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

Gelyma

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SUMMARY

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INTRODUCTION

"The eyes are the mirror of the soul and reflect everything that seems to be hidden and like a mirror they also reflect the person looking into them" - Paulo Coelho.

The skin around the eyes is perceived as an essential feature of facial beauty since it immediately reflects fatigue.

It 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 also can mirror one's lifestyle to a certain extent *e.g*. stress, excessive use of tobacco and alcohol or else 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 (on the order of 0.33 to 0.36 mm *i.e*. 3 to 5 times thinner than the rest of the facial skin).
  It is under constant moving and 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 tend 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 to 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 contribute to swelling/puffiness and therefore it appears of dark circles 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.

Many 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 (*e.g.* irritation/inflammation due to aggressors, crow's feet, swelling/puffiness and dark circles) in order to provide long-term youthful and revitalized look.

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GELYMA proposes PHYCO'DERM® that offers a natural multi-target science based approach for taking care the delicate area around the eyes by improving the aesthetic appearance with major benefits:

- protection against microbial infections and various kinds of environmental aggressors (*e.g.* free radicals, UV radiations, heavy metals) for increasing the cellular stress response, detoxifying the skin and reinforcing skin resistance while reducing sensations of irritation and inflammation of the skin,

- enhancement of firmness and tone for smoothening wrinkles and fine lines and attenuating crow's feet,

- reduction of puffiness and under-eye dark circles for minimizing the look of fatigue.

As results, the periocular area appears refreshed and less fatigued.

PHYCO'DERM® combines, in a glycerin excipient, two potent algal extracts prepared from the brown seaweed *Undaria pinnatifida* and the red seaweed *Corallina officinalis,* chosen because of their particular biochemical composition advantages.

The efficacy of PHYCO'DERM® has been demonstrated by both *in vitro* and clinical tests, detailed hereafter, for proving cosmetic benefits on visible global tiredness of the delicate area around the eyes.

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ALGAL SOURCE

PHYCO'DERM® combines in a glycerin excipient two seaweeds extracts prepared from:

- *Undaria pinnatifida*
- *Corallina officinalis.*

Undaria pinnatifida

➤ Classification

Empire	Eukaryota
Kingdom	Chromista
Phylum	Ochrophyta
Class	Phaeophyceae
Subclass	Fucophycidae
Order	Laminariales
Family	*Alariaceae*
Genus	*Undaria*
Species	*pinnatifida* (Harvey) Suringar 1873.

Basionym

Alaria pinnatifida Harvey.

Origin of species name

Adjective (Latin): pinnately cleft.

Heterotypic Synonym(s)

Undaria pinnatifida f. *typica* Yendo
Undaria pinnatifida f. *subflabellata* Suringar
Alaria amplexicaulis Martens *1866*
Undaria pinnatifida var. *vulgaris* Suringar 1872
Undaria pinnatifida var. *elongata* Suringar 1872
Undaria pinnatifida f. *distans* (Miyabe & Okamura) Yendo 1911.

Common names

Chinese	Qun dai cai
English	Sea mustard, Precious sea grass, Wakame
Japanese	Wakame
Korean	Miyok, Miyeouk.

➤ Morphology & Biology

The thallus of *Undaria pinnatifida* consists of a basal part, a stipe and a lamina (Fig.1).

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

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.

The lamina is usually simple and ovate when young, pinnately sected and coarsely bullate-rugose when adult.

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The sporophyll shows a dilated undulato-folded wing formed on both sides of stipe. It contains numerous sori.

The life cycle is typical as for the other Laminariales: diplohaplontic with alternation of a large sporophyte bearing unilocular meiosporangia with paraphyses (sori) and microscopic dioceious and oogamous heteromorphous gametophytes.

The growth rate has been measured at 1cm per day.

Fig.1 - Morphology of *Undaria pinnatifida* - Drawings from SURINGAR 1872

Fig.2 - Morphology of *Undaria pinnatifida in situ* Photo GELYMA

➤ Ecology & Geographical distribution

This alga is found in the upper part of the infralittoral zone (Fig.2), from low tide down to depths of 10-18 m. It can form dense kelp forests.

Until 1960, this alga was endemic to Japan. Actually, it occurs in Japan on rocks at depths of 15 meters (Saito, 1975 – Advance in Phycology in Japan, 304-320) but also in large aquacultural areas.

It is widely distributed except in the eastern and the northern coasts of Hokkaido (which receive the cold Kurile Current) and the eastern coasts of Kyrishu and Shikoku (which receive the warm Kuroshio).

Currently it also grows along the coasts of China and Korea where it was introduced deliberately for cultivation (Akiyama & Kurogi, 1982 – Bull. Tohuku Re. Fish. Res. Lab. 37: 43-49).

Undaria pinnatifida is also present in:

- New Zealand [Hay & Luckens, 1987 – N. Z. J. Bot. 25:364-366; Stapleton, 1988 – Jap. J. Phycol. 36:178-179; Hay, 1990 – Br. Phycol. J. 25: 301-313; Brown & Lamare, 1994 –Jap. J. Phycol. 42: 63-70].

- Tasmania (Sanderson & Barret, 1989 - Dep. Sea Fish. Tasm. Mar.: Lab. Tech. Rep. 38: 1-35).

In Europe, this alga was introduced accidentally along with oysters to the Bassin of Thau in the south of France as far as back as 1960. Currently, it is well established in this lagoon growing well with a similar phenology to Asian algae (Perez *et al*. 1981 – Science et Pêche 315:1-12). It is being cultivated off the coasts of Brittany (France) (Perez *et al*. 1984 – Science et Pêche 343: 3-15).

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Today, it is spreading in:

- Mediterranean towards Venice (Curiel *et al*. 1994 – Lavori-Soc.Ven.Sc.Nat. 19: 121-126),
- Atlantic towards England (Fletcher & Manfredi, 1995 – Bot. mar. 38: 355-358).

➤ Chemical composition

The chemical composition varies among the plant parts such as the frond, stipe and sporophyll and the environmental conditions.

Major compounds of *Undaria pinnatifida* are regrouped here after (*cf.* CEVA nutritional data 2015).

in g/100 g dehydrated

Minerals	12.6 - 41.1
Proteins	8.9 - 21.4
Dietary fibers	13.6 - 71.0
Lipids	0.4 - 7.2
Polyphenols	0.08 - 0.60

In mg/100 g dehydrated

Potassium	380 - 16 919
Sodium	1463 -10 060
Magnesium	370 - 2789
Phosphorus	155 - 979
Calcium	274 - 2744
Manganese	2.4
Iron	1.4 - 131.1
Copper	0.6
Zinc	0.3 - 6.5
Iodine	2.0 - 143.7
Vitamin A	0.02 - 1.14
Vitamin E	0.2 - 2.4
Vitamin C	2.8 - 137.2
Vitamin B1	0.3
Vitamin B2	0.1 - 1.7
Vitamin B3	0.8 - 11.0
Vitamin B5	0.15 - 0.18
Vitamin B6	0.1
Beta carotene	1 - 402

In µg/100 g dehydrated

Selenium	5.4 - 274.4
Vitamin K	732
Vitamin B8	14.6 - 20.1
Vitamin B9	72.2 - 503.0
Vitamin B12	0.03 - 0.5

Undaria pinnatifida contains alginic acid: 34-40 and fucans: 2-3 (in % DW).

Fucoidans extracted from sporophylls of this alga show a higher sulfate and L-fucose content than others fucoidans (Kim *et al.* 2010 – Food Chem. Toxicol. 48: 1101-1104).

➤ Bioactivities & utilizations

Undaria pinnatifida offers numerous interesting bioactivities such as:

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- antibiotic action: Saito & Sameshina, 1955 – J. Agr. Chem. Soc. Japan 29: 427-430; Kamimoto, 1955 Japan J. Bacteriol. 10: 897-902,

- anti-tumour action: Yamamoto *et al*. 1982 – Bot. Mar. 25 : 45-457; Ohigashi *et al*. 1992 – Biosci. Biotechnol. Biochem. 56: 994-995,

- anti-nicotine action carried out on animals: Watanabe 1968 in Takagi - 1975 - Advance in Phycology in Japan, 321-325,

- anti-leukaemic action: Furusawa *et al*. 1991 – Cancer Lett. 56: 197-205,

- antiviral action: Muto *et al*., 1992 – US Patent 508999481,

- anti-inflammatory action: Patent KR 20070099126.

At least, *Undaria pinnatifida* is known for its medicinal properties *e.g.* in Japan for purifying blood. In Korea, this alga is used traditionally by women before and after childbirth.

Numerous patents concern these therapeutic properties for treating:

- osteoporosis Patent KR2009 0116248,
- hypertension Patent KR 20110022472,
- diabete Patent CN 104187621,
- asthma Patent CN 104353028,
- topic dermatitis Patent US 2015 140112.

Fucoxanthin extracted from *Undaria pinnatifida* would be effective as an anti-obesity agent (Patents W0 2006 126 325; EP 1884238).

Fucans extracted from this alga show high potential commercial value for therapeutic applications against especially:

- cancer [BOO *et al.* 2011- Phytother. Res. 25 (7) : 1082-1086 ; Yang *et al*. 2013-Mar. Drugs 11(6): 1961-1976 ; Patent KR 2014 0135536],
- thrombolysis (Patent KR 2012 0086533).

Undaria pinnatifida plays a major role in East Asian food markets, known as wakame. It is a highly prized delicacy and considered as a sea vegetable. It is an important nutrient source for diet and food additives due to the high content of minerals, proteins and carbohydrates. It is eaten dried, boiled or fresh (Yamanaka & Akiyama 1993- J. Appli. Phycol. 5: 249-253). It brings excellent sources of nutrients (Taboada *et al.* 2013- J. Appl. Phycol. 25:1271-1276).

Wakame is sold in bundles or cut up as "*Kizami-wakame*". It is also presented as:

"*subo boshi wakame*" dried under the sun,
"*shibori wakame*" washed with sea water and then dried under the sun,
"*Shionuki wakame*" washed with soft water then dried under the sun,
"*zoen wakame*" raw, salted then vacuum-packed,
"*iudoshi zoen wakame*" boiled then salted.

In France, this seaweed has been included on edible foods since 1988.

In the United States, it is recognized GRAS (Generally Recognized As Safe- 212 Code of Federal regulation, Part 184). In both countries, it must be in accordance with the Food Chemical Codex, especially for heavy metals and iodine.

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Corallina officinalis

➤ Classification

Empire	Eukaryota
Kingdom	Plantae
Phylum	Rhodophyta
Class	Florideophyceae
Subclass	Corallinophycidae
Order	Corallinales
Family	*Corallinaceae*
Genus	*Corallina* Linnaeus 1758
Species	*officinalis* Linnaeus 1758.

The genus *Corallina* includes 33 species currently accepted taxonomically.

Origin of the species name

from Greek "*corallion* ": a coral and from Latin "*officina*" : a shop.

Heterotypic Synonym(s)

Corallina calvadosii JV Lamouroux 1816
Corallina cretacea Postels & Ruprecht 1840
Amphiroa cretacea (Postels & Ruprecht) Endlicher 1843
Corallina compacta P. Crouan & H. Crouan 1867
Arthrocladia cretacea (Postels & Ruprecht) Weber-van-Bosse 1904
Pachyarthron cretaceum (Postels & Ruprecht) Manza 1937
Bossiella cretacea (Postels & Ruprecht) H.W. Johansen 1969.

Common names

English Sea Common Coral weed.

➤ Morphology & Biology

Corallina officinalis is a calcified or calcareous red marine alga reaching 5-12 cm high.

It shows an erect articulated (geniculate) thallus arising from a firmly attached crustose base up to 70 mm in diameter and bearing tufts of branched and articulated fronds up to 120 mm long. The structure appears multiaxial combined with marked calcification. Numerous calcified segments are separated by uncalcified horny and flexible joints (genicula). These segmented fronds provide flexibility in churning seawater. The branches are irregular, opposite and pinnate, resulting in a father-like appearance (Fig.3).

The color of the alga can be widely varied. The thallus appears dull purple when growing in deep water, becoming red yellow and finally white on exposure.

Corallina officinalis has been the subject of many studies on:

- anatomy [Rosenvinge L.K. , 1917- Kongelige Danske Videnskabernes Selskabs Skrifter, ser.7: 155-283; Suneson, 1937- Lunds Universitets Arsskrift 33 (2): 1-101],

- cytology, growth and development (Cabioch 1971 – Cahiers de Biologie Marine 12: 121-186, 1972- *ibid* 13: 137-288),

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- ultrastructure (Giraud & Cabioch, 1976 – Phycologia 15: 405-414,1977 – Revue Algologique 12: 45-60; Borowitzka & Vesk, 1978 – Marine Biology 46:295-304) and

- calcification (Pentecost, 1978 – British Phycological Journal 13: 383-390.

Corallina officinalis forms calcium carbonate deposits within its cells.

Plants are dioecious with male conceptacles elongated at the apex and female conceptacles, those bearing tetraspores ovoid.

Corallina officinalis grows from 1-2 cm per year (Masaki *et al*. 1981- Proc. Int. Seaweed Symp. 10:607-612).

It is present year around.

Fig. 3- Morphology of *Corallina officnalis*
Drawing from *HARVEY 1849*
Phycologia Britannica vol. II

➤ Ecology & Geographical distribution

Corallina officinalis is widely distributed, especially in temperate areas on rocks, midtidal pools and drainage runnels (Fig.4). It can be found to 33 m deep.

It is a very common species which adapts to 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.

Fig.4 - Morphology *in situ* of *Corallina officinalis*
Photo GELYMA

➤ Chemical composition

The chemical composition of *Corallina officinalis* has been studied by different authors.

According to Marsham *et al*. (2007—Food Chemistry 100:1331-1336), this red alga contains (% DW):

Ash	72.8 ± 1.2	Proteins	6.9 ± 0.1
Lipids	0.3 ± 0.2	Crude fibre	8.3 ± 3.2

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Sebaaly C. *et al*. (2014 – J. Applied Pharmaceutical Sc. 4 (4): 30-37) reported, in *Corallina officinalis* collected from Lebanese coast, high amounts of trace elements (in mg/100 g), specially potassium (3750) sodium (750) calcium (2000) and magnesium (750) and also lipids specially 70.81% saturated fatty acids, 25.54% monounsaturated fatty acids and 3.65% polyunsaturated fatty acid , the major fatty acids being saturated palmitic acid (52.13%) and the monounsaturated cis-vaccenic acid (18.2%).

Other compounds have been also characterized from this red alga:

▶ Peptides specially a pentapeptide (Haas P. & T.S. Hill, 1933 – Biochem. J. 27: 1802-1804),

▶ Pigments such as carotenoids and phycobiliproteins

Carotenoids have been isolated *e.g*. beta-carotene, zeaxanthin, fucoxanthin, fucoxanthinol and the epimeric mutatoxanthins (Palermo J.A. *et al*. 1991- Phytochemistry 30(9): 2983-2986).

It would exist in *Corallina officinalis* two phycoerythrins with different molecular weights and spectral properties (Van der Velde H.H. 1973 – Biochim. Biophys. Acta 303 (2): 246-257; Hildich N. *et al.* 1991- J. Applied Phycol. 3 (4): 345-354).

▶ Polysaccharides

The amount of cellulose reaches 5.1% (In Percival & Mc Dowell 1967- Chemistry & Enzymology of Marine Algal Polysaccharides –Acad. Press, London).

Sulphated xylogalactans are well studied (Cases M.R. *et al.* 1994- Int.J. Biol Macromol. 16 (2): 93-97; Navarro D.A. & C.A. Stortz , 2002- Carbohydrate Polymers 49 (1): 57-62) showing large amounts of xylose and L-galactose (Cases M. R. *et al.* 1992- Phytochemistry 31 (11):3897-3900).

▶ Heterosides specially floridoside (Haas P. & T.S. Hill, 1933 – Biochem. J. 27:1802-1804; Wickberg B. – 1958 – Acta Chem. Scandinavia 12: 1183-1186; Majak *et al*. 1966- Can.J. Bot. 44: 541-549; Craigie *et al*. 1968- Can. J. Bot. 46: 605-611; Kirst G.O. – 1980 – Phytochemistry 19 (6):1107-1110).

▶ Sterols with a total amount of 16.7 mg/Kg DW, chlolesterol being the major one (Gibbons G.F. *et al*. 1967 – Phytochemistry 6: 617-683; A.S. Goldberg *et al.* 1982 – Bot. Mar. 25: 351-355).

▶ Several volatile compounds such as hydrocarbons (aliphatic & cyclic), terpenes, (mono- & diterpenes), aldehydes, ketones, esters (Borik R.M. – 2014 – World Applied Sc. J. 30 (66): 741-746).

▶ Specific enzymes such as bromoperoxidases with important studies relative to vanadium-dependent haloperoxidase (Yamada H. *et al.* 1985 – Agric. Biol. Chem., 49 (10): 2964-2967; YU H. & J.W. Whitaker, 1989 – Biochem. Biophys. Res. Comm 160 (1): 87-92; Rush C. *et al.,* 1995- FEBS Letters 359: 244-246; Littlechild J. & E. Garcia-Rodriguez , 2003- Coordination Chemistry reviews 237 (1-2): 65-78).

➤ Bioactivities

The crude extract of *Corallina officinalis* exhibits antibacterial activities against *Enterococcus foecalis*, *Enterobacter aerogenes* and *Escherichia coli* (Taskin E. *et al.* 2007 – African J. Biotechnology 6 (24):2746-2751).

Polysaccharides present in this red alga show anticoagulant and antibacterial activities (Sebaaly *C. et al*. 2014 – J. Applied Pharmaceutical Sc. 4 (4):30-37) .They also offer antioxidant activities *in vitro* (Yang Y. *et al*. 2011 – Intern. J. Biol. Macromolecules 49(5): 1031-1037).

The lipoidal matters of *Corallina officinalis* exhibit a significant hypolipidaemic activity from alcohol and hexane extracts (Awad N.E. *et al.* 2003- Phytotherapy Research 17 (1): 19-25).

Corallina officinalis offers weak anti-inflammatory activities relative to PLA2 and elastase (Oumaskour K. *et al.* 2013 – Int. J. Pharm. & Pharmaceutical Sc. 5 (3): 145-149).

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➤ Uses

Corallina officinalis extract is a very popular ingredient for:

- personal care (SECMA Patent FR 2674126-1992 ; CODIF Patent FR 2892024- 2007 ; KITASAKI SHINICHI JP Patent JP 2003328852-2003, Patent W0 2005027941- 2005, Patent KR 2006 0085249-2006; COTY Patent US 2009117060- 2009,
- traditional medicine, specially in Asia for treating *e.g.*

- thyroid adenoma	SONG CHUAWEI – Patent CN 10151501515 20150817-2015
- dysmenorrhea	WANG LILI – Patent CN 2014149151420140910-2014
- acne	XU YAN Patent CN 20151310554 20150609-2015
- transient synovitis	LIU YANG Patent CN 2015 1297945 20150527-2015
- vascular cognitive impairment	LI FENGPENG Patent CN 2015 1297932 20150527-2015
- chronic tracheatis	GANNAN CIGARETTE FACTORY *et al*. Patent CN 1986101200 19860222-1987.

This extract is also added in antifungal composition (UNIV SILLA – Patent KR 20070121624 20071127-2008) or in a mulberry root health tea (ANHUI YUNFENG AGRICULTURAL DEV. Co LTD Patent CN 2015 1645119 20120930-2015).

In Europe *Corallina sp.* have been used as a vermifuge towards the end of the XVIII th century (in Chapman V.J. 1950- Seaweeds and their uses, Methuen & Co Ltd, London). This property would be linked to the presence of a peptide including kainic acid residues (Calaf R. *et al.* 1989 – J. Applied Phycol. 1 (3): 257-266). Its addition in animal meals this alga would be appreciated by sheep (in Guiry & Blunden, 1991- Seaweeds Resources in Europe, Wiley).

Other compounds present in *Corallina officinalis* show great industrial interest, specially phycobiliproteins and bromoperoxidases.

- Phycobiliproteins are economically important because they are used as colorants in food and cosmetics. They show therapeutic value by immunomodulating activity and anticancer activity. Because of their fluorescence properties, they are important in the development of phycofluor tags for immunodiagnostics and highly sensitive fluorescence techniques (*cf.* in Sinha R.P. *et al.* 2003 – Trends in Phytochemistry and Photobiology 10: 149-157).

  SIGMA-ALDRICH offers R-phycoerythrin from *Corallina officinalis* (ref. PO 159).

- Bromoperoxidases catalyze the halogenation of various compounds *e.g*. iodine, bromo (Yamada H. *et al.*1985 – Agric. Biol. Chem. 49(10): 2961-2967). The vanadium bromoperoxidase of *Corallina officinalis* presents considerable interest due to the exceptional stability for industrial catalysis in a variety of contexts (Sheffield *et al*. 1994- Biotechnology Techniques 8: 579-582; Vreeland V. & E. Grotkopp Patent US 5,520,727, 1996; Vreeland V. & Kwan L. Ng Patent US 2002/0035245-2002; Littechild J. & E. Garcia-Rodiguez 2003 - Coordination Chemistry Reviews 237 (1-2): 65-75). They have also medical applications *(cf.* in Vreeland V. & Kwan L. Ng Patent US 2002/0035245-2002).

  SIGMA-ALDRICH proposes bromoperoxidases from *Corallina officinalis* (refs. B 2170; 17965).

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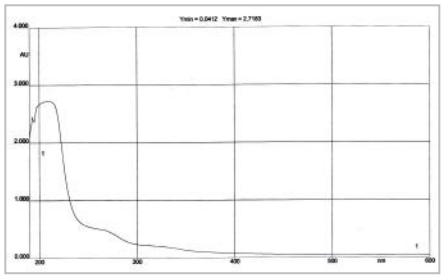
THE ACTIVE INGREDIENT PHYCO'DERM®

Specifications

on a control batch

- Appearance : limpid liquid yellow coloured
- odour : typical
- pH : 6.0 ± 1.0
- density : 1.014 ± 0.02
- dry residual (%) : 50 ± 5

-UV spectrum (5% in water)



- solubility : soluble in aqueous solutions
  : insoluble in oils
- microbiology : bacteria < 100 germs / ml
  : yeasts, moulds < 100 germs / ml
  : pathogens free

Regulatory data

INCI names	CAS n°	EINECS n°	Amounts (%)
water	7732-18-5	231-791-2	30.0
glycerin	50-81-5	200-289-5	50.0
Undaria pinnatifida extract	-	-	18.5
Corallina officinalis extract	89997-92-2	289-730-0	1.5
Preservative	as required	-	-

Addition of preservative by selection: Phenoxyethanol or Microcare SB.

ECOCERT/COSMOS compliant with none preservative or with addition of Microcare SB.

CHINA compliant (list 2015).

06260	水	WATER
02421	甘油	GLYCERIN
05477	裙带菜（UNDARIA PINNATIFIDA）提取物	UNDARIA PINNATIFIDA EXTRACT
05972	珊瑚藻（CORALLINA OFFICINALIS）提取物	CORALLINA OFFICINALIS EXTRACT

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Distinctive items

PHYCO'DERM® combines glycerin with two seaweeds extracts prepared from
- *Undaria pinnatifida*
- *Corallina officinalis.*

These seaweeds have been chosen because of their specific biochemical advantages.

Benefits provided by glycerin

The glycerin here used is obtained from natural sources. It helps replenish moisture in the *stratum corneum* for keeping the skin hydrated and supple.

Benefits provided by seaweeds extracts

Undaria pinnatifida extract

The extract of *Undaria pinnatifida* present in PHYCO'DERM® is a water extract containing the sulfated polysaccharide fucoidan, known to have important biological properties influenced by the molecular size of molecules.

The structure and bioactivities of fucoidans are well known (Li B. *et al.* 2008 – Molecules 13: 1671-1695; Tutor-Ale M. *et al.* 2011 – Mar. Drugs 9: 2106-2130; Sinurat E. & E. Marraskuranto2012 – Squalen 7 (3): 131-138 ; Morya V.K. *et al*. 2012 – Appli. Microbiol. Biotechnol. 93: 71-82).

It is well demonstrated that the fucoidan extracted from *Undaria pinnatifida* offers:

- antioxidant properties (Kang *et al.* 2008- Biotechnology & Bioprocess Engineering 13: 168-173; Mak W. *et al.* 2013 – Carbohydrate Polymers 96: 606-614),
- anti coagulant properties (Kim *et al.* 2007- Algae 22(3): 247-252),
- anti-inflammatory activities (Khan *et al.* 2008- Phytother; Res. 22: 634-639; Kim *et al*. 212 – Fitoterapia 83(8) : 1628-1635),
- anti-angiogenic activity (Liu *et al*. 2012- Phytomedecine 19(8-9): 797-803).

It induces apoptosis in carcinoma cells (Yang L. *et al.* 2013 – Mar. Drugs 11:1961-1976), inhibits the activity of herpes simplex virus type L (Hayashi K. *et al.* 2008 – Int. Immunopharmacol. 8: 109-116) and enhances stem cell mobilization (Stemtech Int. Inc. Patent US 2016/0136225).

In addition it shows anticancer activities (You *et al*. 2010- Food Chemistry 110 (2): 554-559).

Fucoidans are used for cosmetics preparation for skin and hair care (Wu H.K. *et al.* Patent US 2004/0043961; Athwal G. Patents CA 27 01 378, US 2011/0262505, US 2014/0242130; US 2016/0008267) Mizutani S. *et al.* Patents US 2003/0039670, US 2006/0093566 ; Gupta S.K. Patents US 2004/0219124, US 2006/0198805 ; Cappello J.V. Patent US 2013/0115195).

To our knowledge, a combination of an extract of *Undaria pinnatifida* rich in fucoidan with a concentrated extract of *Corallina officinalis* has never been used for cosmetic purposes.

Corallina officinalis extract

The extract of *Corallina officinalis* here present is a water concentrated extract. It brings numerous minerals such as calcium, sodium, potassium and magnesium.

The effects of *Corallina officinalis* extract for skin care are well documented for improving the overall skin condition. It has been used in sun-protection products (Coty Patent US 78 92 523) and slimming preparations (Codif Patent FR 2 892 024).

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Specific mineral composition of PHYCO'DERM®

Analysis performed by In Vivo Labs (France).

➢ Minerals (mg/100g)

Potassium	:	530.3
Sodium	:	420.4
Calcium	:	142.9
Magnesium	:	60.7
Phosphorus	:	8.9
Iodine	:	1.9 mg/Kg

➤ **Potassium** participates in several vital functions *e.g.* cell growth, maintenance of cell volume, DNA and protein synthesis, enzymatic activity, acid base balance and cellular apoptosis. It acts on cellular membrane permeability and works closely with **sodium** to maintain cellular osmotic balance.

➤ **Magnesium** is involved in many physiologic pathways *e.g.* energy production, nucleic acid and protein synthesis and cell signalling.

➤ **Calcium** plays optimal role in cellular regulation and acts on all skin components. It strengthens cell membranes. It is also involved in the production of energy and the maintenance of immune function.

➤ **Phosphorus** takes a share in several biological processes *e.g.* energy production, cell signalling through phosphorylation reactions and regulation of acid-base homeostasis.

➤ **Iodine** is known to stimulate the breakdown of fat tissue by activating lipases and thus resulting in slimming.

➢ Heavy metals (ppm)

Arsenic	:	1.383	(< 1.5 ppm)
Lead	:	0.086	
Cadmium	:	0.029	
Mercury	:	< 0.010	

➤ PHYCO'DERM® offers good balanced mineral composition for maintaining the skin healthy.

Storage

PHYCO'DERM® should be stored in the original sealed drums, under clean conditions between 15 to 25°C.
In order to avoid microbial secondary contamination, it is recommended to use the whole content of the drum once opened.
If stored under the recommended conditions, PHYCO'DERM® remains stable for at least 18 months.

Pack size: 1kg - 5 kg - 10 kg.

Safety

No animal experimentation

Standard safety testing proves that PHYCO'DERM® is safe for cosmetic use.

- Ocular irritation : practically non irritant (Het Cam test)
- Cutaneous irritation : very good skin compatibility (Human patch test).

diluted at 10% in water.

cf. annex pp. 42-43.

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EFFECTIVENESS EVALUATION

PHYCO'DERM

Takes care of the delicate area around the eyes

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

Up regulation of the expression of genes related to multiple defense systems

- Genes involved in the anti-microbial defense & associated inflammation → Protects against microbial infections & related inflammations
- Genes involved in the anti-oxidant defense → Prevents the oxidative stress Regulates cellular redox state
- Genes involved in the detoxification systems → Strengthens the detoxification systems

PHYCO'DERM® improves dermis properties for reversing skin ageing

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

- Genes involved in cell adhesion
- Genes involved in the hyaluronan synthesis
- Genes involved in MMPs action

→ Preserves efficient extracellular matrix (ECM) maintenance

- Effects of PHYCO'DERM® on fibroblasts → Stimulation of human fibroblasts proliferation → Activates metabolism for promoting neosynthesis of ECM components
- Effects of PHYCO'DERM® on Type I collagen synthesis → Increase of Type I collagen synthesis → Counteracts the thinning of the periocular skin and tone eyelids

Clinical studies & subjective evaluation

- PHYCO'DERM® attenuates fine lines & wrinkles of crow's feet → Skin profilometry by means 3D LEFEVIZ MicroTM → Brings smoothing effect in the periocular area
- PHYCO'DERM® minimizes the appearance of dark circles under the eye → Color by colorimetry → Erases the signs of fatigue
- PHYCO'DERM® reduces the under-eye bags → Evaluation of volume by using Primos 3D technology → Alleviates puffiness of the skin

PHYCO'DERM® improves the aesthetic appearance of the periocular area for maintaining youthful and refreshed appearance

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For demonstrating the different ways of action of PHYCO'DERM®, we have used different methods, specially transcriptomic analysis (collaboration Strati CELL-Belgium), other *in vitro* studies (collaboration SEPhRA PHARMA-France) and clinical observations (collaboration FARCODERM-Italy).

Here after, for a better understanding of the results, we are going to detail the transcriptomic analysis.

The other methods will be explained at the top of the relative paragraphs.

Transcriptomic study

The transcriptomic analysis demonstrates the action of PHYCO'DERM® on the gene expression of several important markers.

Collaboration: Strati CELL–Belgium.

Method

The transcriptomic used method was conducted with Affymetrix DNA microarrays that allows to identify and characterize a large number of genes/pathways that are up-regulated or down-regulated in specific conditions.

The study was performed on normal dermal fibroblasts NHDFs cultured in DMEM medium without any serum. The tested dose of PHYCO'DERM® has been 5% (no cytotoxic dose) applied for 24 h (n=3).

Data were analysed through the "StratiCell Skin Knowledge database".

Results

Results concerning the implication of PHYCO'DERM® in different pathways are explained here after.

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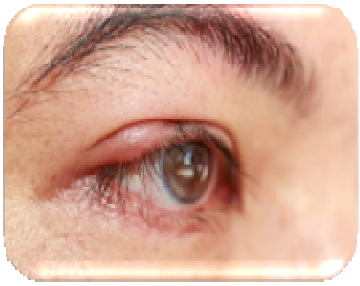
PHYCO'DERM® stimulates major defense systems for increasing resistance against environmental stressors

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 rays and various pollutants) and also of physiological factors (*e.g.* fatigue, stress).



The look of the periorbital area can reveal signs of irritation.

Therefore, it requires particular care.

Skin cells respond to these various stressors through a wide range of stress response pathways acting at the cellular level.

Up- regulation of the expression of genes related to multiple defense systems

The goal of such responses is to help cells to defend against and to recover from these insults.

As it exists many kinds of stress, cell responses will depend on the type and level of the insult. Consequently, different defense mechanisms may be activated.

➤ PHYCO'DERM® is able to regulate several defense systems linked to the anti-microbial defense, the anti-oxidant defense, the HSP response and detoxification systems.

Genes involved in the microbial defense & associated inflammation

The skin around the eyes is in direct contact with the external environment. Thus it is continuously exposed to large numbers of microorganisms. It is very reactive due to its richness in inflammatory cells (mast cells) which represent a site subject to allergy reactions.

To cope with the substantial microbial exposure, skin cells react by the synthesis, expression and release of various peptides and proteins that directly can kill or inhibit the growth of microorganisms.

➤ PHYCO'DERM® is able to up-regulate the gene expression of both chemokines and toll-like receptors involved in protective immune defense strategies.

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Action on the gene expression of chemokines

Chemokines are a class of cytokines acting as secondary inflammatory mediators induced by numerous cells *e.g.* keratinocytes and fibroblasts after different signals (*e.g.* growth factors, bacterial infections, inflammatory mediators such as IL-1 and TNFα) (*cf.* in Graves D.T. 1999 - Clin. Infect. Dis. 28 (3): 482-490).

These small proteins provide an essential first-line defense against invading microbes. Some chemokines are considered pro-inflammatory, and their release can be induced during an immune response at a site of infection, while others are considered homeostatic and are involved in controlling of cells migration during tissue development or maintenance.

There are two families of chemokines based on the first cysteine residue. Chemokines with two consecutive cysteines are referred to as members of the CC subfamily (CCL1-28), whereas chemokines with two cysteines separated by one amino acid are referred to as members of the CXC subfamily (CXCL1-17).

As a general rule, CC chemokines are chemotactic for monocytes and a small subset of lymphocytes whereas CXC chemokines are chemotactic for neutrophils. However both CC and CXC chemokines have the capacity to stimulate migration in other cell types besides monocytes and neutrophils respectively (Graves D.T. 1999 - Clinical Infections Diseases 28: 482-490).

Chemokines are also grouped into two main functional subfamilies: inflammatory and homeostatic chemokines. Inflammatory chemokines control the recruitment of leukocytes in inflammation and tissue injury, whereas homeostatic chemokines fulfill housekeeping functions such as navigating leukocytes to and within secondary lymphoid organs as well as in the bone marrow and the thymus during hematopoiesis (Wagner W. *et al*. 2007- Stem Cells.25: 2638–2647).

➤ PHYCO'DERM® is capable to overexpress the gene expression of 3 chemokines: CCL2 -CX CL1 and CCL11 highly involved in the inflammatory response.

Gene names	Fold change	P-value
CCL2 / MCP1 / MCP-1 / MCAF / SMC-CF / GDCF-2 / HC11 / MGC9434 chemokine (C-C motif) ligand 2 / monocyte chemotactic protein 1, homologous to mouse Sig-je / monocyte chemoattractant protein-1 / monocyte chemotactic and activating factor / monocyte secretory protein JE / small inducible cytokine subfamily A (Cys-Cys), member 2	3,13	2,40E-06
CXCL1 / SCYB1 / GROa / MGSA-a / NAP-3 chemokine (C-X-C motif) ligand 1 (melanoma growth stimulating activity, alpha)	2,62	2,60E-05
CCL11 / eotaxin / MGC22554 chemokine (C-C motif) ligand 11 / eotaxin-1	2,34	3,80E-02
CXCL2 / SCYB2 / GROb / MIP-2a / MGSA-b / CINC-2a chemokine (C-X-C motif) ligand 2	1.23	6,60E-04

CCL2 is important in coordinating the immune response following bacterial infection by regulation T-Cell polarization as well as leukocyte migration (Held K.S. *et al*. 2004- Virology 329: 251-260).The recruitment of monocytes/macrophages in response to inflammation is a vital response to eliminate invading pathogens through phagocytosis. (Deshmane S. *et al.* 2009- J. Interferon Cytokine Res. 29 (6): 313-326).

CXCL2 gene encodes a protein known to play a pivotal role in inflammation and as chemoattractant for neutrophils.

In response to the presence of allergens, the chemokine CCL11 (eotaxin) directly promotes the accumulation of eosinophils, a prominent feature of allergic inflammatory reactions.

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Action on the gene expression of Toll-like receptors

Toll-like receptors represent a family of pattern recognition receptors (PRRs) that recognize distinct, conserved microbial components and permit cells to recognize self from nonself in immune activation (L. S. Miller -2008 -"*Advances in Dermatology* 24: 71–87; Netea M.G. *et al*. – 2004 *Journal of Leukocyte Biology*, 75 (5): 749–755). Different TLRs are associated with an array of skin diseases. TLR agonists and antagonists have great potential for the treatment of allergic and inflammatory diseases (*cf*. in Hari A. *et al.* 2010 – ID 437 246 – doi 10.1155/2010/437246).

➤ PHYCO'DERM® is capable to overexpress the gene expression of two toll-like receptors: TLR6 and TLR 3.

Gene names	Fold change	P-value
TLR6 / CD286 toll-like receptor 6	1,79	7,90E-04
TLR3 / CD283 toll-like receptor 3	1,38	3,40E-02

TLR 6 is known to be highly involved in inflammation and functional responses of zinc oxide nanoparticles *via* several pathways (Roy R. *et al.* 2014- Immunology 142 (3): 453-464).

The protein encoded by TLR3 gene plays a fundamental role in pathogen recognition and activation of innate immunity.

➤ The combined activation of genes related to CC and CXC chemokines and to toll-like receptors induced by PHYCO'DERM® may induce complementary effects for enhancing natural adaptive immune responses in the skin.

Protection against inflammation

Skin irritation is an important consideration for periocular skin care.

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.

➤ PHYCO'DERM® is able to influence the gene expression of major negative regulators of inflammatory process: TNFAIP3, NFKBIA and BIRC3.

Gene names	Fold change	P-value
TNFAIP3 / A20 / OTUD7C tumor necrosis factor, alpha-induced protein 3	1.31	3,80E-02
NFKBIA / IKBA / MAD-3 / kappa Balp nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	1.32	1,80E-02
BIRC3 / cIAP2 / hiap-1 / MIHC / RNF49 / MALT2 / c-IAP2 baculoviral IAP repeat containing 3 / apoptosis inhibitor 2 / TNFR2-TRAF signaling complex protein / mammalian IAP homolog C / inhibitor of apoptosis protein 1	1.42	1,70E-02

The TNFAIP3 gene (also known as ubiquitin-editing enzyme A 20) encodes an important immune regulator that has functions in the negative regulation of NF-kappa B signalling in response to multiple stimuli (Vereecke L. *et al.* 2009- Trends Immunol. 30 (8): 383-391).

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The NF-kappa B family of transcription factors plays a key role in controlling inflammatory and immune responses. Its activation can proceed by two distinct signalling cascades. Canonical NF-kappa B signalling is induced in responses to pro-inflammatory cytokines and microbial infection and induces the expression of mainly anti-inflammatory and survival genes whereas non-canonical NK-kappa B signalling is initiated by several receptors and mainly regulated by adaptive immune responses (*cf.* in Vereecke L. *et al.* 2011- Biochem Soc. Trans 39: 1086-1091).

In addition to the TNFAIP3 gene, two other negative regulatory genes are upregulated by PHYCO'DERM®: NFKBIA and BIRC3.

NFKBIA is one of the most important negative regulators of NF- kappa B signaling in that it interacts directly with the NF-kappa B transcription factor subunits, p65/p50 heterodimers, sequestering them in the cytoplasm and preventing their translocation to the nucleus.

The BIRC3 (cIAP2) gene is an inhibitor of apoptosis and whose expression in epithelial cells is likely to protect against cytokine-induced apoptosis (Yan F. *et al*. 2011. J. Clin. Invest. 121: 2242–2253).

➤ In addition to the combined action of chemokines and toll-like receptors, PHYCO'DERM® may regulate inflammatory response related to the transcription NF-kappa signalling also known as a crucial player in immunity processes.

Therefore, by increasing the production of anti-microbial peptides PHYCO'DERM® is also able to 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.

Genes involved in the anti-oxidant defense

Oxidative stress is the main common cause of ageing. It leads to the formation of free radicals that can react with a large number of potential target molecules in the skin (*e.g*. DNA, proteins and lipids). It follows a wide spectrum of responses depending upon damage severity, dose magnitude and exposure duration.

In defense against oxidative stress, the skin engages all of distinct mechanisms: reparation, prevention, stabilization and antioxidant defense where enzymes and scavengers react directly with ROS, preventing them from reaching their biological target (Kohen R. 1999 - Biomed. Pharmacother. 53: 181-192; Shindo Y. *et al.* 1993- J. Invest. Dermatol. 100: 260-265).

Oxidative stress activates numerous major signalling pathways resulting in changes in gene expression which influence cell survival.

One of the most important defense regulators against oxidative stress is the transcription factor nuclear factor-erythroid –derived 2-related factor 2 (Nrf 2). Genes regulated by Nrf 2 encode proteins that help to protect cells against oxidative damage and control cellular redox state.

These proteins include

- several enzymes *e.g*. NQO1, GCLM,
- other antioxidant proteins *e.g*. HSP proteins, thioredoxins, glutaredoxins and sulfiredoxins systems,

(*cf* in Lieder F. *et al.* 2012- J. Biol. Chem. 287: 33001-33013).

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Action linked to several enzymes & HSP proteins

➤ PHYCO'DERM® over expresses the gene expression of NQO1, GCLM, SLC7A11, G6PD and HSPA2 highly involved in the antioxidant response.

Gene names	Fold change	P-value
NQO1 / DHQU / QR1 / DTD NAD(P)H dehydrogenase, quinone 1	1,59	1,90E-04
GCLM glutamate-cysteine ligase, modifier subunit / gamma-glutamylcysteine synthetase	2,21	2,80E-05
SLC7A11 / xCT solute carrier family 7 (anionic amino acid transporter light chain, xc- system), member 11	1,83	4,90E-05
G6PD / G6PD1 glucose-6-phosphate dehydrogenase	1,33	1,90E-03
HSPA2 heat shock 70kDa protein 2	1,37	6,70E-03

NQO1 plays major antioxidant role, probably *via* the reduction of endogenous quinones which subsequently help protect cellular membranes against oxidative damage. It is thought to help preserve certain endogenous antioxidants in their reduced and active forms.

In addition to its catalytic role related to quinones reduction, NQO1 has been found to scavenge superoxide directly, this activity providing efficient protection. (*cf.* in Atia A. *et al.* 2014 - J. Appl. Pharm Sc. 4 (12): 118-122).

The first and the rate-limiting step in the synthesis of GSH is carried out by glutamate-cysteine ligase (GCLM) (Dringen R. 2000 - Progress in Neurobiology 62 (6): 649-671).

GSH (tripeptide glutathione -γ-glutamyl-cysteinyl-glycine) is one of the most abundant cellular thiols. It plays diverse functions including detoxification, antioxidant defense, thiol status maintenance and cell proliferation modulation (Lu S.C. 2009- Mol. Aspects Med. 30 (1-2): 42-59).

Concerning cellular defense against reactive oxygen species, it is known to scavenge both singlet oxygen and hydroxyl radical. It is used by glutathione peroxidases and glutathione transferases to limit the level of certain reactive aldehydes and peroxides within the cell (Dringen R. 2000 - Progress in Neurobiology 62 (6): 649-671; Franco R. *et al. 2*007- Arch. Physiol. Biochem. 113 (4-5): 234-258). GSH displays as key determinant of redox signalling, vital in detoxification of xenobiotics and regulation of cell proliferation, apoptosis and immune function (Lu S.C. 2013- Biochim. Biophys. Acta 1830 (5): 3143-3153).

SLC7A11 encodes a subunit of the xCT cystine /glutamate amino-acid transport system. It plays a critical role in glutathione generation and cellular protection from oxidative stress (Martin L. & L.B. Gardner 2015- Oncogene 34: 4211-4218).

The increase of the expression of SLC7A11 gene is directly associated to the regulation of cellular levels of glutathione and antioxidant protection (Kabayashi S. *et al*. 2012- Free Radic. Biol. Med. 53 (2): 197-203).

Glucose-6-phosphate dehydrogenase (G6PD) appears as the first and rate-limiting enzyme of the pentose phosphate pathway, indispensable to the maintenance of the cytosolic pool of NADPH and consequently to cellular redox balance (Ho HY *et al*. 2007 – Redox Rep. 12 (3): 109-118).

G6PD converts α-D-glucose-6-phospate into D-glucono-1,5 -lactone-6-phosphate. It is involved in the generation of NADPH required for the generation of reduced glutathione, important against oxidative damage protection (Efferth T. *et al*. 2006 - Cell Death & Differentiation 13: 527-528).

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HSP 70 proteins are central components of cellular network of molecular chaperones. They assist a large variety of protein folding processes in the cell by transient association of their binding domain with short hydrophobic peptide segments within their substrate proteins (*cf.* in Mayer M.P. & B. Bukau 2005 – CMLS Cell. Mol. Life Sci. 62: 670-684).

Up-regulation in heat shock protein (HSP) expression, particularly HSP 70, have been shown to enhance cells survival and prevent apoptosis during a wide variety of stress conditions (Creagh *et al*. 2000- Leukemia 14: 1161–1173) including direct oxidative damage (*i.e*., hydrogen peroxide or hypoxia reperfusion injury) as well as a variety of other stresses in which generation of ROS is implicated in cytotoxicity (*i.e.,* chemotherapeutic agents, heat stress, cytokines) (Creagh and Cotter, 1999 – Immunology 97: 36–44.; Ding and Keller, 2001- J. Neurochem. 77: 1010–1017).

In addition, elevated HSP expression not only improves cell survival, but also reduces oxidative damage to proteins, DNA, and lipids (Su *et al*.1999 – Biochem. Biophys. Res. Commun. 265: 279–284.; Yamamoto *et al.* 2000 Res. Exp. Med. (Berl.) 199: 309–318, Martindale J.L. & N.J. Holbrook 2002- J. Cell Physiol. 192: 1-15).

Action linked to redox sensitive proteins

Redox reactions are essential requirements for cell metabolism most notably in the form of biological energy transduction in inner mitochondrial membranes.

Many key regulators of redox signalling are members of several proteins families such as thioredoxin (TRX), glutaredoxin (GLRX), sulfiredoxin (SRX) playing as pivotal players both as transducers and regulators of second-messenger levels (Hanschmann E.M. *et al.* 2013- Antioxidants & Redox Signaling 19 (13): 1539-1605).

In the proteins, all amino acids may be oxidized, specially sulphurous amino acids (cysteine and methionine) and aromatic amino acids (tryptophane and tyrosine) being the most sensitive.

Cysteine oxidation is influenced by enzyme repair systems such as thioredoxin and glutaredoxin systems. These systems control cellular redox potential, keeping a reducing thiol-rich intracellular state, which on generation of reactive oxygen species signals through thiol redox control mechanisms (Holmgren A. *et al.* 2005- Biochem. Soc. Trans. 33: 1375-1377).

➤ PHYCO'DERM® is also able to stimulate the gene expression of the redox sensitive proteins *e.g*. thioredoxin, sulfiredoxin and glutaredoxin systems.

Gene names	Fold change	P-value
TXNRD1 / TXNR / GRIM-12 / Trxr1 thioredoxin reductase 1	1,36	1,90E-03
SRXN1 / Npn3 / SRX1 / YKL086W / dJ850E9.2 sulfiredoxin 1	1,47	2,90E-03
SRXN1 / Npn3 / SRX1 / YKL086W / dJ850E9.2 sulfiredoxin 1	1,36	4,80E-03
GLRX / GRX / GRX1 glutaredoxin (thioltransferase)	1,23	1,10E-02

The thioredoxin system is composed of NADPH, thioredoxin reductase and thioredoxin.

It is a pivotal key antioxidant system in defense against oxidative stress through its disulfide reductase activity regulating protein dithiol/disulfide balance.

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The Thioredoxin system provides electrons to thiol-dependent peroxidases (peroxiredoxins) to remove reactive oxygen and nitrogen species with fast reaction rate.

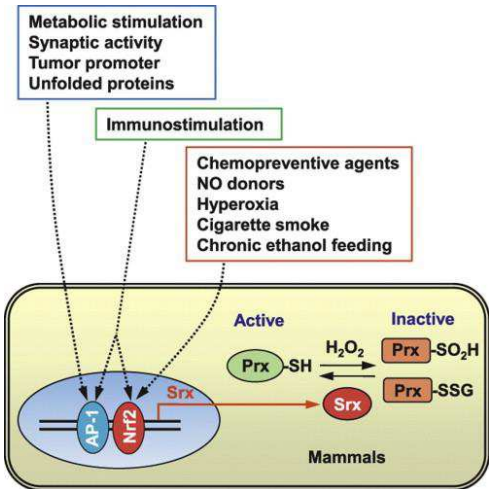
Thioredoxin antioxidant functions are also involved in DNA and protein repair *via* the reduction of ribonucleotide reductase and methionine sulfoxide reductases and in the regulation of many redox-sensitive transcription factors.

Moreover, thioredoxin systems play critical roles in the immune response, virus infection, and cell death through interactions with thioredoxin-interacting protein (Lu J. & A. Holmgren 2014 - Free Radic. Biol. Med. 66: 75-87).

The isoform 1 of TRX system is known to be under the control of Nrf 2 factor, playing pivotal role in anti-oxidant defense (Lee S. *et al.* 2013 - Antioxid. Redox Signal. 18 (10): 1165-1207).

Besides the TRX system, it exists the peroxiredoxin/sulfirodoxin system (SRX).

It is well known that the induction of the SRXN genes are under the control of the transcription factor AP-1 and Nrf 2 (Jeong W. *et al.* 2012 - Free Radic. Biol. Med. 53 (3): 447-456).



Glutaredoxins (GRX) like thioredoxins, are involved as alternative pathways in cellular functions such as formation of deoxyribonucleotides for DNA synthesis (by reducing the essential enzyme ribonucleotide reductase), the generation of reduced sulfur (*via* 3'-phosphoadenylylsulfate reductase), signal transduction, and oxidative stress defense.

Glutaredoxins also catalyze the formation of mixed disulfides (glutathionylation), which is an important redox regulatory mechanism, particularly in mammalian cells under oxidative stress conditions, to sense cellular redox potential (Fernandes A. P. & A. Holmgren 2004 - Antioxid. Redox Signal 6(1): 63-74).

➤ PHYCO'DERM® is also able to influence the Nrf 2 factor known as a master regulator of cellular antioxidant systems *via* the activation of several pathways in order to increase cytoprotective protection against oxidative stress.

➤ PHYCO'DERM® also helps regulate cellular redox state by acting through several signalling and transcriptional processes in cells from the detrimental effects of reactive oxygen species.

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Genes involved in detoxification systems

Major air pollutants including solar ultraviolet radiation affects the skin, specially the periocular area skin.

Depending of the nature of these pollutants and skin integrity, their modes of penetration differ. However their effects may contribute to skin ageing, atopic dermatitis and skin cancer.

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

➤ PHYCO'DERM® is able to over express genes involved in protective detoxification mechanisms mediated by specific proteins named metallothioneins and also by several enzymatic pathways specially the cytochromes P 450 (CYP) and glutathione -S- transferase (GST).

Metallothioneins (MT) are ubiquitous, cysteine-rich metal binding proteins. They are known to play pivotal role in several fundamental processes like detoxification of heavy metals (*e.i.* mercury and cadmium), homeostasis of essential metals (*e.i.* copper and zinc), antioxidation against reactive oxygen species, protection against DNA damage, oxidative stress and cell survival (Higashimoto Minoru *et al.* 2009 - Life Sciences. 84: 569-575. 10.1016/j.lfs.2009.01.022.; Takahashi S. 2012 - J. Hematology & Oncology 5: 41; Ruttkay-Nedecky B. *et al.* 2013 - Int J. Mol. Sci. 14: 6044-60--; Ottaviani M.M. 2014 - Metallothioneins - Univ. Torino).

The metallothioneins (MT) appear to act against large spectrum of free radical species, with the ability to scavenge superoxide, as well as hydroxyl radical, nitric oxide and peroxynitrite (Nielsen *et al.* 2007 - Biomark Insights.1: 99–111). MT might also influence cellular respiration and thus have unique dual influence on both the metabolic generation of free radicals as well as their detoxification (Ye *et al.* 2001 – Proc. Natl. Acad. Sci. USA. 98: 2317–22).

They play in normal cellular homeostasis and cellular responses to stressors. In addition to their important immunomodulatory functions, they can protect essential cellular compartments from toxicants, serve as a reservoir of essential heavy metals, and regulate cellular redox potential (Lynes M.A. *et al.* 2014 - Cell Stress & Chaperonnes DOI 10.1007/s 12192-014-0501-z).

➤ PHYCO'DERM® is able to over-express gene expression of three isoforms of the MT1

Gene names	Fold change	P-value
MT1G / MT1K metallothionein 1G / metallothionein 1K	13,85	2,60E-06
MT1H metallothionein 1H	5,34	1,40E-03
MT1F metallothionein 1F	1,94	2,40E-04

These MT1 isoforms, known to regulate copper and zinc, are involved in cell transcription and heavy metals detoxification.

Recent studies paid attention to the possible role of MTs within the concept of ageing, especially in pro-longevity interventions. Consequently MTs are going to become an attractive target from the standpoint of ageing research (Yang *et al*. 2006 - FASEB J. 20: 1024–6; Bahadorani *et al.*2010 – Neurobiol. Aging. 31: 1215-26; Sato *et al*. 2010 - FASEB J. 24: 2375–84; Swindel W.R. 2011 – Ageing Res. Rev. 10 (1): 132-145).

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It is also proved that MTs expression decreases with old ageing. This phenomenon can be at least partially attributed to diminished cell proliferation. The modulation of MT may be beneficial for restoring immune function at old age (Malavolta M. *et al.* 2007- Rejuvenation Research 11(2): 455-459).

Other studies concerns the role of metallothioneins against photoprotection. This role has been suggested by Hanada K. and collaborators as early as 1991 demonstrating that increasing MT synthesis suppressed UVB-mediated sunburn cell formation in mouse skin (1991 - Photodermatol. Photoimmunol. Photomed.8: 111-115.) confirmed some years after (Hanada K. *et al.* 1998 - J. Invest. Dermatol. 110:259-262). The mediators of cutaneous MT induction by UV radiation would be interleukin-6 (Nishimura N. *et al.* 2000- J. Invest. Dermatol. 114: 343-348). The decrease in sun-exposed skin of the expression of MT-1 and MT-2 seems to be associated to the decrease of keratinocyte proliferation (Ma C. *et al.* 2011 – Br. J. Dermatol. 164 (3): 479-482).

➤ By increasing the gene expression of metallothioneins (MTs), PHYCO'DERM® comforts its pivotal role at once in the detoxification process related to heavy metals, UV radiation and free radical induced-damage and in the restoration of immune function during skin ageing.

It exists other detoxification systems.

➤ PHYCO'DERM® is also able to over-express gene expression of enzymatic pathways related to detoxification mechanisms.

Gene names	Fold change	P-value
CYP1B1 / CP1B cytochrome P450, family 1, subfamily B, polypeptide 1	1,74	3,80E-04
GSTM5 glutathione S-transferase mu 5	1,66	3,60E-04

The cytochromes P 450 belong to a multigene family that code for an array of enzymes catalyzing the mixed function oxidation of large varieties of endogenous compounds and environmental pollutants. Therefore they are referred to as detoxification enzymes.

CYP 1B1 is involved in the metabolic activation of many environmental pollutants. Especially this gene plays a major role in the metabolism of polycyclic aromatic hydrocarbures present in the atmosphere pollution (Ahmad & H. Mukhtar 2004 – J. Invest. Dermatol. 123 (3): 417-425).

Glutathione-S-transferase (GST) plays an important role in cellular defense against electrophilic chemical species and radical oxygen species.

GST might contribute to the protection against oxidative stress by direct inactivation of peroxides lipids or DNA (Tan K.H. *et al.* 1988- Biochem. J. 254 ; 841-845 ; Ketterer B. & D J. Meyer 1989 – Mutat. Res. 214 : 33-40 ; Berhane K. *et al.* 1994- Proc. Natl. Acad. Sc. USA 91: 1480-1484).

➤ By activating the expression of important genes involved in pivotal pathways of defense systems, PHYCO'DERM® increases detoxification process of skin cells and therefore puts up skin resistance of the periocular area against various harmful external stressors.

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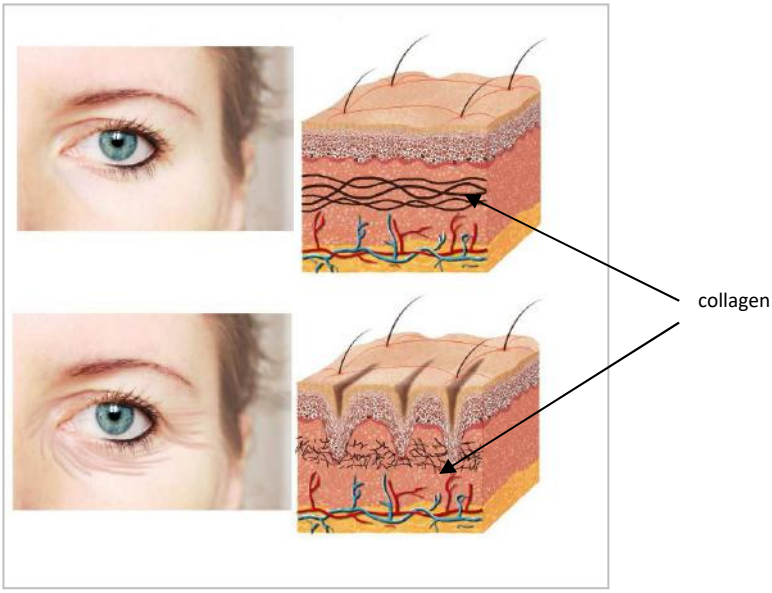
PHYCO'DERM® improves dermis properties for reversing skin ageing

The periocular area exhibits the most fragile skin on the entire face and reveals the passage of time.

Therefore it appears prematurely fine lines and trace of fatigue due specially to the breakdown of collagen.



So it is important to use a specific treatment in order to reduce fine lines and wrinkles around the eyes.

The dermis is a connective tissue consisting of various cell types of which fibroblasts are the majority cells. However a substantial part of the tissue volume is occupied by a complex network referred to as the extracellular matrix (ECM).

The extracellular matrix (ECM) is composed by a wide variety of different macromolecules which carry distinct domains with defined structural and/or biological activities. Cells are known to interact with these molecules *via* specific receptors.

Cell-matrix interactions, not only control the shape and orientation of cells but can also directly regulate cellular functions, including migration, differentiation, proliferation, and the expression of different genes. They act in several biological processes, especially morphogenesis and differentiation, but also play an important role during pathological situations such as wound healing. (*cf.* in Eckes B. *et al.* 1999 - Springer Semin. Immunopathol. 21 (4): 415-429).

When we age, the early signs of skin ageing include the first stages of visible fine lines, especially around the eyes and the beginning of uneven skin tone.

In fact skin variations result from the modifications of cell functions and progressive changes of the extracellular matrix structure.

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It happens:

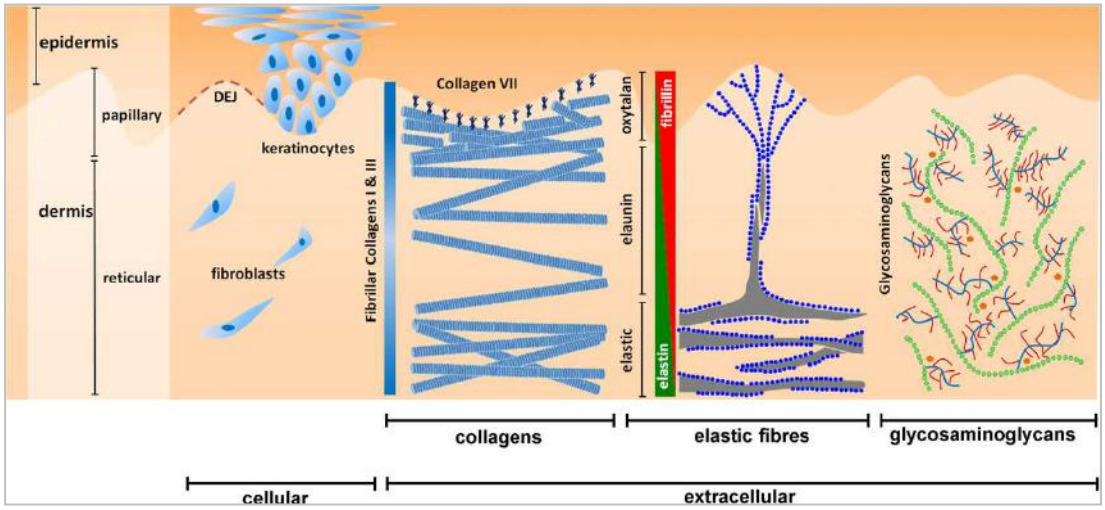
- decline in the number of fibroblasts that can induce general atrophy of the extracellular matrix,
- decrease in macromolecules organization, especially collagens and elastin.

Biochemically, the components of the ECM can be divided into three major classes of biomolecules:

1. structural proteins: *e.g*. collagens, fibrillins, and elastin,
2. specialized proteins: *e.g*. fibronectin, laminins and integrins,
3. proteoglycans: these are composed of a protein core to which is attached long chains of repeating disaccharide units termed of glycosaminoglycans (GAGs) forming extremely complex high molecular weight components of the ECM.

Each of which has diverse subcategories of components with varying physical and biochemical properties.

Some of the ECM proteins (*e.g*. fibrillar collagens and elastin) form fibrils from protein monomers and contribute to major tensile strength and viscoelasticity of the tissue. Other proteins (*e.g*. fibronectin, laminin) also participate in building the matrix network as connectors or linking proteins (Vakonakis I. & I.D. Campbell 2007 – Curr. Opin. Cell Biol. 19: 578–583; Daley W.P. *et al*. 2008 – J. Cell Sci. 121: 255–264).



From Naylor E.C. *et al*. 2011 – Maturitas 69: 249-256.

In fact, the ECM performs many functions in addition to its structural role, displaying both direct and indirect signalling properties, since it can act directly by binding cell surface receptors or by non-canonical growth factor presentation (Hynes R.O. 2009 -Science 326: 1216–1219).

Importantly, all these characteristics and properties are strongly interconnected and one can influence the others. This becomes even more evident when considering that cell–ECM connection is a reciprocal interaction in which cells continually remodel the ECM present in their microenvironment, and these dynamic modifications of the ECM direct cell behavior.

Therefore it is not surprising that alterations in a specific ECM component can have a remarkable impact on the biochemical, biomechanical and physical properties of the ECM, leading to disorganized network (*cf.* in Gattazzo *et al.* 2014 - Biochimica et Biophysica Acta 1840 : 2506–2519).

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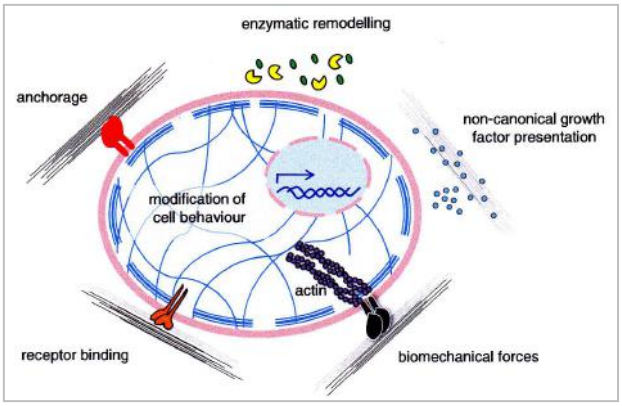
➤ PHYCO'DERM® is able to improve dermis properties by:

- modulating the expression of major genes involved in the morphological organization and physiological function,
- activating fibroblast proliferation and
- stimulating collagen synthesis while protecting it against degradation.

Modulation of the expression of genes related to the dynamic structure of the ECM

This pleiotropic aspect of ECM function depends on its highly dynamic structure and its remodelling as an effective mechanism whereby diverse cellular behaviors can be regulated.



The ECM can directly bind different types of cell surface receptors, thus mediating cell anchorage and regulating some pathways involved in intracellular signalling. Moreover the ECM can act by non-canonical growth factor and be pre-modeled by the action of enzymes (yellow pic) which can release functional fragments green).

From Gattazzo F. *et al.* 2014- Biochem. Biophysic. Acta 1840: 2506-2519.

This concept is particularly important when considering processes and cell behaviors that need to be deployed promptly and transiently and wherein cell–cell and cell–matrix interactions are constantly changing (Daley WP *et al*. 2008 - J Cell Sci. 121: 255–264).

➤ PHYCO'DERM® is capable to influence the dynamic behavior of the ECM.

Genes involved in cell adhesion

➤ PHYCO'DERM® up-regulates the expression of genes related to cell-matrix receptors, specially ITGB3 and SDC1.

Gene names	Fold change	P-value
ITGB3 / CD61 / GPIIIa integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) / platelet glycoprotein IIIa	1,66	5,40E-04
SDC1 / CD138 / syndecan / SYND1 syndecan 1 / syndecan proteoglycan 1	1,25	2,60E-02

Integrin beta-3 is a member of the Integrin receptor family. The integrin family has been shown to be instrumental in cell adhesion to extracellular matrix proteins and can be found in certain cell to cell adhesions. Integrins are also important in transmembrane connections and intracellular signaling

Syndecan-1 (SDC1) is an integral transmembrane (Type I) heparan sulfate proteoglycan. It participates in cell proliferation, cell migration and cell-matrix interactions *via* its receptor for extracellular matrix proteins.

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It plays important roles in several mediating key events because it regulates a number of important processes, including cell adhesion, cell migration and endocytosis (Bauvais D. *et al*. 2009- J. Exp. Med. 206 (3): 691-705).

Especially cell adhesion is regulated by up-expression of syndecan 1.

➤ PHYCO'DERM® activates the gene expression of major membrane receptors, therefore PHYCO'DERM® helps increase cell adhesion to the extracellular matrix proteins.

Genes involved in hyaluronan synthesis

➤ PHYCO'DERM® up-regulates the expression of genes CD 44 – HAS2 and HAS3.

Hyaluronic acid (also called hyaluronan) is unique among the GAGs in that it does not contain any sulfate. It is not found covalently attached to proteins forming a proteoglycan. It regulates water balance, osmotic pressure and ion flow.

Gene names	Fold change	P-value
CD44 / IN / MC56 / Pgp1 / CD44R / HCELL / CSPG8 CD44 molecule (Indian blood group) / hematopoietic cell E- and L-selectin ligand / chondroitin sulfate proteoglycan 8	1,44	6,80E-03
HAS2 hyaluronan synthase 2	1,17	3,80E-02
HAS3 hyaluronan synthase 3	1,16	3,10E-02

The protein CD44 encoded by this gene is a receptor for hyaluronic acid (HA). It is a cell-surface glycoprotein involved in cell-cell interactions, cell adhesion and migration. It can also interact with other ligands, such as collagens, and matrix metalloproteinases (MMPs). This protein participates in a wide variety of cellular functions.

Hyaluronan synthesis is catalyzed by a family of hyaluronan synthases (HAS). There are three members of the mammalian HAS gene family, HAS1, HAS2, and HAS3.

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

➤ PHYCO'DERM® over expresses the expression of major genes involved in hyaluronan synthesis that help fight against skin ageing when occurs and prevent loss of moisture.

Genes involved in MMPs regulation

➤ PHYCO'DERM® is able to act on the gene expression of several MMPs: MMP1 – MMP11 – MMP 14.

Matrix metalloproteinases (MMPs) consist of several well defined groups based on their similarities in terms of structure and substrate specificity (Woessner J.F. 1991- Faseb J. 5: 2145).

According to their structural and functional characteristics, MMPs family members have been classified into different but closely related subgroups with fairly characteristic but often overlapping substrate specificities.

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This classification considers collagenases that degrade fibrillar collagens (MMP-1 or interstitial collagenase, MMP-8, or collagenase neutrophil MMP-13 or collagenase 3, MMP-18 or collagenase 4), gelatinases that degrade type IV collagen or any form of denatured collagen (MMP-2 or gelatinase A (72 kDa), MMP-9 or gelatinase B (92 kDa)), stromelysins (MMP-3 or stromelysin 1, MMP-10 or stromelysin 2, MMP-11 or stromelysin 3) whose broad spectrum of activity is directed to proteins of the extracellular matrix such as glycoproteins (fibronectin, laminin), proteoglycans, etc., matrilysin (MMP-7), the metalloelastase (MMP-12) or the membrane metalloproteases (MMP-14, MMP-15, MMP-16 and MMP-17).

MMPs are integrated in the network of multidirectional communication within tissues and cells as important regulators of cell proliferation and differentiation, tissue homeostasis, immune response and several other processes. Since uncontrolled MMP activity can easily become destructive and lead to breakdown of homeostasis (Löffek S. *et al.* 2011 – Eur. Resp. J. 38: 191-208).

Therefore, matrix metalloproteinases (MMPs) play crucial role in the maintenance of the normal balance between extracellular matrix (ECM) synthesis and degradation in tissues. They have predominant role in skin ageing.

➤ PHYCO'DERM® is able to down express genes related to MMP 1 - MMP14 and MMP 11.

MMP-1 is known to break down collagens, especially Type I and III (Krieg T. & M. Aumailley 2011 – Exp. Dermatol. 20 (8): 689-695).

MMP-14 (also known as MT1-MMP) seems to be a pivotal collagenase *in vivo*. Its expression in fibroblasts plays crucial role in collagen remodelling in adult skin and largely contributes to dermal homeostasis underlying its pathogenic role in fibrotic skin disease.

MMP-14 is also implicated in the degradation of Type I collagen and the activation of pro-MMP2 (Zigrino P. *et al.* 2016 - J. Invest. Dermatol. 136: 1575-1583).

Matrix metalloproteinase (MMP)-11, or Stromelysin 3, is a particular member of MMP family, a group of zinc-dependent endopeptidases involved in matrix degradation and tissue remodelling. However despite intense efforts since its first characterization 15 years ago, its role and target substrates in different diseases remain largely unknown (Matziari M. *et al*. 2007- Med. Res. Rev. 27 (4): 528-552).

Gene names	Fold change	P-value
MMP1 matrix metallopeptidase 1 (interstitial collagenase)	-1,76	7,40E-04
MMP14 / MT1-MMP matrix metallopeptidase 14 (membrane-inserted) / membrane type 1 metalloprotease	-1,60	1,70E-04
MMP11 matrix metallopeptidase 11 (stromelysin 3)	-1,83	2,10E-04

➤ By down regulating MMP1 and MMP14, PHYCO'DERM® helps avoid the Type I collagen degradation while regulating collagen homeostasis.

➤ By influencing the dynamic behaviour of pathways, PHYCO'DERM® helps the extracellular matrix direct essential morphological organization and physiological function in order to preserve an efficient maintenance.

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Stimulation of human fibroblasts proliferation

Fibroblasts play fundamental role in dermal metabolism.

Their primary function is the maintenance of structural integrity within connective tissue. They secrete extracellular matrix precursors required for the formation of connective tissue and various fibres.

Fibroblasts produce glycosaminoglycans, collagens, elastic fibers, reticular fibres and glycoproteins present in the extracellular matrix (ECM).

When we age, their number decreases that can promote general atrophy of the ECM and decrease in macromolecules organization.

So it is important to increase the biosynthetic capacity of fibroblasts in order to induce the enhancement of cell activity and therefore the synthesis of dermal molecules, especially collagens.

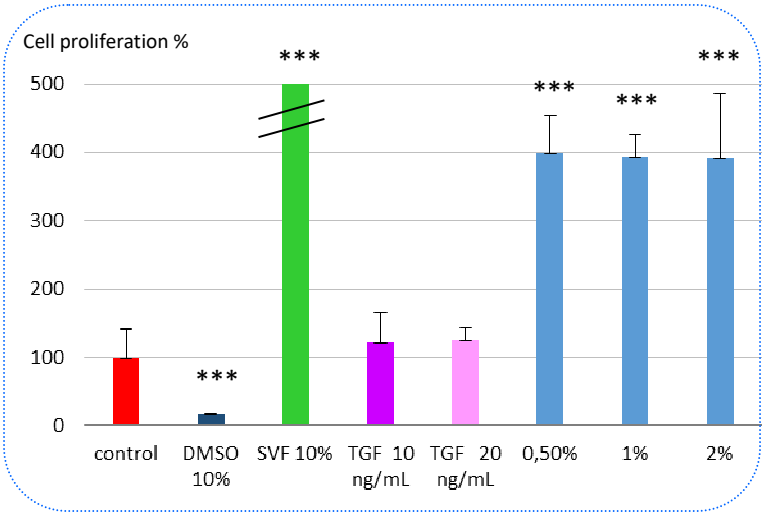
Collaboration: SEPhRA-PHARMA - France

Method

Normal human fibroblasts are cultured for 72 hours in a medium without any serum. Results have been validated with the addition of DMSO (10%) or foetal calf serum (10%) and TGF- β1 (10 and 20 ng/ml) as positive control. PHYCO'DERM® has been tested at 3 doses: 0.5%- 1% - 2% by using the BrdU assay. This assay is based on the detection of BrdU incorporated into the genomic DNA of proliferating cells. Thus monitoring DNA synthesis is an indirect parameter of cell proliferation, as well as being suitable for the study of the regulation of DNA synthesis.

Results

Results are illustrated here after.



With

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

versus control.

➤ PHYCO'DERM® maintains significantly active fibroblasts proliferation.

Therefore the cell metabolism is activated that promotes neosynthesis of extracellular matrix macromolecules.

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Increase of Type I collagen synthesis

Various collagens constitute major proteins of the extracellular matrix (about 70% of the dermal constituents).

Types I, II and III collagens are the most abundant and all three types form fibrils of similar structure.

Of these three major types of collagen, Type I is by far the most abundant, constituting nearly 85-90 % of all the collagen in the human body.

Collagens are predominantly synthesized by fibroblasts.

Firmness and resistance to pressure are dependent on dermal fibrous collagens.

Type I collagen homeostasis in tissues is finely regulated by a continuous balance of synthesis and degradation, the alteration of which leads to various pathologies such as scleroderma, keloid, hypertrophic scar formation, and skin degeneration during ageing (Ogawa R. & CK Hsu 2013 - J Cell Mol. Med. 17: 817-822.)

The major factor contributing to facial ageing is damage to and loss of dermal collagen. So it is important to stimulate collagen production.

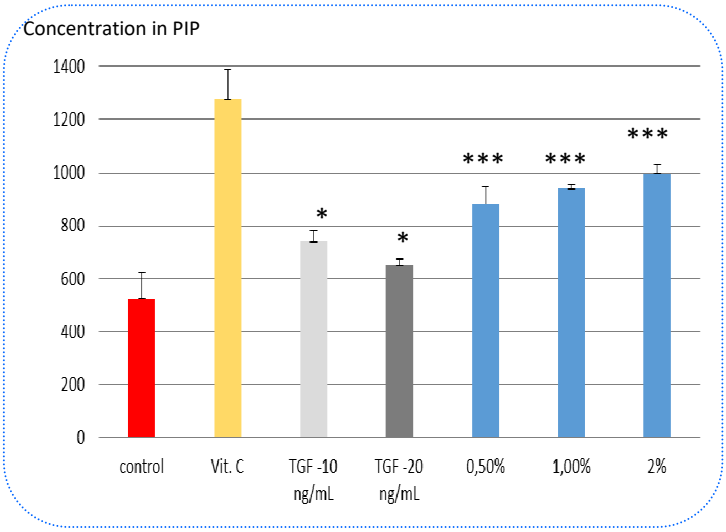
Collaboration: SEPhRA-PHARMA - France

Method

- Determination of cell viability by using the XTT test
- Evaluation of Type I pro collagen production by using ELISA test (Takara MK 101) after 48 h treatment with different doses of active (0.5% - 1% and 2%).
- Standards: vitamin C (dose: 50µg/mL) - TGF β-1 (doses: 10ng/mL and 20 ng/mL).

Results

Results are given in concentration of pro collagen I (PIP) concentration in ng/mL. and allows to calculate collagen production.



The collagen production equals

+ 52 % with TGF β1 -10 ng/mL

+ 32 % TGF β1 -20 ng/mL

+ 81 % 0.5 % PHYCO'DERM®

+ 92 % 1 % PHYCO'DERM®

+ 95 % 2 % PHYCO'DERM®

versus control.

➤ PHYCO'DERM® increases significantly Type I collagen synthesis that help:
- counteract the thinning which characterizes the skin around the eyes and
- tone eyelids.

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Clinical studies

Collaboration: FARCODERM-Italy

Method

Several clinical studies have been performed on two groups of women volunteers type Caucasian
-Panel of 30 subjects from 42 to 66 years old for the evaluation of a basic gel with 4% active
-Panel of 30 subjects from 37 to 71 years old for the evaluation of the placebo.

Formulation of the basic gel *(collaboration: Laboratoire CNB-France)*

Trade name	Supplier	INCI names	%
Demineralised water	-	Aqua (water)	QSP
Sepimax Zen	SEPPIC		1.50
Phenoethol	CLARIANT	Phenoxyethanol	0.90
Sensiva SC50	SHELCKE	Ethylhexylglycerin	0.10
Perfume	TECHNICOFLOR	Fragrance	0.30
PHYCO'DERM			4.00
NaOH 30%	UNIVAR	Aqua & sodium hydroxide	Qs
Citric acid 40%	UNIVAR	Aqua & citric acid	Qs

Treatments have been applied twice a day for a period of 28 days.

The instrumental evaluation of the parameters under study and digital pictures of the treated area have been performed after 28 days of product use. Moreover, volunteers filled a self-assessment questionnaire of the tested products.

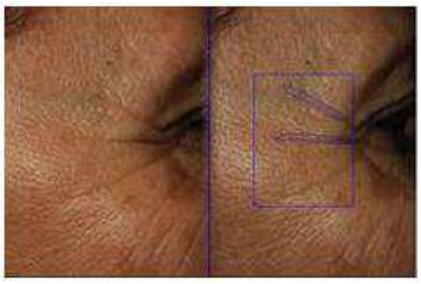
PHYCO'DERM® attenuates fine lines and wrinkles of crow's feet

The area around the eyes is typically susceptible to wrinkling caused in part by the natural slowdown of repair of the extracellular matrix.
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 also develop after excessive sun exposure, smoking, squinting, frowning and smiling.

Method

Evaluation of skin profilometry: wrinkle depth on volunteers

Skin surface is quantitatively assessed by LIFEVIZ MicroTM (QUANTIFICARE). LIFEVIZ MicroTM is a non-contact *in vivo* skin measurement device based on structured light projection. In conjunction with a comprehensive 3-D measurement and evaluation software, the sensor allows to evaluate skin surface properties, especially wrinkle depth.



The technique 3D LIFEVIZ MicroTM is an imaging CRO providing life-like and reproducible images. The technology includes dual beam pointers to measure the exact same distances from the patient, which ensure perfect image reproducibility and compatibility though time. It integrates a cross-polarized flash which guarantees the same light and image quality. A stereovision algorithm is applied to reconstruct and quantitatively analyze the skin surface in 3D.

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Before the study start at day 0 (T 0) and at the end of the study at day 28 (T 28) pictures of the treated area are acquired. Photographs are done using a digital professional reflex camera (NIKON D600 digital camera, Nikon Corporation Tokyo, Japan) equipped with macro objective (AF-S Micro NIKKOR 60mm f/1:2.8G ED, Nikon Corporation Tokyo, Japan) and with an autonomous flash system (WIRELESS REMOTE SPEEDLIGHT SB-R200, Nikon Corporation Tokyo, Japan). These images allow the evaluation of macro-effects due to the product use during the study.

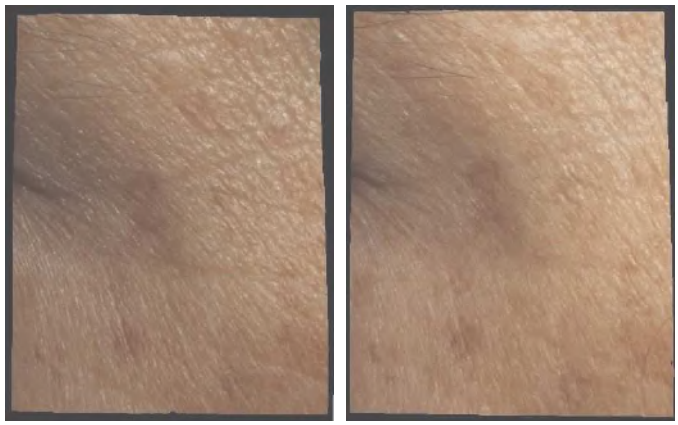
Results

The mean between day 0 (T0) and day 28 (T 28) are presented here after.

	Δ TO / T 28	Maximum Δ / T 28	Minimum Δ / T 28
Placebo	**1.5 %**	- 27.6 %	17.7 %
Gel 4 % PHYCO'DERM®	**- 6.1 %**	- 46.7 %	56.6 %

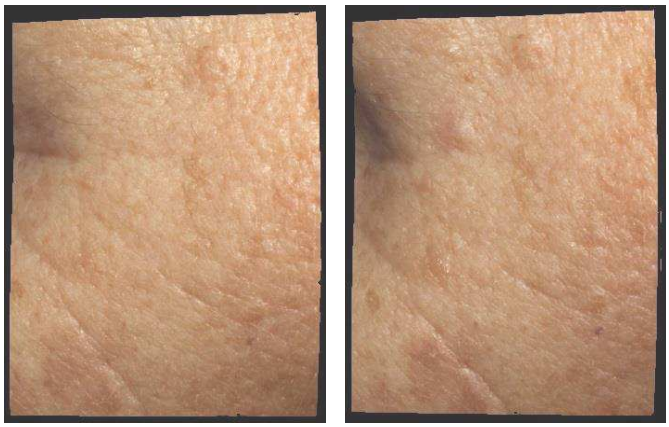
➤ PHYCO'DERM® in a basic gel at 4% is able to reduce wrinkles depth in comparison with the placebo gel after 28 days of treatment.

Volunteer 1



T 0 T 28

Volunteer 2



T 0 T 28

➤ PHYCO'DERM® helps erase fine lines and wrinkles of the crow's feet inducing smoothing effect in the periocular area.

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

Dark circles are changes in skin color around the eyes. They result from a combination of factors linked for example to UV exposure, ageing, superficial vascularity, hyperpigmentation, skin translucency, allergic reactions, fatigue and pregnancy or menstruation. They make people look tired, exhausted, unhealthy and older.

A complex superficial vascular network exists within the dermis of the skin around the eyes. When these tiny capillaries become visible they lend a reddish discoloration of the area. Hyperpigmentation may result from various conditions.

The skin of the lower eyelid is contiguous with that of the upper cheek area. With ageing, the mid-face descends due to the loss of volume and support of the lateral component of the orbitomalar ligament. With this descent, a tear through deformity is created and therefore a "dark circle".

Method

Measurement of under-eyes dark circles color

The measurement of under-eyes dark circles color is carried out by means of a spectrophotometer /colorimeter CM-700d (Konica Minolta). The instrument is able to evaluate the colour according to a standard method defined by the International Lighting Commission (CIE). CIELab is a standardized color space in which the color is defined - under standard illumination conditions (illuminant) and observer angle - by three colorimetric parameters called a*, b* and L*. a* and b* values define hue and color saturation and L* value is related to the color brightness.

The colorimetric parameters a* and b* (respectively red and blue component) that are related with the dark-circle intensity, are evaluated.

A decrease of a* and an increase of b* are related to an improvement in the dark circles appearance.

For more information about the process of measurement and data analysis see figures here after.

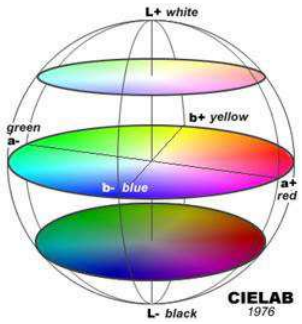

Figure a

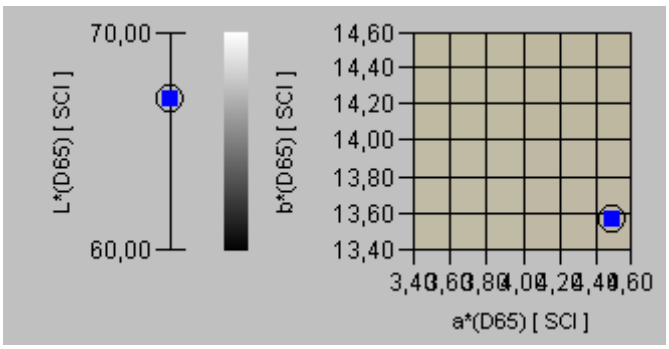

Figure b

The spectrophotometer CM-700d measures the skin color in the CIELab chromatic space (a). CIELab is a standardized color space in which the color is defined - under standard illumination conditions (illuminant) and observer angle - by the values called a* and b* that defines hue and color saturation and by the value called L* that defines the color brightness. The instrument, which principle of function is the reflectance spectrophotometry, emits an intense white light that is collected by 36 photodiodes each with different spectral sensitivity (from 400 nm to 700 nm) when it is re-emitted from the object (at an angle of 10 °). The sensitivity of the photodiodes is regulated according to a "standard observer" simulating the sensitivity of the human eye. This information is then elaborated by a microprocessor and graphically displayed as shown in Figure b).

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Results

The mean between day 0 (T0) and day 28 (T 28) for the both parameters are presented here after.

The parameter "a" concerns the green-red component.

Parameter « a »

	Δ T 0 / T 28	Maximum Δ / T 28	Minimum Δ / T 28
Placebo	**- 1.5 %**	- 21.4 %	14.4 %
Gel 4 % PHYCO'DERM®	**- 7.5 %**	- 16.8 %	4.0 %

When "a" decreases, redness decreases.

The parameter "b" concerns the blue-yellow component.

Parameter « b »

	Δ T 0 / T 28	Maximum Δ / T 28	Minimum Δ / T 28
Placebo	**0.2 %**	3.6 %	- 3.0 %
Gel 4 % PHYCO'DERM®	**3.0 %**	13.1 %	- 6.1 %

When "b" increases", dark circles are less blue.

➤ PHYCO'DERM® in a basic gel at 4 % decreases the intensity of coloration that diminishes the sensation of darkness under the eyes.

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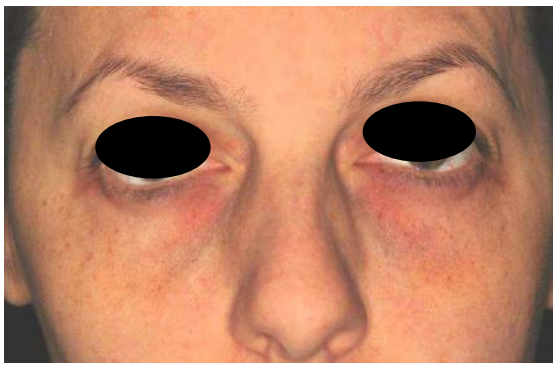
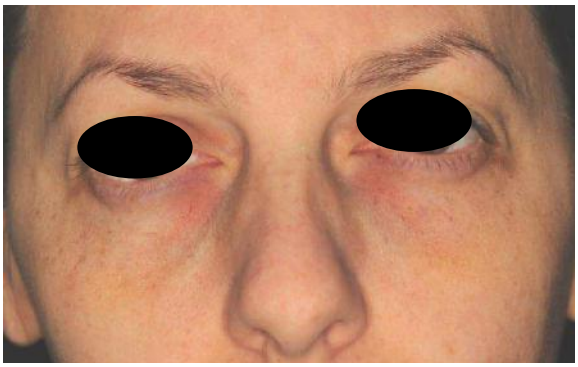
The images here after confirm these results.

Volunteer 25

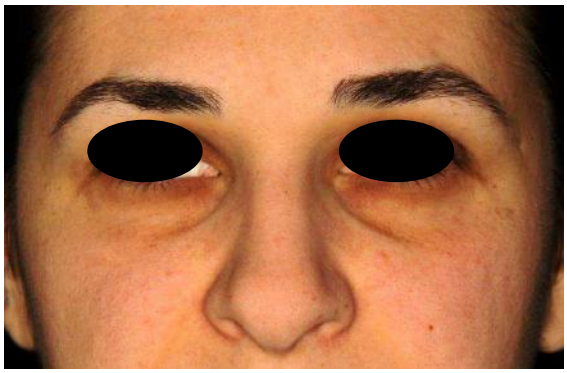


T0

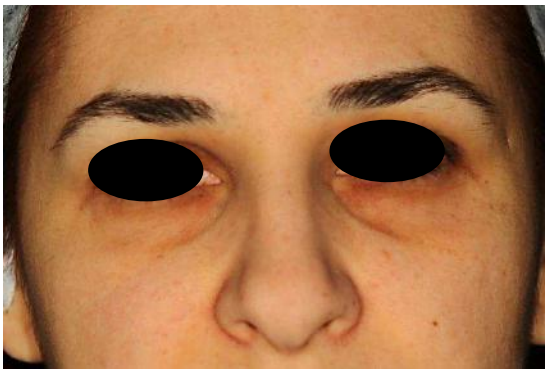


T28

Volunteer 26

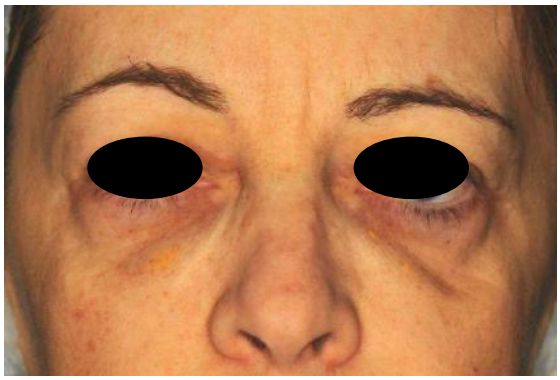


T0

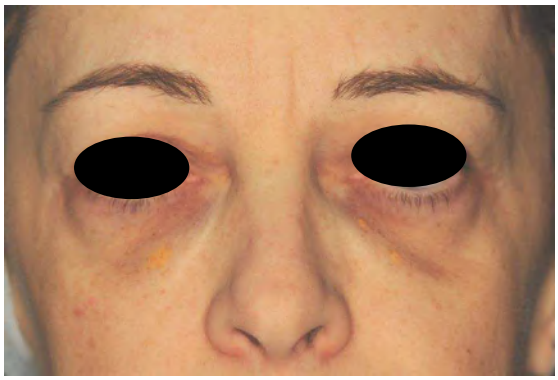


T28

Volunteer 30



T0



T28

➤ PHYCO'DERM® disguises the appearance of dark circles under the eyes, helps increase skin luminosity under the eyes and erases the look of fatigue.

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

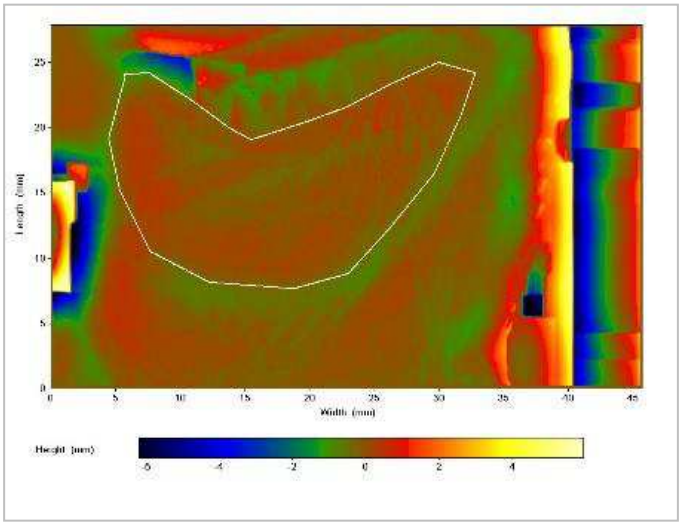
Periorbital puffiness is the appearance of swelling in the tissues around the eyes. It appears as a common cosmetic problem occurring when the skin of the lower eyelid is slightly swollen. That may be caused by various factors such as sleep deprivation, fluid retention, diet, excessive caffeine and alcohol 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 properties: under-eye bags

Skin surface is quantitatively assessed by Primos 3D (GFMesstechnik GmbH). Primos 3D is a non-contact *in vivo* skin measurement device based on structured light projection. In conjunction with a comprehensive 3-D measurement and evaluation software, the sensor allows to evaluate skin surface properties (*i.e*. wrinkle depth, volume, roughness etc.). In this study under-eye bags volume are evaluated. *cf*. figure here after.



The technique Primos 3D is a 3D scanner that create a point cloud (set of vertices in a three-dimensional coordinate system) of geometric samples on the surface of the subject. These points are then used to extrapolate the shape of the subject (a process called reconstruction). Like cameras, 3D-scanner have a cone-like field of view, and like cameras, they can only collect information about surfaces that are not obscured. While a camera collects color information about surfaces within its field of view, 3D scanners collect distance information about surfaces within its field of view. The "picture" produced by a 3D scanner describes the distance to a surface at each point in the picture.

This technique allows to:

a) take a high resolution image of the skin,
b) take a 3 dimensional image and
c) analyze by means of image analysis the information of the 3D image.

It gives information of the eye bags volume.

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Results

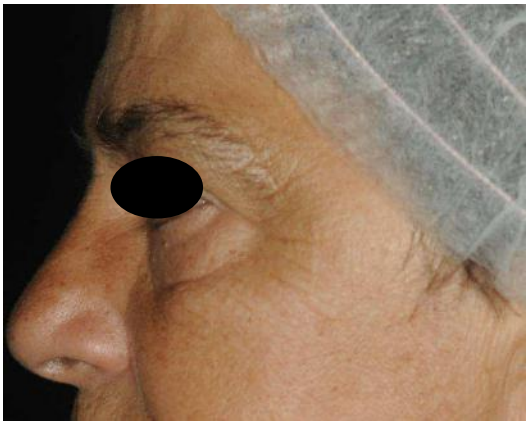
The mean between day 0 (T0) and day 28 (T 28) are presented here after.

	Δ T 0 / T 28	Maximum Δ / T 28	Minimum Δ / T 28
Placebo	**11.7 %**	- 23.0 %	55.9 %
Gel 4% PHYCO'DERM®	**- 9.1 %**	- 31.9 %	10.1 %

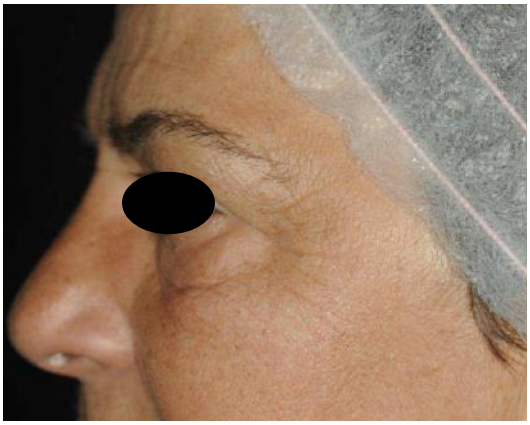
➤ PHYCO'DERM® decreases significantly the volume of under-eye bags compared to placebo (by up to 19%).

The images here after confirm this result.

Volunteer 4

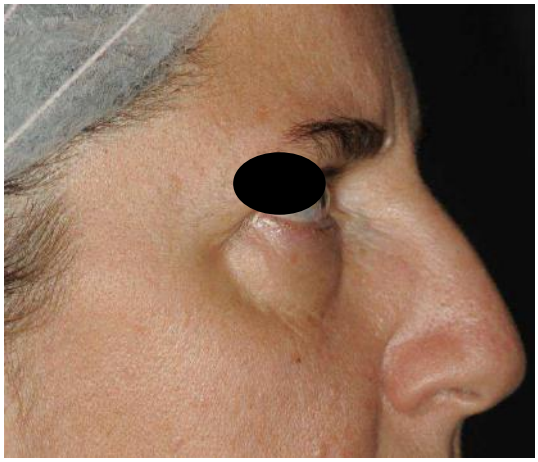


T 0

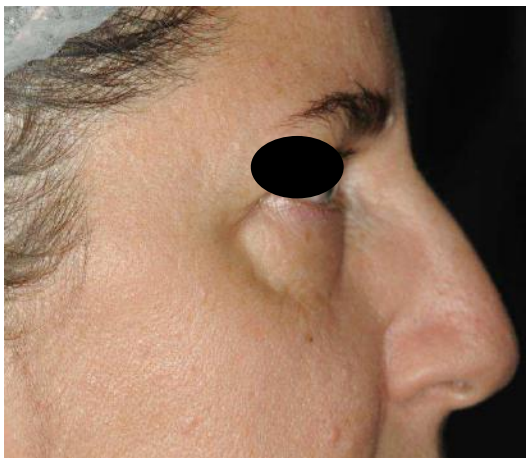


T 28

Volunteer 7



T 0



T 28

➤ PHYCO'DERM® alleviates puffiness of the skin and helps restore a smooth skin contour to puffy skin beneath the eye.

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PHYCO'DERM® - Subjective evaluation

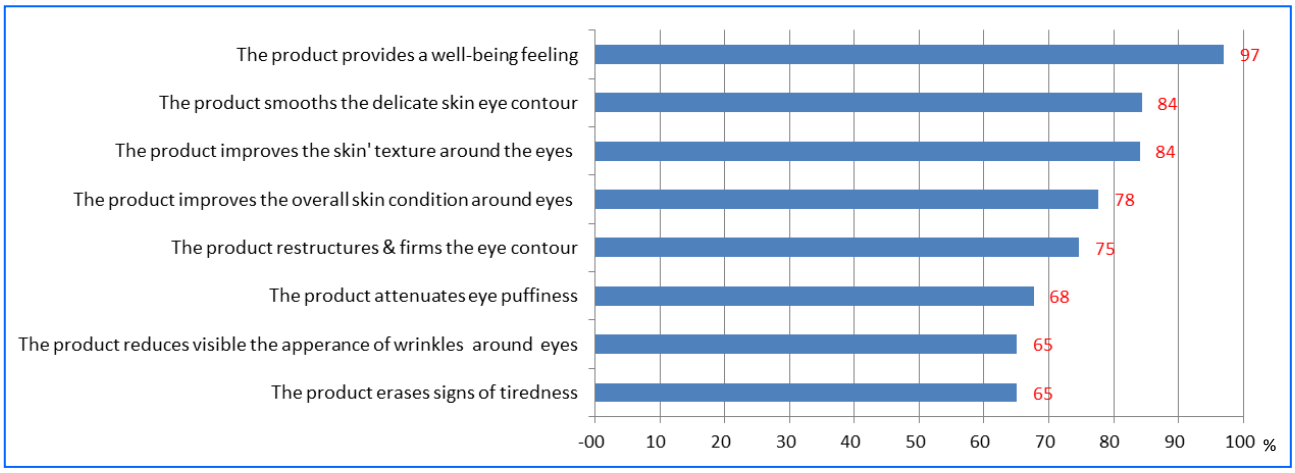
Method

Subjective evaluation of PHYCO'DERM® by 30 Caucasian women panelists (from 42 to 66 years old) after twice daily application of a gel with 4 % active for 28 days.

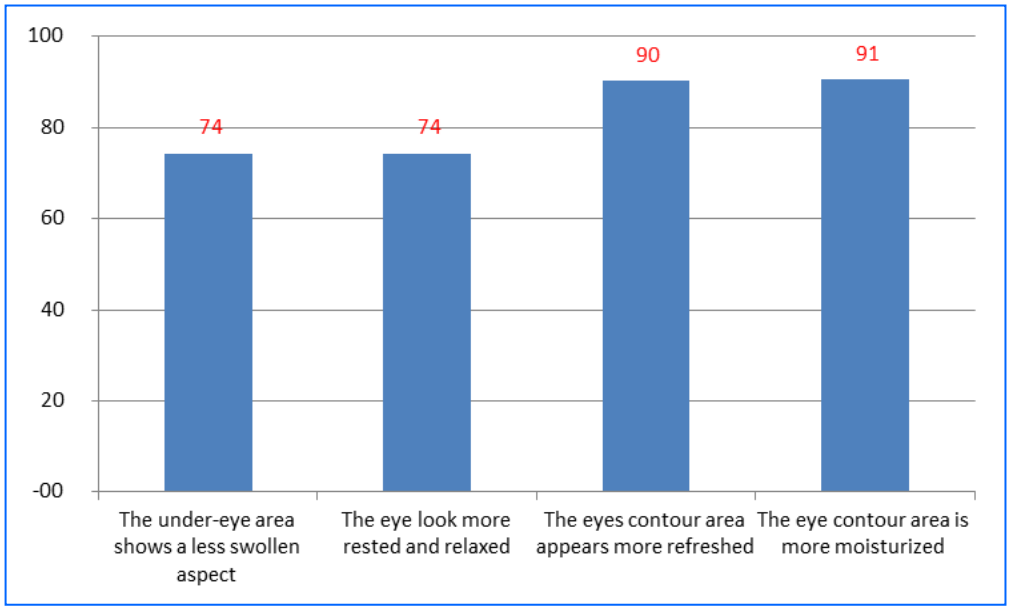
Results

The evaluation of panelists through a subject questionnaire has been particularly positive for a general assessment.

➤ It provides conclusive evidence of important benefits of using PHYCO'DERM®.



➤ The panelist's 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.

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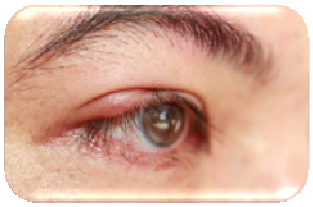
CONCLUSION & COSMETIC BENEFITS

PHYCO'DERM® is a natural marine complex designed to take care the delicate area around the eyes. It associates two seaweed extracts in a glycerin excipient that helps combat dryness for keeping the skin hydrated and supple.

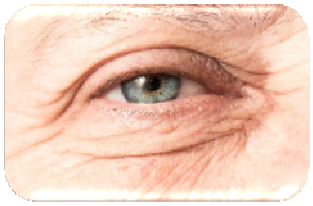
PHYCO'DERM® conceals imperfections of the periocular area by providing numerous desirable benefits proved by different *in vitro* and clinical studies:



➡ 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.

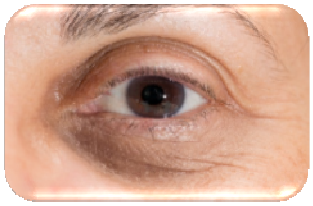


➡ Improvement of dermis properties

- 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.

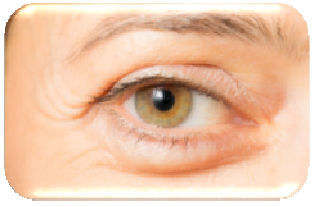
➡ Attenuation of fine lines and wrinkles of crow's feet for inducing smoothing effect of the periocular area.



➡ 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.

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Clinical studies have demonstrated the positive effects of PHYCO'DERM® after only 28 days of treatment versus placebo 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

Collaboration: Nature en textures- France

Serum eye contour care

Phase	Ingredients	Quantity (g)	INCI names
A	Eau	70	Aqua (Water)
	Glycérine	2	Glycerin
	Solagum AX	1,5	Acacia Senegal Gum (and) Xanthan Gum
B	Simulgreen 18-2	3	Hydroxystearyl Alcohol (and) Hydroxystearyl Glucoside
	Dub Inin	5	Isononyl Isononanoate
	Huile de Macadamia	4	*Macadamia ternifolia* Seed Oil
	Huile de Jojoba	3	*Simmondsia chinensis* (Jojoba) Seed Oil
	Isopropyl Myristate	3	Isopropyl Myristate
C	Vitamine E acétate	0,2	Tocopheryl Acetate
	Geogard 221	0,8	Dehydroacetic Acid (and) Benzyl Alcohol
	Sensiva SC50	0,3	Ethylhexylglycerin
	Parfum SHG6611	0,3	Perfume
	PHYCO'DERM®	4	
	Acide citrique à 50%	0,4 Qs pH 5,4 - 5,8	Citric Acid
	Eau	Qs 100	

This formula is presented in good faith, and we believe it is correct, but no warranty as to accuracy of results, or fitness for a particular use is given, nor is freedom from patent infringement to be inferred. It is offered solely for your consideration, investigation and verification. We are unable to guarantee the stability of this formula in view to limited stability studies.

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ANNEX

Evaluation of ocular irritation

Etude HC - B16 0622/16-1324

EVALUATION DU POTENTIEL IRRITANT D'UN ELEMENT D'ESSAI PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE EMBRYONNE - HET-CAM -

ASSESSMENT OF THE IRRITANT POTENTIAL OF A TEST ITEM AFTER APPLICATION TO THE EMBRYONIC HEN'S EGG CHORIOALLANTOIC MEMBRANE - HET-CAM -

RESUME/ *SUMMARY*

- **PRINCIPE DE L'ETUDE/ *PRINCIPLE OF THE STUDY***

L'étude a été basée sur l'observation, par une personne qualifiée, des effets irritants (hyperhémie, hémorragie, coagulation) pouvant survenir dans les cinq minutes suivant le dépôt d'un élément d'essai sur la membrane chorio-allantoïdienne (MCA) d'œufs de poule embryonnés au dixième jour d'incubation.
Le potentiel irritant a été scoré selon une échelle allant de 0 à 21. L'élément d'essai a été classé dans l'une des catégories définies en fonction du score moyen obtenu.

The study was based on the observation, by a trained person, of the irritant effects (hyperhemia, haemorrhage and coagulation) occurring during the five minutes after application of test item to the chorioallantoic membrane (CAM) of embryonic hen's eggs on the tenth day of incubation.
The irritant potential was scored according to a scale from 0 to 21. The test item was classified in one of the categories defined according to the mean score obtained.

Score moyen/ *Mean Score* (Scm/ *MSc*)	**Classification/ *Classification***
Scm/*MSc* < 1	Pratiquement non irritant/*Practically non irritant*
1 ≤ Scm/*MSc* < 5	Faiblement irritant/ *Slightly irritant*
5 ≤ Scm/*MSc* < 9	Modérément irritant/*Moderately irritant*
Scm/*MSc* ≥ 9	Irritant/*Irritant*

- **DATE(S) DE DEBUT ET DE FIN D'EXPERIMENTATION / *EXPERIMENTAL STARTING DATE AND EXPERIMENTAL COMPLETION DATE*** : 27 et 30 juin 2016 */ June 27 and 30, 2016*

- **RESULTATS/ *RESULTS* :**

Elément d'essai *Test item*	**Concentration testée** *Tested concentration*	**Score moyen sur 4 œufs ± écart type** *Mean score on 4 eggs ±standard deviation*	**Classification** *Classification*	**Comparaison par rapport à des éléments d'essai appartenant à la même catégorie** *Comparison with test items belonging to the same category*
PHYCO'DERM Réf. Gelyma 06 a-2016 - Lot : 16 06 070	Dilué à 10% dans l'eau p.p.i. / *Diluted at 10% with water for injection*	0.8 ± 1.5	pratiquement non irritant / *practically non irritant*	pas de comparaison disponible / *no available comparison*

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51, avenue de Paris - F94300 VINCENNES - Tél. 33 (0)1 41 74 40 23 - Fax 33 (0)1 41 74 40 24 - E-mail : evic-paris@evic.fr
EUROFINS EVIC PRODUCT TESTING FRANCE SAS au capital de 475 000 € –RC 70B70 Bordeaux – SIREN 470 200 700 – FR 79470200700

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Evaluation of cutaneous irritation

eurofins | EVIC

Rapport d'étude – version n° 1 du 11/07/2016 – PT Dermato
PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070
EUROFINS EVIC france 16-1103/0 – Etude I16 0486 / 16-1369

HUMAN PATCH TEST UNDER DERMATOLOGICAL CONTROL

Résumé en anglais / English synopsis

STUDY OBJECTIVE	To confirm the skin compatibility of the investigational product in a panel of healthy human subjects after single application under maximising and controlled experimental conditions.
TYPE OF THE STUDY	Monocentric randomised clinical study performed in single blind and defined as a non interventional clinical research according to the French law 2004-806 of 09/08/2004 relating to the policy of public health. The test subject was used as own control.

RESULTS

Characteristics of the included panel

Number of included subjects: 10
Number of exclusions: 0
Number of withdrawals: 0
Number of valid cases: 10

- Age: 24 to 69 (Mean= 51 years old)
- Sex: female
- Phototype: II to IV
- All types of skin on the back

Checking of the skin compatibility

No reaction was noted on the control site

For the investigational product:

Control time after patch removal	Type of reaction	Number of reactive test subjects	% of reactive test subjects	Mean daily irritation score MDIS	Skin compatibility of the product
T15-30 minutes (D3)	/	0	0 %	0	**Very good skin compatibility**

Legend: / = none

OVERALL CONCLUSION

Under the experimental conditions adopted:

single application of the product diluted at 10% in water for injection, under semi-occlusive patch, on a panel of 10 women, aged between 24 and 69 years old, with phototype II to IV and with all types of skin on back,

the product **PHYCO'DERM - Réf. Gelyma 06 a-2016 - Lot : 16 06 070** has a **very good** skin compatibility.

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afaq ISO 9001 Qualité

Mat. R_HC_06_14_F

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