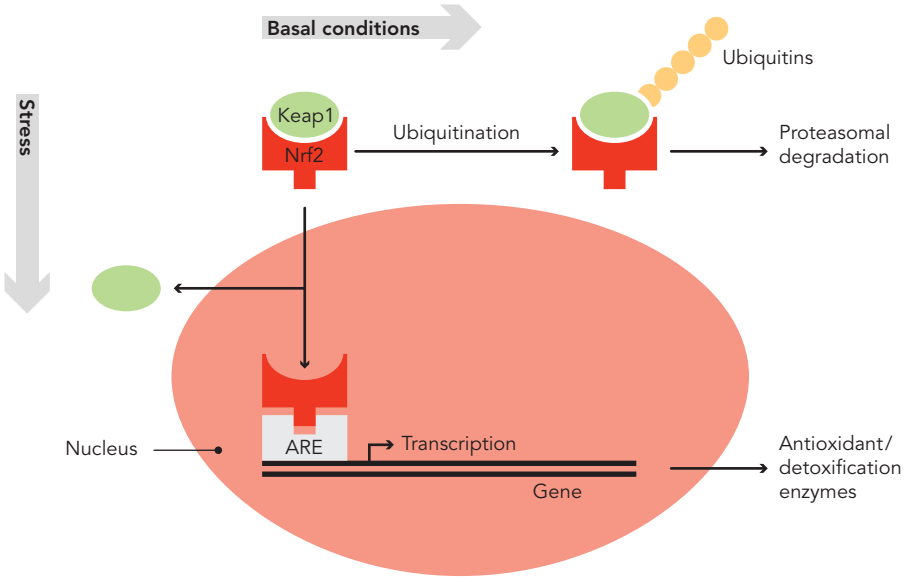
However, this cellular self-protection system cannot cope with an excess of detrimental chemical species. Furthermore, its activity declines with age.

Nrf2 Activates Cytoprotective Enzymes

In unstressed cells, Nrf2 activity is repressed by the Keap1 protein (Kelch-like ECH-associated protein 1). This sees Keap1 binding to Nrf2 in the cytoplasm and initiating its degradation through ubiquitination followed by proteasomal degradation. Nrf2/Keap1 interaction takes place via the ETGE amino-acid motif that is located in the Neh2 domain of Nrf2 and the Kelch domain in Keap1.

In cases of stress, the Nrf2/Keap1 complex dissociates and Nrf2 translocates to the nucleus. Nrf2 induces the transcription of several genes coding for cell protecting enzymes that are characterized by a specific gene sequence that is called the antioxidant response element (ARE). By binding to these ARE sequences, Nrf2 increases the expression of these cell protecting enzymes that include phase II and antioxidant defense enzymes, such as NAD(P)H:quinone oxidoreductase 1 (NQO1), heme oxygenase-1 (HMOX1) and peroxiredoxin1 (PRDX1).

Activation of Nrf2



PerfectionPeptide P7
A new peptide designed to increase Nrf2 activity

An Alternative Strategy to Activate the Cellular Self-Protection System

Topically applied antioxidants such as vitamins C and E can neutralize reactive chemical species, although the vitamins are consumed only after one reaction.
In addition, they are not stable in cosmetic formulations. Consequently, their antioxidant power is limited, which means that the stimulation of the skin's own cell protecting enzymes would appear to be a better strategy.

Natural Nrf2 activators such as sulforaphane are shown to modify Keap1 conformation and result in the interruption of ubiquitination and therefore the prevention of Nrf2 from degradation.

PerfectionPeptide P7 offers an alternative strategy to activate Nrf2; it disrupts the Nrf2/Keap1 interaction by competing with Nrf2.

Design of a Biologically-Active Peptide

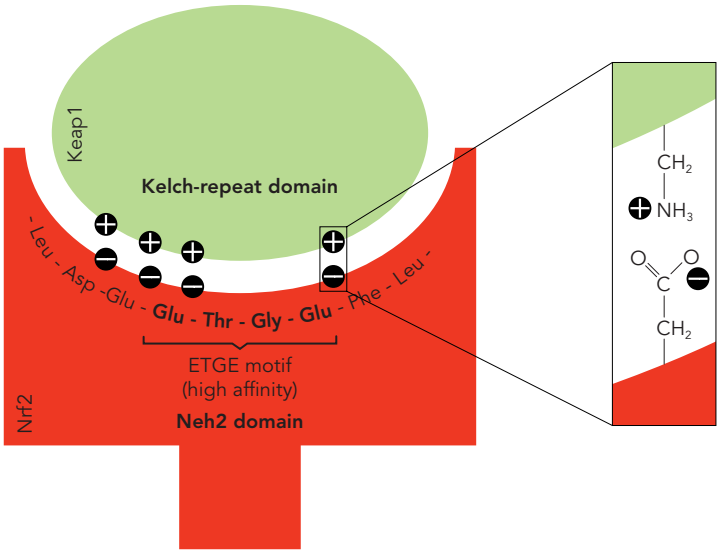
Under normal circumstances, Nrf2 is blocked as a Nrf2/Keap1 dimer via the ETGE amino acid sequence of Nrf2. In order to specifically compete with this interaction, Mibelle Biochemistry has designed a heptapeptide that contains the ETGE sequence of Nrf2.

However, the stratum corneum is poorly permeable to hydrophilic and high molecular weight compounds such as peptides. Consequently, the heptapeptide (acetyl-DE**ETGE**F) was incorporated into a soft sphere carrier system based on shea butter in order to:

- increase its penetration into the skin
- enhance its uptake by keratinocytes
- enable a controlled-release delivery
- protect the peptide molecules in the formulation against degradation.

Clinical studies (results not shown in this brochure) have confirmed the efficiency of this carrier system.

Nrf2/Keap1 Interaction



PerfectionPeptide P7
Mechanism and advantages

Mechanism of PerfectionPeptide P7
PerfectionPeptide P7 boosts the cellular self-protection system of the skin and preserves it from an excess of oxidative stress, as well as from age-related decline, by enhancing Nrf2 activity.

Indeed, PerfectionPeptide P7 mimics the binding sequence of Nrf2 to Keap1 and in doing so effectively acts as a decoy. In this way, PerfectionPeptide P7 releases Nrf2 from the Keap1 complex.

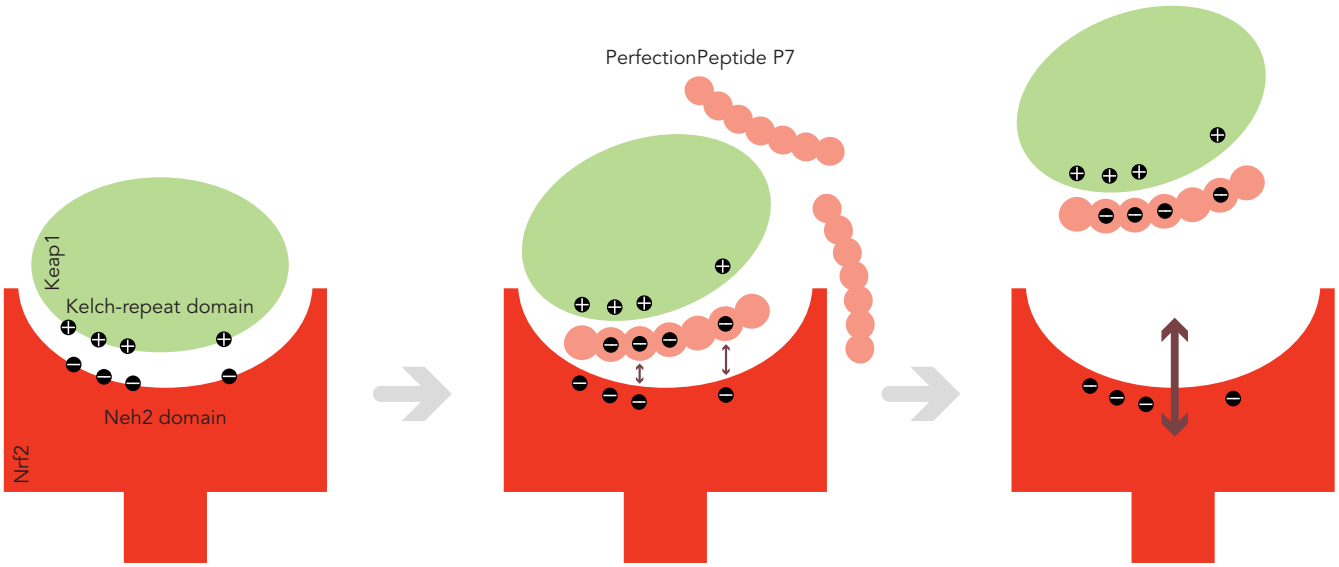
In cell culture assays, PerfectionPeptide P7 was found to stimulate Nrf2-regulated cell protecting enzymes.

Ex vivo and clinical studies have confirmed the capacity of PerfectionPeptide P7 to protect the skin against oxidative stress at the cellular level:

- DNA damage, which is known to be a consequence of the UVA-induced formation of reactive oxygen species, was significantly reduced.
- UV-induced formation of sunburn cells (apoptotic keratinocytes, markers of excess UV radiation) and the depletion of Langerhans cells (involved in the skin immune system) were also significantly and greatly reduced.

Advantages of PerfectionPeptide P7
- New strategy to activate Nrf2
- Biomimetic process
- Targeted and continuous delivery
- Low concentration but high efficacy and tolerability.

Competition of PerfectionPeptide P7 and Nrf2 for Binding Keap1



PerfectionPeptide P7
Study results

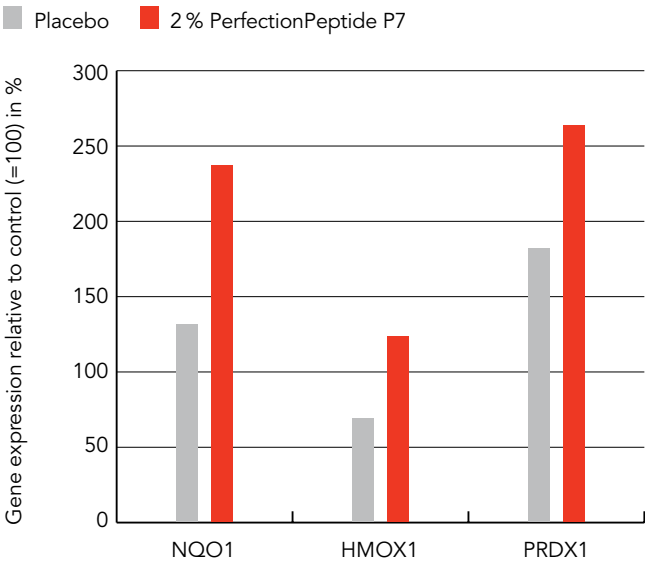
Stimulation of Cell Protecting Enzymes
The effect of PerfectionPeptide P7 on Nrf2-dependent antioxidant enzymes was evaluated on skin explants from abdominal plastic surgery by quantitative PCR.

Skin explants were either treated or not treated with the test products (2 % PerfectionPeptide P7 in o/w cream and the corresponding placebo) for 6 hours.

The expression of markers was analyzed by using quantitative PCR on mRNA extracted from the skin samples.

Results showed a strong stimulation of the genes coding for the phase II and antioxidant defense enzymes:
- NAD(P)H:quinone oxidoreductase (NQO1) which reduces quinones, in so doing diminishing their toxicity.
- Heme oxygenase-1 (HMOX1) involved in heme catabolism.
- Peroxiredoxin-1 (PRDX1) which reduces peroxides.

Stimulation of Nrf2-dependent Antioxidant Enzymes



PerfectionPeptide P7
Study results

Analysis of a DNA Damage Marker in Suction Blister Biopsies

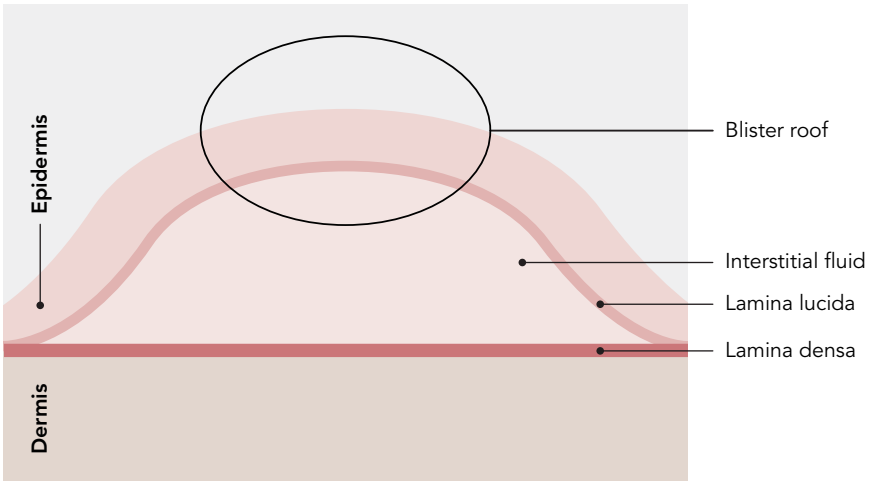
The protective effect of PerfectionPeptide P7 against oxidative damages was evaluated on human skin by using the suction blister technique. This is an in vivo method that gives access to the epidermis.

The degree of oxidative stress was determined by measuring the concentration of the DNA damage marker 8-hydroxy-2'-deoxyguanosine (8-OHdG) in the epidermis after UVA irradiation.

The test products (a cream with 2% PerfectionPeptide P7 and a corresponding placebo) were applied twice daily for 14 days to the inner side of the forearm of 10 volunteers aged from 42 years to 64 years (mean age = 51.9 years).

Two hours after the final application, the skin was irradiated with 20 J/cm^2 UVA. Subsequently, suction blisters were induced artificially within a period of approximately 2 hours by using a suction chamber and a small vacuum pump. During this step, the epidermis was slowly detached from the underlying dermis. From there, following the removal of the chamber, the blister roof (epidermis) was removed using sterile instruments.

Suction Blister Technique



Suction blister technique (non-invasive in vivo treatment): A plexiglass chamber with round openings is attached to the forearm of a volunteer and connected to a vacuum pump. After approximately 2 hours, blisters are formed. The lamina lucida is cleaved from the underlaying lamina densa, separating the complete, viable epidermis from the dermis. The induced blisters fill with fluids from the surrounding tissue (interstitial fluid).

The blister roofs were collected, sliced into thin layers and immunohistologically treated for the detection of the 8-OHdG DNA damage marker.

The slices were microscopically analyzed and the number and intensity of stained 8-OHdG positive cells were counted and scored.

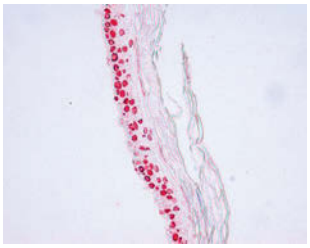
The concentration of 8-OHdG in the blister roofs was then calculated based on the stained 8-OHdG positive cells.

The results showed that:

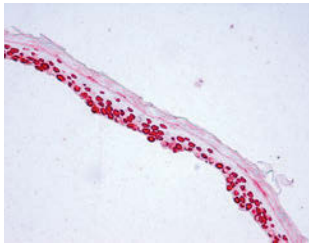
- irradiation with UVA light led to a statistically significant increase of 8-OHdG concentration in the human epidermis.
- no difference was detected between the irradiated control (untreated) and the irradiated placebo.
- however, skin treated with PerfectionPeptide P7 showed a statistically significant 20% decrease in DNA damage as compared to the placebo.

Therefore, PerfectionPeptide P7 efficiently protects the epidermis against DNA damage induced by oxidative stress.

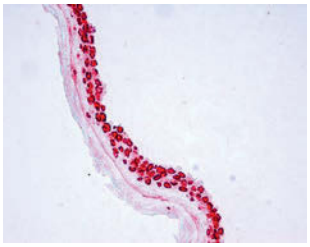
8-OHdG Positive Cells in Suction Blister Biopsies



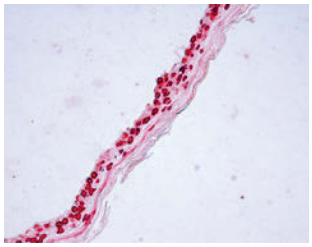
Untreated, unirradiated



Placebo, irradiated

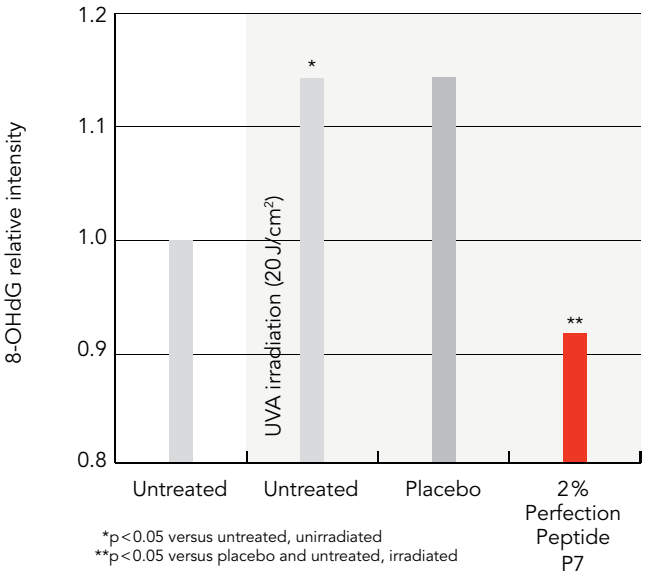


Untreated, irradiated



2% PerfectionPeptide P7, irradiated

8-OHdG Concentration in the Epidermis



*p<0.05 versus untreated, unirradiated
**p<0.05 versus placebo and untreated, irradiated

PerfectionPeptide P7
Study results

Multi-Cellular Protection against UV-Induced Stress

The capacity of PerfectionPeptide P7 to protect the skin against UV-induced stress was also assessed in skin explants by evaluating:

- the formation of sun burn cells (SBC). These apoptotic keratinocytes form in the epidermis as a result of excess UV radiation. Their presence indicates severe UV-induced cell damage.
- the number and morphology of Langerhans cells (LC). These antigen-presenting cells are involved in the skin's immune system. Following contact sensitization, these dendritic cells migrate from the epidermis to the dermis in order to activate helper T lymphocytes. LC are very sensitive to UV irradiation, which decreases their number in the epidermis.

Skin explants were either treated or not treated (control) for 24 hours with the test products (PerfectionPeptide P7 and SPF 30 sun cream [positive reference]).
Following this pre-treatment, the skin explants were irradiated with UV (UVB 1.25 J/cm^2 + UVA 18.7 J/cm^2) and then treated again with the test products for 24 hours.
At the end of the treatment, skin explants were sectioned transversally and stained for the detection of SBC and LC.

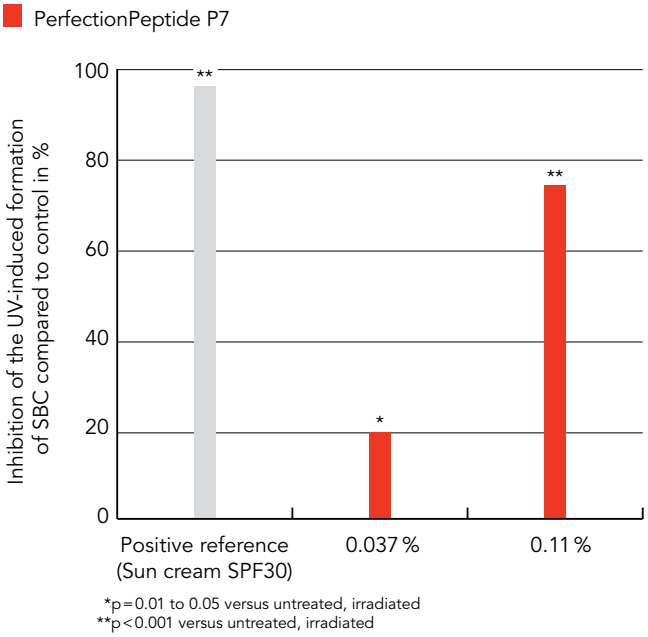
Results showed that UV-irradiation of the skin explants induced the appearance of SBC and led to a reduction of the number of LC. As expected, the application of the SPF 30 sun cream significantly protected the skin explants against UV-induced SBC formation and LC depletion.

The application of PerfectionPeptide P7 on the skin explants led to a significant and dose-dependent protection against UV irradiation:

- 0.037 % PerfectionPeptide P7 inhibited the formation of SBC by 20 % and the depletion of LC by 6 %.
- 0.11 % PerfectionPeptide P7 inhibited the formation of SBC by 75 % and the depletion of LC by 39 %.

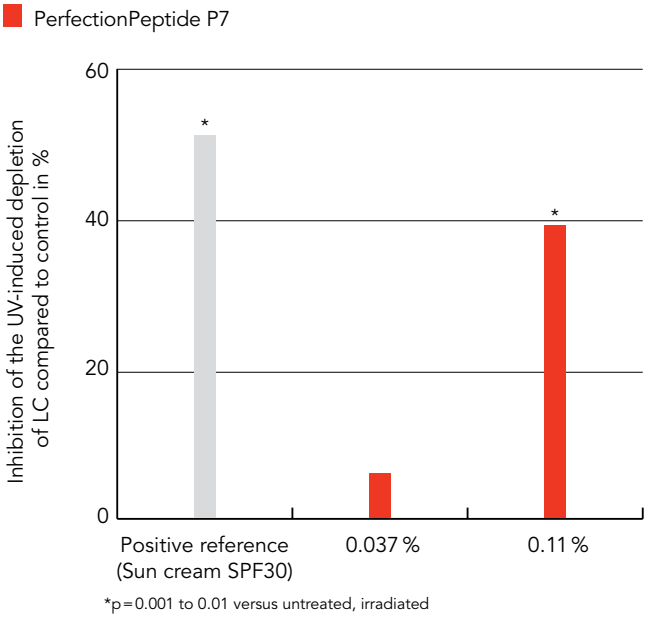
Therefore, PerfectionPeptide P7 can greatly protect the skin against UV-induced cell damages that occur at the DNA level and impact the immune system.

Protective Effect against the Formation of SBC



*p = 0.01 to 0.05 versus untreated, irradiated
**p < 0.001 versus untreated, irradiated

Protective Effect against the Depletion of LC



*p = 0.001 to 0.01 versus untreated, irradiated

PerfectionPeptide P7
Cellular defense regenerator

Claim Ideas for PerfectionPeptide P7
- Activates the skin's self-protection capacity
- Fights both environmental and chronological aging
- Protects DNA against oxidation
- Neutralizes free radicals and empowers the skin's own antioxidant capacity

Applications
- DD creams (daily defense)
- Protecting and regenerating skin care
- Age prevention products
- Sun protection

Marketing Benefits
- Activation of the cellular self-protection system has been comprehensively proven – even in vivo
- Novel peptide
- Preservative-free

It is much more than just an antioxidant as it also neutralizes free radicals and activates the cellular self-defense capacity.

Innovating for your success
Mibelle Biochemistry designs and develops innovative, high-quality actives based on naturally derived compounds and profound scientific know-how. Inspired by nature – Realized by science.

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