PHYCO’DERM®

Takes care of the delicate area around the eyes

* Multiple ways of action

Stimulates the major defense systems for increasing resistance against environmental stressors

Improves the dermis properties for smoothing fine lines and wrinkles of crow’s feet

Minimizes the appearance of under-eye dark circles for erasing the look of fatigue

Reduces the volume of under-eye bags for alleviating skin puffiness
The skin around the eyes is perceived as an essential feature of facial beauty since it immediately reflects fatigue.

It also appears as the first visible area to exhibit the changes of ageing that include dark under-eye circles, “bagging” or loss of elasticity, “crow’s feet” or wrinkling and loss of opacity of the under-eye skin.

It can too reflects one's lifestyle to a certain extent e.g. stress, use of tobacco and exposition to ultraviolet radiations.

In fact, the skin of the periocular area is distinct from other parts of the facial skin.

Why?

- This area is extremely thinner than anywhere else on the face and under constant moving. So it is rapidly affected by the signs of time. Smiling, squinting and blinking promote wrinkles from the repetitive contraction of the underlying muscle, leading to the development of crow's feet. Moreover when we age, the firmness and elasticity of the skin of eyelids decrease.

- This area lacks the natural moisture because the fewer presence of sebaceous glands. So it often appears dehydrated. The stratum corneum on eyelids presents a high trans-epidermal water loss (TEWL). With ageing, it exists a trend towards lower skin dehydration making the skin barrier in the periocular area more vulnerable.

- This area is more sensitive to injuries than the rest of the skin face due to constant exposure of environmental stressors (e.g. UV, smoke, fumes, wind..) that can be exacerbated by stress, lack of sleep, poor diet and allergies.

- This area can often show fragility of the fine and dense capillary network and a deficiency of the lymphatic system that lead to dark-circles and puffiness under the eyes which are considered to be unattractive.

Thus the skin around the eyes mandates earlier and more aggressive protection to avoid early ageing damage and combat the look of fatigue.

Several facial rejuvenation procedures help reverse the signs of facial ageing by the cosmetic, pharmaceutical, medical and plastic surgery industries. However many such methods and devices involve substances that are toxic (e.g. botulinum toxin), require injection (e.g. hyaluronic acid) or surgical procedures (e.g. blepharoplasty). Many of them are costly, less effective and not suitable for long-term use due to undesirable side effects.

Therefore it occurs a growing demand for non-invasive, effective and risk-free cosmetic agent which can reduce and or minimize the appearance of defects of the region around the eyes in order to provide a short and long-term youthful and revitalized look.

GELYMA proposes PHYCO’DERM® that offers a natural multi-target science based approach for taking care of the delicate area around the eyes by improving the aesthetic appearance with major benefits (e.g. protection against harmful environmental aggressors, enhancement of firmness and elasticity, attenuation of fine lines and wrinkles of crow’s feet, reduction of puffiness and dark circles under the eyes). As results the periocular area appears refreshed and less fatigued.

PHYCO’DERM® combines, in a glycerin excipient, two potent seaweed extracts prepared from the brown seaweed Undaria pinnatifida and the red one Corallina officinalis, chosen because of their particular biochemical benefits. Glycerin helps replenish moisture in the stratum corneum for keeping the skin hydrated and supple.
Mechanisms of action

The mechanisms of action of PHYCO’DERM® has been demonstrated by using the transcriptomic analysis on normal human fibroblasts for the gene expression analysis using the DNA chip technology from AFFYMETRIX (collaboration Strati CELL-Belgium) completed by other in vitro testing (collaboration SEPHRA PHARMA-France) and clinical studies (collaboration FARCODERM-Italy) in order to provide valuable data.

PHYCO’DERM® stimulates major defense systems for increasing resistance against environmental stressors

Because of its extreme thinness, the skin around the eyes is particularly sensitive to adverse impact of external factors (e.g. bacterial infection, oxidative stress, UV and various environmental pollutants) that may cause irritation and itching sensation.

Protection against microbial infections & associated inflammation

The periorbital region may be affected by inflammation due to various infections, especially infections of the dermis and associated tissues around the eyes. Swelling of eyelids is a common symptom of inflammation and allergy.

Chemokines are a class of cytokines acting as secondary inflammatory mediators induced by numerous cells (e.g. keratinocytes and fibroblasts) after different signals, specially bacterial infections. It exists two families of chemokines: CC and CXC chemokines.

PHYCO’DERM® is capable to overexpress the gene expression of both chemokines families highly involved in inflammatory response.

Toll-like receptors (TLRs) represent a family of pattern recognition receptors known for their role in inflammation and innate immunity.

PHYCO’DERM® is capable to overexpress the gene expression of two TLRs.

Their complementary action with chemokines helps enhance or restore natural adaptive immune responses in the skin.

The genes TNFAIP3 and NFKBIA play a key role in the negative regulation of NF-kappa B signalling in response to multiple stimuli. The gene BIRC3 is an important inhibitor of apoptosis.

PHYCO’DERM® is able to influence major negative regulators of inflammatory process. Therefore it reduces inflammation that can also lead to vessel fragility in the periocular area.

PHYCO’DERM® would be able to:

1- enhance immune adaptive response against microbial aggressions by combining the chemotactic activity of CC and CXC chemokines with the recognition of microbial components linked to toll-like receptors (TLRs),

2- regulate the inflammatory response related to the transcription NF-kappa B signalling known as a crucial player in inflammation and immunity processes.

Therefore, PHYCO’DERM® may also help neutralize irritation and reinforce the barrier function of the skin by eliminating bacterial infections and fighting against possible allergic and inflammatory diseases that may occur on the eyelids.
Protection against oxidative stress & regulation of cellular redox state

The periorbital region may be highly affected by constant exposure of environmental factors e.g. UV radiations, exhaust fumes, cigarette smoke that cause tissue damage and ageing. Skin cells respond to these harmful stressors through a wide range of interlinked defense mechanisms.

Oxidative stress activates numerous major signalling pathways resulting in changes in gene expression which influence the ability of cells to survive.

One of the most important regulators is the Nrf2 factor.

Genes that are regulated by Nrf2 encode proteins that include:

- several enzymes e.g. NQO1, GCLM
- other antioxidant proteins e.g. HSP Proteins (specifically HSP70 known to enhance cell survival, prevent apoptosis & reduce oxidative stress) and thioredoxins, glutaredoxins sulfiredoxins systems known to have pivotal role in redox signalling for controlling cell metabolism.

PHYCO’DERM® is able to influence the Nrf2 factor via the activation of several pathways in order to:

- increase the cytoprotection against oxidative stress
- regulate the cellular redox state from the detrimental effects of reactive oxygen species by acting through several signalling & transcriptional processes in cells.

Reinforcement of detoxification systems

Major air pollutants affects the skin, especially the periorcular area. Depending of the nature of these pollutants and skin integrity, their modes of penetration differ but their effects contribute to skin ageing, atopic dermatitis and skin cancer.

These environmental factors stimulate skin response through a wide range of interlinked defense mechanisms.

Metallothioneins (MT) are ubiquitous proteins known to play a pivotal role in several fundamental processes e.g. detoxification of heavy metals, homeostasis of essential metals, protection of DNA against oxidative damage and cell survival. They are also implicated in photoprotection. Their expression decreases with age. It is thought that their up-regulation would be profitable for restoring immune function at old age. MT are going to become an attractive target for ageing research.

Several enzymatic mechanisms play major role in the cellular defense specially CYP1B1 in the metabolism of polycyclic aromatic hydrocarbons present in the atmosphere and GST against electrophilic chemical species and radical oxygen species.

PHYCO’DERM® is able to over-express major genes involved in the detoxification systems such as three isoforms of MT-1 metallothioneins and specific enzymatic pathways.

By activating the expression of important genes involved in the major defense systems, PHYCO’DERM® increases detoxification process of skin cells and therefore put up skin resistance of the periorcular area against harmful external stressors.
The periocular area exhibits the most fragile skin of the entire face, revealing the passage of time and trace of fatigue. It is thinner than the rest of the face skin and it moves a lot more. When we age, skin variations result from modifications of cell functions and progressive changes of the extracellular matrix (ECM) structure. They are responsible for creating wrinkles in this area as collagen breaks down.

- **Modulation of the expression of genes related to the dynamic structure of the extracellular matrix (ECM)**

The ECM is a highly dynamic structure constantly remodelled. Its molecular components are subjected to numerous post-translational modifications. Through these characteristics, the ECM generates specific biochemical and mechanical properties.

- **PHYCO’DERM® is able to influence the dynamic structure of the extracellular matrix.**

- **Up-regulation of the expression of genes involved in cell adhesion**

Integrin beta-3 is a member of the integrin receptor family playing important role in cell adhesion to extracellular matrix proteins.

Syndecan-1 participates to numerous processes including cell adhesion, cell migration and endocytosis.

- **PHYCO’DERM® activates the gene expression of major membrane receptors (Integrin beta 3 & Syndecan 1).**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene abbreviation</th>
<th>Expression change*</th>
<th>P value (t-test)</th>
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<td>Integrin beta 3</td>
<td>ITGB3</td>
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<tr>
<td>Syndecan 1</td>
<td>SDC1</td>
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*relative expression compared to untreated control

Therefore PHYCO’DERM® helps increase cell adhesion to extracellular matrix proteins.

- **Up-regulation of the expression of genes involved in the hyaluronan synthesis**

The protein CD 44 is a receptor for hyaluronic acid. It is involved in cell-matrix interactions, cell adhesion and migration.

Hyaluronan regulates water balance, osmotic pressure and ion flow. Its synthesis is catalyzed by hyaluronan synthases (HAS) specially HAS1-HAS2-HAS3.

HAS3 and CD44 are down regulated during ageing. Therefore their upregulation is considered anti-ageing against the atrophy of the epidermis and the dermis.

- **PHYCO’DERM® over-expresses the expression of major genes involved in the hyaluronan synthesis (CD 44 & hyaluronan synthases) that help fight against skin ageing when occurs and prevent loss of moisture.**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene abbreviation</th>
<th>Expression change*</th>
<th>P value (t-test)</th>
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<td>Hyaluronan synthes 2</td>
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<td>3.10E-02</td>
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*relative expression compared to untreated control

- **Down-regulation of the expression of matrix metalloproteinases (MMPs)**

MMPs play crucial roles in the maintenance of the normal balance between ECM synthesis and degradation in tissue.

MMP1 is known to break down collagens, especially Types I & III.

MMP14 plays a crucial role in collagen remodelling and contributes to dermal homeostasis.

MMP11 (stromelysin 3) is involved in matrix degradation.

- **PHYCO’DERM® down regulates the gene expression of MMPs specially MMP1 and MMP14, therefore helps avoid collagen degradation while regulating collagen homeostasis.**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Gene abbreviation</th>
<th>Expression change*</th>
<th>P value (t-test)</th>
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</thead>
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</table>

*relative expression compared to untreated control

By influencing the dynamic behaviour of pathways, PHYCO’DERM® helps the ECM direct essential morphological organization and physiological function in order to preserve an efficient maintenance.
The effect of PHYCO'DERM® on fibroblast proliferation

Fibroblasts play fundamental roles in the maintenance of structural integrity within connective tissues. When we age, their number decreases that can promote general atrophy of the extracellular matrix and decrease in macromolecules organization.

Method: Evaluation of the proliferation of fibroblasts after 72h cultivation by using BrdU Elisa assay. Reference molecules: DMSO (10%) SVF (10%) - TGF β1 (10 – 20 ng/mL) - vitamin C (50 µg/mL).

Results

The effect of PHYCO'DERM® on collagen Type I synthesis

The major factor contributing to facial ageing is damage to and loss of dermal collagen. So it is important to stimulate the collagen production for increasing skin firmness and resistance.

Method: Evaluation of Type I pro-collagen production (PIP) by using Elisa test after 48h treatment with different doses of active. Reference molecules: TGF β1 (10 – 20 ng/mL) - vitamin C (50 µg/mL).

Results

With

- 0.5 % PHYCO'DERM® ➔ + 400 % proliferation
- 1 % PHYCO'DERM® ➔ + 393 % proliferation
- 2 % PHYCO'DERM® ➔ + 392 % proliferation

versus control

PHYCO'DERM® increases significantly the synthesis of Type I collagen that helps counteract the thinning which characterizes the skin around the eyes and tone eyelids.

PHYCO'DERM® induces wrinkle smoothing effect in the periocular area that has been confirmed by clinical studies.
Clinical studies: performed on two groups of 30 women volunteers type Caucasian each, one for the evaluation of a basic gel with 4% active (42-66 years old), the other for the evaluation of the placebo gel (37-71 years old). Treatments were applied twice a day for a period of 28 days. Digital pictures of the treated area were performed at day 0 (T 0) and day 28 (T 28).

PHYCO’DERM® attenuates fine lines and wrinkles of crow’s feet

The area around the eyes is typically susceptible to wrinkling caused in part by the natural slowdown of repair of the extracellular matrix of the skin.
Crow’s feet are clusters of tiny wrinkles and fine lines that form around the outer corners of the eyes. They develop with age following the loss of skin’ elasticity due to the reduced collagen synthesis. They may appear after excessive sun exposure, smoking, squinting, frowning and smiling.

Method: Evaluation by skin profilometry by means of 3D LIFEVIZ MicroTM (QUANTICARE) analysis after 28 days application twice a day of a gel with 4% PHYCO’DERM® versus placebo.

Results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Δ DO / D 28</th>
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<tbody>
<tr>
<td>Placebo</td>
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<tr>
<td>Gel 4% PHYCO’DERM®</td>
<td>-6.1%</td>
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</table>

 PHYCO’DERM® is able to reduce wrinkles depth in comparison with the placebo eye gel after 28 days of treatment.
 PHYCO’DERM® helps erase fine lines and wrinkles of the crow’s feet inducing smoothing effect in the periocular area.

PHYCO’DERM® minimizes the appearance of dark circles under the eyes

Dark circles are changes in skin colour around the eyes. They results from a variety of factors linked for example to superficial vascularity, hyper vascularisation, skin translucency, fatigue and structural shadowing. They make people look tired, exhausted, unhealthy and older.

Method: Evaluation of color intensity by means of CM-700d spectrophotometer analysis after 28 days application twice a day of a gel with 4% PHYCO’DERM® versus placebo.

Results

<table>
<thead>
<tr>
<th>Parameter a</th>
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<td>Gel 4% PHYCO’DERM®</td>
<td>-7.5%</td>
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The parameter “a” concerns the green-red component
When “a” decreases, redness decreases

<table>
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<tr>
<th>Parameter b</th>
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<tr>
<td>Placebo</td>
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<td>Gel 4% PHYCO’DERM®</td>
<td>3.0%</td>
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The parameter “b” concerns the blue-yellow component
When “b” increases”, dark circles are less blue

 PHYCO’DERM® decreases the intensity of coloration that fades dark circles and therefore increases skin luminosity under the eyes and erases the look of fatigue.
Periorbital puffiness is the appearance of swelling in the tissues around the eyes. It appears as a common cosmetic problem occurring when the skin of the lower eyelid is slightly swollen. That may be caused by various factors such as sleep deprivation, fluid retention, diet, excessive caffeine and alcohol consumption, tobacco use, allergies, skin disorders and normal ageing.

The skin located here lost firmness and elasticity. Fluid accumulation in this area gives rise to an edema which the consumer perceives as anti-aesthetic.

Method: evaluation of skin surface of under-eye bags by means of Primos 3D analysis after 28 days application twice a day of a gel with 4% PHYCO’DERM® versus placebo.

Results

PHYCO’DERM® reduces the volume of under-eye bags

PHYCO’DERM® decreases significantly the volume of under-eye bags compared to placebo.

<table>
<thead>
<tr>
<th></th>
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<tr>
<td>Placebo</td>
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<tr>
<td>Gel 4% PHYCO’DERM®</td>
<td>-9.1 %</td>
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PHYCO’DERM® alleviates puffiness of the skin and helps restore a smooth skin contour to puffy skin beneath the eye.
Subjective evaluation of PHYCO’DERM® by 30 Caucasian women panelists (from 37 to 71 years old) after twice daily application of a gel (4% active) for 28 days.

The evaluation of panelists through a subject questionnaire has been particularly positive. It provides conclusive evidence of important benefits of using PHYCO’DERM®.

The panelist’ responses also concerned the action of PHYCO’DERM® on eye look (in % satisfaction).

The appearance of the periorbital area is particularly enhanced for producing a more youthful look.
**Cosmetic benefits**

PHYCO’DERM® is a natural marine complex designed to take care the delicate area around the eyes. It combines two seaweed extracts in a glycerin excipient that helps combat dryness.

PHYCO’DERM® conceals imperfections of the periocular area by providing numerous desirable benefits proved by different *in vitro* and clinical studies.

- Protection of the periocular skin cells against microbial infections and various kinds of environmental aggressors (free radicals, UV radiations, heavy metals)
  - enhancement of natural adaptive immune response
  - regulation of the inflammatory processes *via* NF-Kappa B signalling
  - neutralization of irritation risks
  - prevention of cellular damage against oxidative stress
  - regulation of cellular redox state *via* Nrf 2 factor activation
  - stimulation of pivotal detoxifying defense systems

  for enhancing natural immune responses, stimulating cellular stress response, reducing sensations of irritation and inflammation of the skin and reinforcing skin resistance to harmful external stressors.

- Improvement of dermis properties
  - modulation of the expression of genes related to the dynamic structure of the extracellular matrix (cell adhesion, hyaluronan synthesis and MMPs regulation)
  - activation of fibroblasts metabolism
  - stimulation of Type I collagen synthesis

  for enhancing skin firmness and elasticity, counteracting the skin thinning of the periocular area and toning eyelids.

- Attenuation of fine lines and wrinkles of crow’s feet for inducing smoothing effect of the periocular area.

- Alleviation of the look of dark circles
  - decrease of the intensity of coloration

  for diminishing the sensation of darkness under the eyes, increasing skin luminosity under the eyes and erasing the look of fatigue.

- Decrease of skin puffiness under the eyes
  - reduces the volume of bags

  for restoring smooth skin contour to puffy skin beneath the eyes.

Clinical studies have demonstrated the positive effects of PHYCO’DERM® after only 28 days of treatment on visible global fatigue of the eye contour.

- The periocular area looks more refreshed and less fatigued.
Cosmetic applications

All products intended for eye contour care.
Recommended use levels: 3% - 5%.

Indicative formulation - Serum eye contour care

<table>
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<th>Phase</th>
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<td>Solagum AX</td>
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<td>B</td>
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<td>PHYC’DERM®</td>
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<tr>
<td></td>
<td>Acide citrique à 50%</td>
<td>0,4 Qs pH 5,4 - 5,8</td>
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<tr>
<td></td>
<td>Eau</td>
<td>Qs 100</td>
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Technical data summary

Aspect : limpid liquid yellow coloured with typical odour
Odor : characteristic
pH : 6.0 ± 1.0
Soluble : in aqueous solutions
Insoluble : in oils.

Regulatory data

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<tr>
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<td>Corallina officinalis extract</td>
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About PHYCO’DERM®

The two seaweed extracts present in PHYCO’DERM® *Undaria pinnatifida* and *Corallina officinalis* have been chosen because of their particular benefits.

*Undaria pinnatifida* is a seaweed belonging to the kingdom *Chromista*, the class *Phaeophyceae* and the family *Alariaceae*.

Its thallus consists of a basal part, a stipe and a lamina. The stipe vertically arises from hapters which are dichotomously divided and fixed to rocky substratum. It appears terete-compressed. It continues upwards as a prominent midrib through the lamina, winged on both sides with a narrow continuation of the lamina.

This alga can reach 3 meters in height, 60 cm in width. Its colour is yellowish-brown, turning to greenish-olive when drying.

*It* offers a balanced composition in minerals and vitamins. It contains alginic acid and fucans with high potential commercial value.

*Corallina officinalis* is a seaweed belonging to the kingdom of *Plantae*, the classe *Florideophyceae* and the family *Corallinaceae*.

It is a calcified or calcareous red marine alga reaching 5-12 cm high. The thallus, erect articulated (geniculate), arises from a firmly attached crustose base and shows articulated fronds. Its color can be widely varied, from purple in deep water to yellow-white on exposure.

This alga is widely distributed and common in a wide range of habitats. It is present in Atlantic Ocean from Norway to Morocco and from Greenland to Argentina. It is found in North America from Labrador to Maryland in the United States. It is reported in W–Baltic, Mediterranean, Japan, China, Australia, South Africa and the Arctic Sea.

*Corallina officinalis* includes numerous specific compounds e.g. a special pentapeptide, two characteristic phycobiliproteins (phycoerythrins), sulphated xylagalactans, floridoside and bromoperoxidases.

*Corallina officinalis* is known as Common Coral weed in England. In Europe it have been used as a vermifuge towards the end of the XVIIIth century and serves still now in traditional medicine in Asia. Phycobiliproteins are economically important due to their colorant and fluorescence properties. Bromoperoxidases offer considerable interest due to their exceptional stability for industrial catalysis in a variety of contexts. They have also medical applications.

The extract of *Corallina officinalis* here present is a water concentrated extract, rich in numerous minerals such as calcium, sodium, potassium and magnesium which contribute to reinforce the skin barrier and improve the overall skin condition.

Its innovative association with *Undaria pinnatifida* extract offers efficient balanced composition for maintaining the skin healthy.

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