OCEA DEFENCE®

Marine skin immunity booster

*

Reinforces the cutaneous shield

Reduces UV-induced erythema with a short term efficacy

Improves the comfort of fragile & vulnerable skin

Gelyma

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SUMMARY

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INTRODUCTION

The skin is one of the most important organ of the body. It is the first line of defence against a variety of physical, chemical and microbiological assaults which can lead to structural and biological damage. These cumulative damage to our skin increase with age and can result in oxidative stress and chronic inflammation.

In addition to its mechanical and physical barrier, the skin provides a biological and chemical barrier that shields the body from harmful chemicals, physical trauma, environmental aggressions, UV radiations and harmful microorganisms, protecting the skin through different mechanisms operating *via* the immune system that is naturally present in the skin and responds by producing natural antimicrobial peptides (AMPs).

The immune system is composed of two major subdivisions: the innate or non-specific immune system and the adaptive or specific immune system.

The innate immune system is our first line of defense against invading organisms.

The adaptive immune system acts as a second line of defense and also offers protection against re-exposure of the same pathogens.

Indeed disruption of the skin by inflammation, infection, sun damage, disease, burns or by chemical procedures generates a signal to the natural immune system and initiates responses that may or may not be effective in

- preventing and impending invasion from surrounding microbes by producing antimicrobial peptides that play the role of natural antibiotics,

- triggering the regeneration of new healthy cells to replace damaged cells.

These aggressions weaken the immune system, depriving the skin from its basic defense and increasing the possibility of inflammation and acceleration of skin aging.

With a reduced immune defense, the skin becomes less reactive, more fragile and vulnerable. That occurs specially in sensitive and reactive skin, also in aged skin and needs specific care.

So it is important to

- bring a good protection with active ingredients that can decrease the effect of such stresses,

- protect the skin by:

  - stimulating the immune system in order to improve the first line of defense between the skin and the environment in order to soothe skin irritation,

  - preventing damages caused by the oxidative stress and inflammation in order to strengthen fragile skins,

  - maintaining cellular homeostasis

  - stimulating the regeneration of tissue in order to prevent ageing.

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GELYMA launches OCEA DEFENCE®, an oily extract that combines the properties of

- the solvent caprylic/capric triglycerides
- the brown seaweed *Laminaria ochroleuca* collected along the seashore of Brittany.
- tocopherols (vitamin E) from vegetable origin.

This combination of natural ingredients is able to:

- targets the immune system for up-regulating of several markers of innate immunity such as the antimicrobial peptides: RNAse 7, S 100 A7 & toll-like receptor 4, that allows to
    - reinforce the cutaneous innate immune epithelial shield,
    - prevent cutaneous aggressions and infection against pathogens,
    - maintain a perfect and healthy skin
- protect skin against oxidative damage by activating several markers such as metallothioneins and the extracellular SOD
- control and reduce inflammation by down regulating the gene expression of the pro-inflammatory cytokines IL1 and IL6
- protect the matrix against degradation by down modulating genes coding several MMP: MMP1 , MMP3 and MMP9 and plasminogen activator urokinase (PLAU) that allow activates skin repair and restructuring processes.

Furthermore, OCEA DEFENCE® formulated at 2% reduces significantly UV-induced erythema in human volunteers with a short term efficacy (from 30 min).

Thanks to the results we obtained, OCEA DEFENCE® acts as a purifying active ingredient which responds to consumer's expectations for a visible effect of healthier and purer skin. It is designed for improving cutaneous comfort for sensitive skin and preserving the youthful appearance of fragile and vulnerable skin.

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ALGAL SOURCE *Laminaria ochroleuca*

➤ Classification

Laminaria ochroleuca belongs to

Empire	*Eukaryota*
Kingdom	*Chromista*
Infrakingdon	*Heterokonta*
Phylum	*Ochrophyta*
Class	*Phaeophyceae*
Order	Laminariales
Family	*Laminariaceae*
Genus	*Laminaria* J.V. Lamouroux 1813
Species	*ochroleuca* Bachelot de la Pylaie 1824

Synonyms

Laminaria lejolisii Sauvageau 1916
Laminaria pallida var. iberica G. Hamel 1928
Laminaria iberica (G. Hamel) Lami 1934

Common names

Folha-de-carriola in Portugal
Golden kelp in Britain
Kalkon melen, Tali bamboo, Tali laez, Tali bour in Brittany.

➤ Morphology

Laminaria ochroleuca is a yellow-brown digitate *Laminaria* up to about 1.5 m in length. (Fig.1). It shows a distinct yellow area at the junction of the stipe and the blade which appears as a key distinguishing feature.

The stipe is round and rigid without any attached epiphytes.

The blade is broad and flattened, dividing into numerous strap like digits.

Fig. 1 – Morphology of *Laminaria ochroleuca* in Brittany (*Photo Gelyma*).

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➤ Biology & Geographical distribution

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 .

In nature it shows a strong seasonality with rapid growth in spring and sorus formation in summer to autumn.

Fig. 2 – Population of *Laminaria ochroleuca*

This species is a warm-temperate, Lusitanian species present mainly in the upper midsublittoral zone between 0-2m above low water (Fig.2) and also depths in excess of 100m. It can forms large dense laminarian forest (John, 1971 – Mar. Biol., 11: 90-97, Braud J.P., 1974 –Rev. Trav. Inst. Pêches Marit. 38: 115-204).

However the population density varies between sites only in relation to water quality and exposure to wave action.

The young sporophytes of *Laminaria ochroleuca* are susceptible to UV damage which could effectively limit the upper distributional range of this species (Roleda M.Y. *et al.*, 2004 – Phycologia 43: 603-3613

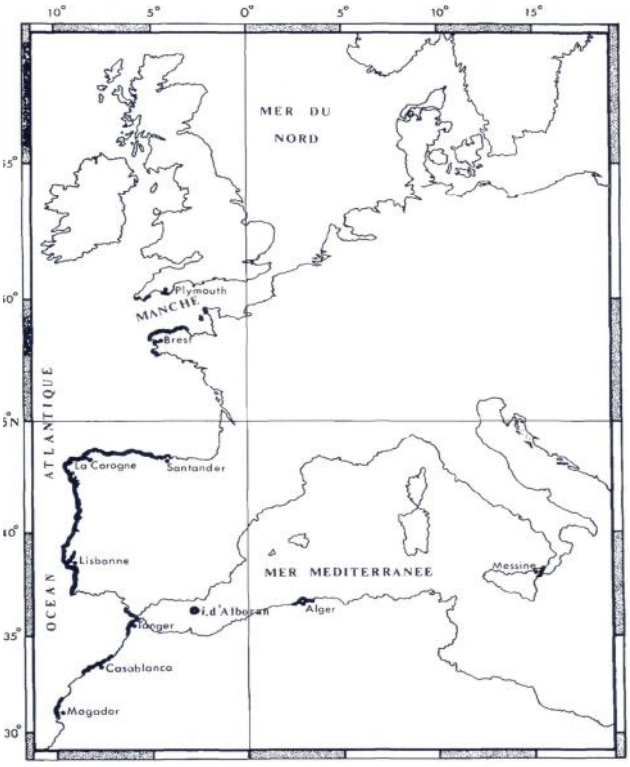

Fig.3 - Repartition of *Laminaria ochroleuca* *cf* in J.P. Braud 1974

L. ochroleuca is found from Morocco (and possibly the Azores) where it is the kelp forest species in place of *L. hyperborea*, northwards on the Atlantic coasts as far as the English Channel and Lundy in the Bristol Channel (Fig. 3).

In the northern part of its range it is found with *L. hyperborea* in a mixed kelp forest.

Since 1940, this species has been found on the coasts of southern England and is apparently indicative of a slow northward extension of warmer waters.

It is also found on the shores of the south-west Mediterranean and in the Straits of Messina where it is forbidden to collect. There, *Laminaria ochroleuca* is a protective species according Bern Convention.

In Mediterranean, *L. ochroleuca* can reach 5-6 m long. Along the Strait of Messina, a dense population can be observed between 50 and 100 m depth.

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Scientists think that *L. ochroleuca* may be similar to a warm temperature ancestor of both *L. digitata* and *L. hyperborea* which crossed the equator in the Pleistocene (*cf* in Levring , K. –1990 -Seaweeds : their environment, biogeography and ecophysiology, Wiley ,NY.)

➤ Chemical composition

It exists a few chemical studies about this species. The following data came from the work of J.P. Braud (1974).

The content in alginic acid varies between 21.8% and 15.8% according to the season, the minimum being registered in may-july. As for the other species of *Laminaria*, the content is higher in stipe than in frond

18.38+/- 2.15 in frond
22.46 +/- 0.79 in stipe

This coloured alginic acid shows a high viscosity: 5 040 cp in frond and 6 720 cp in stipe.

Phloroglucinol has been identified. Also other phenolics compounds.

➤ Bioactivities & utilizations

Laminaria ochroleuca is used for cosmetic purposes. *Laminaria ochroleuca* extract is known as skin conditioning agent in Cosmetics. Applied topically it helps reduce inflammation and show some moisture-binging properties.

The oily extract of *Laminaria ochroleuca* is shown to reduce skin inflammation and to able to improve irritant contact dermatitis (Patent J. N. Thorel FR2912658, Bonneville M. *et al*., 2007- J. European Acad. Dermatol & Vener. 21: 1124-1125).

Laminaria ochroleuca extract associated with:

- rice extract would increase the synthesis of GAGs (Patent L'Oreal EP 1 656 970).

- rose fruit extract would suppress skin roughness and dryness by suppressing production of cytokines that is a potential inflammatory factor (Patent PIAS ARISE, KK JP 2010222306)

- different ingredients such as collagen or *Pelvetia canaliculata* extract would hydrate skin from both the interior and the exterior (Patent CI Labo Dr Shirono Yoshinori WO2005 JP12479 20050706).

- polysaccharide would fight skin ageing by decreasing senescent cells (Patent Laboratoire d'Innovation Cosmetique et Dermatologique , FR 2 919 805).

Laminaria ochroleuca has also been studied for pharmaceutical purposes. The methanolic extract of *L. ochroleuca* is a central nervous system depressant with slight analgesic activity (Vazquez-Freire M.J. *et al*., 1994 – PTR Phytotherapy research 8 (7): 422-425).

Laminaria ochroleuca is collected in Brittany together with *Laminaria digitata* for the manufacture of alginates.

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THE ACTIVE INGREDIENT OCEA DEFENCE®

Specifications

on a control batch

- Appearance : oily limpid transparent liquid
- odour : typical
- solubility : soluble in oils
- microbiology : bacteria : < 100 germs / ml
  : yeasts, moulds : < 100 germs / ml
  : pathogens : free.

Composition

INCI names	CAS n°	EINECS n°	Amounts (%)
Caprylic/capric triglycérides	73398-61-5/ 65381-09-1	277-452-2/ 265-724-3	94.75
Laminaria ochroleuca extract	92128-82-0	295-780-4	5.0
Tocopherol	59-02-9 16698-35-4 54-28-4 119-13-1	200-412-2 240-747-1 200-201-5 204-299-0	0.25

Contents in iodine and heavy metals *(mg/Kg - data from a control batch).*

Iodine	0.23
Arsenic	< 0.25
Cadmium	< 0.25
Lead	< 0.25
Mercury	< 0.25

Storage

OCEA DEFENCE® 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, OCEA DEFENCE® remains stable for at least 18 months.

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

Safety

No animal experimentation

Standard safety testing proves that OCEA DEFENCE® is safe for cosmetic use.

- Ocular irritation : moderately irritant (Het Cam test)
- Cutaneous irritation : nonirritant (Human patch test).

cf. annex pp 27-28.

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EFFECTIVENESS EVALUATION

OCEA DEFENCE®

The best protection for sensitive & vulnerable skins

Cosmetogenomic study

OCEA DEFENCE® up regulates genes involved in the immune system by boosting the synthesis of innate immunity proteins	Genes linked to the markers RNASE 7 – S 100 A7 - TOLL-Like receptor 4
OCEA DEFENCE® reactivates genes involved in the protective mechanisms linked to cellular homeostasis & oxidative stress	Gene linked to the metallothionein MT-1 & the extracellular SOD (SOD3)
OCEA DEFENCE® down-regulates genes involved in the degradation of the basal membrane & the extracellular matrix components	Gene linked to MMP1-MMP3 & MMP9 Gene linked to the plasminogen activator-urokinase PLAU
OCEA DEFENCE® down-regulates genes involved in the pro-inflammatory reactions	Genes linked to IL1α and IL6

In vivo testing

OCEA DEFENCE® soothes & attenuates the effects of UV-induced erythema	Reduction of UV-induced skin erythema with a short term efficacy

The ideal marine answer
for
regulating the level of tolerance of
sensitive skin & vulnerable skin
against external cutaneous disorders

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I- *C*osmetogenomic study

Collaborations: BIOALTERNATIVES – SEPHRA-PHARMA - France

In order to identify the mode of action of OCEA DEFENCE®, we have used different techniques, firstly cosmetogenomic study that allows look how actives interact with the cell's DNA.

Gene expression may be measured by the determination of RNA levels in cells using the polymerase chain reaction (PCR).

The claims are related to the over- or the down expression of specific genes related to inflammatory processes.

Methods

The experiments have been carried out ion reconstituted human epidermis (RHE model - 11 days old) with a topic application (3 mg/cm²) during 24 hours.

Six reconstituted human epidermis have been stimulated or not topically with PMA (0.3µg/ml) in the presence or the absence of active for 24 h.

All experiments have been performed in duplicate (n = 2).

After mRNA extraction, the expression of makers has been evaluated by RT-qPCR, according 64 markers (with 3 reference genes).

Result s

The RT-PCR assay results showed that OCEA DEFENCE® regulates different genes specially those related to:

- the innate immune system
- the oxidative stress
- the chemokines
- the extracellular matrix .

They have been validated by using t-test with p value:

0,05	*
0,01	**
0,001	***

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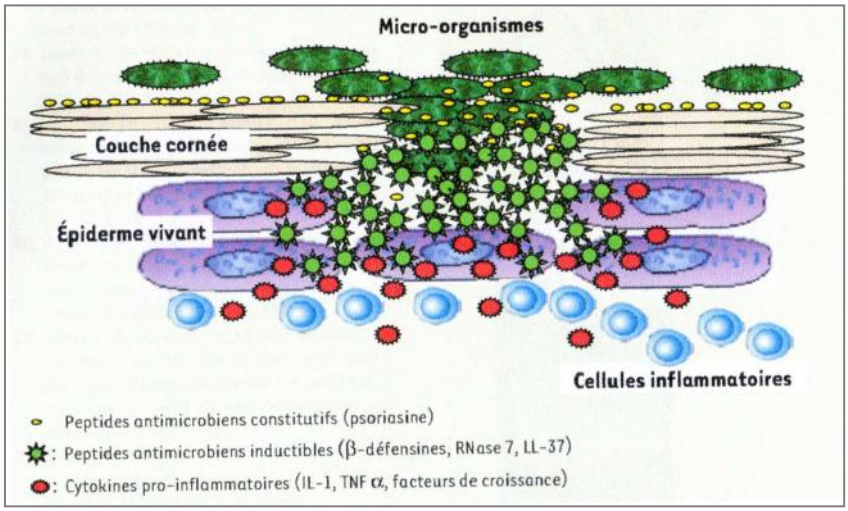
OCEA DEFENCE® up regulates genes involved in the immune system by boosting the synthesis of innate immunity proteins

Human skin is continuously exposed to a wide range of potential pathogenic bacteria. Despite these threats, human skin is normally not infected. In fact human skin provides in addition to its physical barrier, also a chemical barrier based on the release of antimicrobial proteins or peptides named AMPs included in the innate immune system (Braff M.H. *et al.* 2005 – J. Invest. Dermatol. 125: 9-13; Schröder J.M. & Hard J. 2006 – Cell Mol Life Sci. 63: 469-486).

These antimicrobial peptides take part to the innate immune system that appears as the first line of defense against invading microbes (Akira S. *et al.*, 2001 Nat. Immunol. 2: 675-680 ; 2006 – Cell 124 : 783-801).

They form an innate epithelial chemical shield to prevent cutaneous infection, control microbial growth and inhibit infection at the surface of healthy skin. Under resting conditions, low levels of AMPs are synthetized at sites of potential microbial entry into the skin and provide a further impediment to infection. After injury, AMPs levels rise rapidly in the skin due to increased synthesis by keratinocytes (Graff M.H. *et al.,* 2005 – J. Invest. Dermatol. 60:17-24).

Schröder J.M. & J. Harder (2004 – Médecine/Sciences 22: 153-157) have schematized the organisation of the cutaneous chemical barrier.



Bacteria present at the surface of the *stratum corneum* are taken on by constitutive AMPS (specially S 100 A7). When the physical barrier is broken, bacteria induce the production of other AMPs (specially RNAse 7) and pro-inflammatory cytokines (specially IL1, TNF α and growth factors) by keratinocytes

Among the skin immunity markers, we can list various anti-microbial peptides.

The present study concerns RNAse 7, S 100A7 and Toll-like receptor 4.

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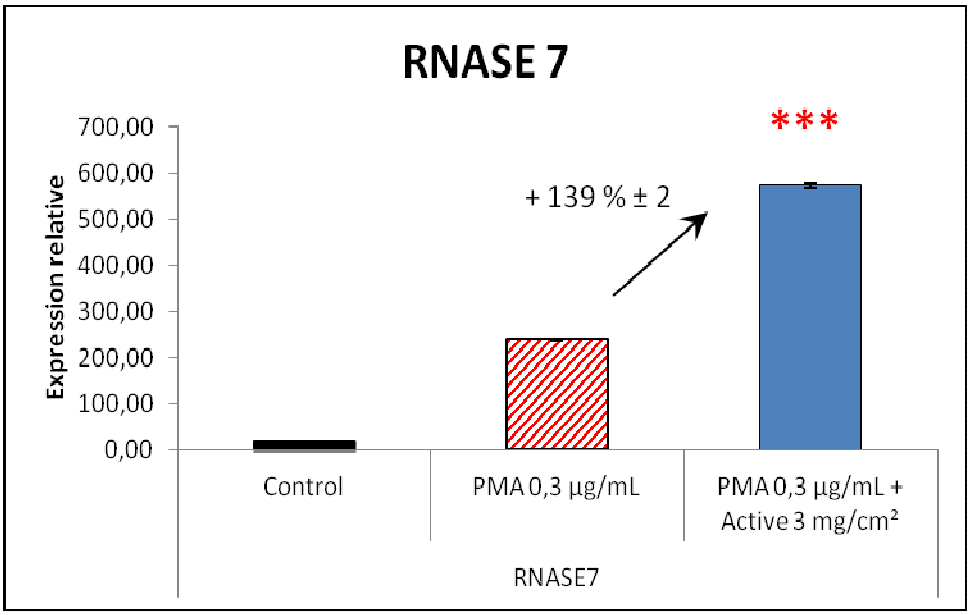
Gene encoded RNAse 7

The RNAse 7 is a potent antimicrobial peptide playing an important role in cutaneous defense (Simanski M. *et al.* 2012 – J. Innate Immun. 4 (3) 241-7). It shows a large spectrum of antimicrobial activity (Harder J. *et al.*, 2002- J. Biol. Chem. 277:1677-84). Its content in the *stratum corneum* varies between 4 to 8 mg/g (Schröder J.M. & J. Harder (2004 – Medecine/Sciences 22: 153-157).

It is known to protect healthy skin from *Staphylococcus aureus* colonization (Simanski M. *et al.*, 2010 – J. Invest. Dermatol. 130 (12):2836-8).
S. aureus is a commensal found on the skin of about one-third of people. It can cause a wide range of major and minor human infections of the skin and soft tissues such as boils, would infections, impetigo, cellulitis and folliculitis.

RNAse 7 also acts in the protection of human skin against *Enterococcus faecium*, a Gram-positive cell that can cause nosocomial bacteremia and various infections (Köten, B. *et al.*, 2009 – PloS One 4 (7) e 6424).

The use of RNAse 7 as cosmetic agent has been investigated by L'Oréal (Patent FR 2908 784). It appears that the expression of RNAse is lower in the *stratum cornuem* of dry skin and aged skin, specially post menopaused women. So the use of RNAse may be useful to counteract cutaneous troubles such as the dryness of epidermis.



➤ OCEA DEFENCE® increases the gene expression of RNAse 7 by 139%, very highly significantly.

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Gene encoded S 100 A7

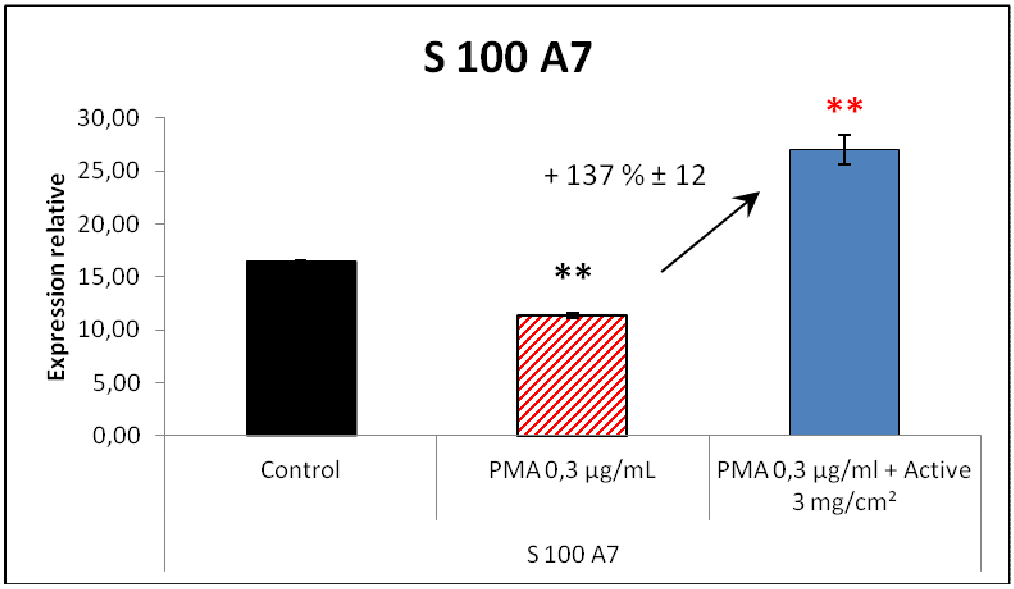
S 100 A7, also known as psoriasin, belongs to the S 100 protein family included about 20 different proteins (Marenholtz I. *et al.*, 2004 – Biochem. Biophys. Res. Comm. 322 (4) 1111-1122).

It is a calcium-regulated protein that regulates fundamental biological processes. It is viewed as a key molecule of the cutaneous barrier (Gläser R. et al., 2011 – J. Dtsch. Dermatol. Ges. 9 (11) 897-902).

S 100 A7 acts both as an antimicrobial peptide and as a chemotactile factor for neutrophils and CD4 T Cells. Specially it has been shown to directly kill bacteria and protect human skin from *Escherichia coli* infection (Gläser R *et al.*, 2005- Nat Immunol. 6 (1): 57-64) and activate neutrophils to produce a range of cytokines, chemokines and AMPs (Zheng Y. *et al.*- 2008- Immunology 124 (3) : 357-367 ; Wolf R *et al* . 2008 – J. Immunol. 181 (2) : 1499-1506).

It is markedly increased in epidermal hyperproliferative disorders. (Eckert *et al.,* 2004- J. Invest. Dermatol.123: 23-33; Eckert R. & K.C. Lee, 2006 – J. Invest. Dermatol. 126: 1442-1444).

A recent study shows that, the production of S100A7 is repressed in seasonal allergic rhinitis which in turn might render patients more susceptible to microbial colonization or infection (Kvarnhammar A. M *et al.* 2012 – Respiratory Research 13 : 2-10).



➤ OCEA DEFENCE® increases the gene expression of S 100A7 by 137% highly significantly.

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Gene encoded the toll like receptor 4

Toll-like receptors (TLRs) are a class of conserved receptors that recognize pathogen-associated molecular patterns present in microbes.

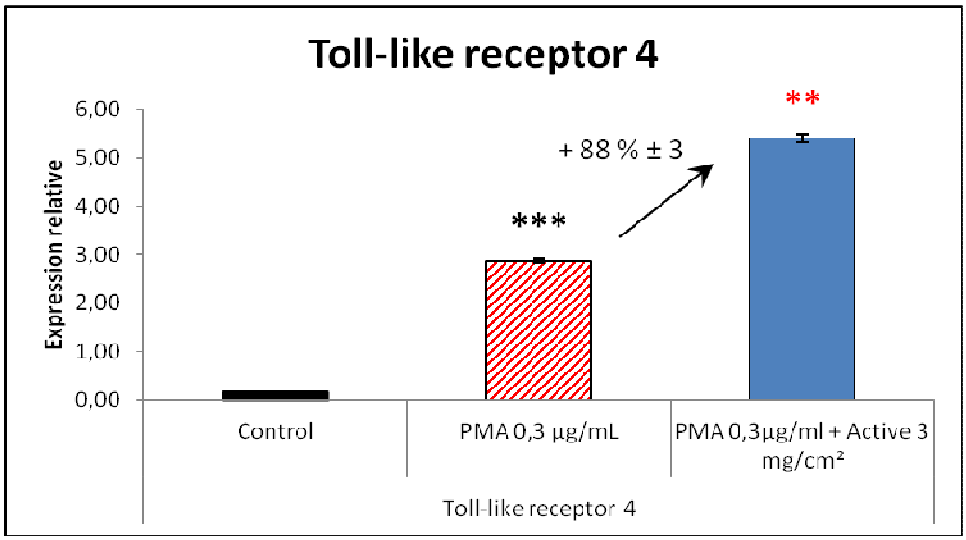
They are viewed as important and functional components that help the immune system to function properly (Aswin Hari *et al*., 2010 Mediators of Inflammation Article ID 437246).

These clever molecules interact with various endogenous and exogenous ligands and antigens. Also they influence acquired immune responses as well as various intracellular signalling processes (Kawai T. *et al*., 2007 – Semin. Immunol. 19: 24-32; Jang S. *et al*., 2012-Chonnam Med. 48: 7-14). They modulate the cytokinerproduction that is necessary for the immunity's efficacy.

In humans, a total of more than 10 TLRs have been discovered. The TLRs are expressed in a variety of cell types and in two general cellular locations, plasma membrane (TLR 1,2,4-6) or intracellular compartments such as the endoplasmic reticulum and endosomes (TLR 3).

Toll-like receptor 4 (TLR4) detects lipopolysaccharides from Gram negative bacteria.

It plays a key role in the initiation of innate immunity and in the regulation of adaptative immune responses. It would be an important regulator of wound inflammation (Chen L. *et al*. 2012 – J. Immunol. 188: 180).



➤ OCEA DEFENCE® increases the gene expression of toll-like receptor 4 by 88% highly significantly.

➤ OCEA DEFENCE® boosts the production of several antimicrobial peptides, specially RNAse 7, S 100A7 and Toll-like receptor 4 .

➤ OCEA DEFENCE® is able to protect skin against bacterial colonization and infection and against epidermis dryness by reinforcing the skin's innate immunity.

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OCEA DEFENCE® reactivates genes involved in the protective mechanisms linked to cellular homeostasis & oxidative stress

OCEA DEFENCE® is able to increase the gene expressions of different genes encoding for metallothioneins and superoxide dismutase linked to homeostasis and oxidative stress.

Gene encoded the metallothionein MT-1

The metallothioneins (MTs) are a class of small cysteine-rich, heavy-metal binding proteins produced in response to a variety of stresses, inflammation and as components of the acute-phase response. They are involved in heavy metals homeostasis and detoxification and in antioxidant defence.

The metallothioneins (MTs)

►are involved in the management of essential heavy metal divalent cations (*e.g*. copper and zinc) (Bremmer I – 1987 – Experientia Suppl. 52: 81-107; Schmidt C. & D. Beyersmann – 1999 Arch. Biochem. Biophys. 364: 91-98).

►serve to interfere with the toxic effects of other heavy metals (*e.g*. mercury and cadmium) (Masters BA *et al*. 1994 – Proc Natl. Acad Sci. US 91 : 584-588) and free radicals (Miura T. *et al*. 1997 – Life Sci; 6 : 301-309).

►maintain the normal cell cycle as an antioxidant against excessive endogenous reactive oxygen species that are generated synchronously with the cell cycle and disturb the normal cell cycle (Takahashi Y. *et al*., 2004 – J. Health Science 50 (2) : 154-158).

►play a major role in the prevention of tissue damage and in cellular homeostasis (Lynes M. *et al.,* 2006 – Exp. Biol. Med 231 (9) 1548-1554).

The photoprotection of MT against UVB radiation is also well demonstrated (Kobayashi S. *et al.,* 1994 – Photochem Photobiol. 59 (6): 650-6; Reeves V.E. *et al*. 2000- Immunoloy 100 (3): 399-404.; Ablett E. *et al*. 2003 – J. Invest. Dermatol. 120 : 318-324).

This photoprotection appears to be associated with the potential of MT to reduce superoxide and hydroxyl radicals in support of its suggested role as an endogenous antioxidant (Hanada K. *et al*., 1993- Photoderm Photoimmunol Photomed 9: 209 ; 1998 – J. Invest. Dermatol. 111: 582-5).

There are different metallothionein isoforms. These MT isoforms are classified based on various factors like molecular weight, metal which bind, encoded genes, binding atomes.

The major groups include MT-1 and MT-2 expressed in almost all tissues. MT-3 and MT-4 are minor groups found only in specialized cells.

MT-1 is present in all cells throughout the body. The different subtypes have been named MT-1 A, E, F , G , H …. accounting for the microheterogeneity of the MT-1 protein (Thirumoorthy N. *et al*., 2000- World J. of Gastroenterology 13 : 993-997).

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MT-1 regulates copper and zinc. It is involved in cell transcription, detoxify heavy metals and play a role in immune function.
Its protective role against DNA damage and apoptosis induced by external stress would be linked to the perinuclear localization of MT-1 mRNA (Levadoux-Martin M. *et al.*, 2001- Biochem.J. 355: 473-479).

Recently it has been demonstrated that the protein levels of MT-1 and MT-2 decrease significantly with increasing age of sun-exposed skin and sun protected skin (r = -0.73 and -0.98 respectively , p<0.01) but was more prominent in sun-exposed skin (Ma C. *et al.*, 2011 – Br. J. Dermatol. 164 : 479-482). The expression of MT-1 and MT62 declined with the decrease of keratinocyte proliferation in the process of skin ageing and tis decline was more significant in sun-exposed skin. So MT-1 and MT-2 supplementation could be interesting to inhibit skin ageing especially photoageing.

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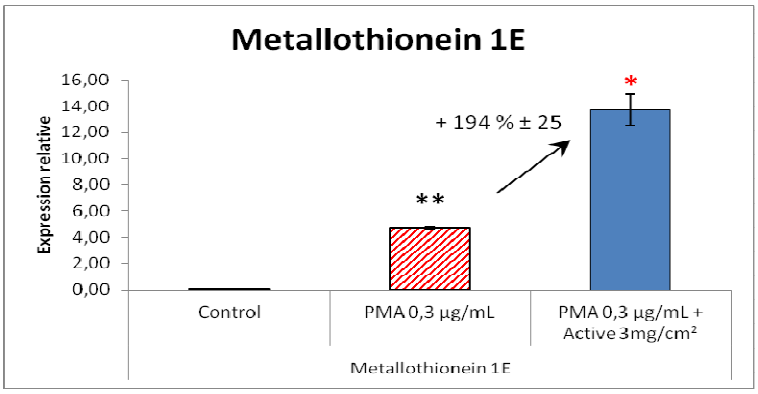
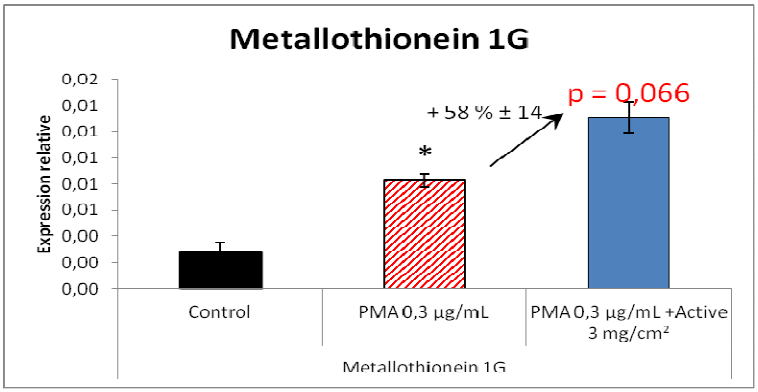
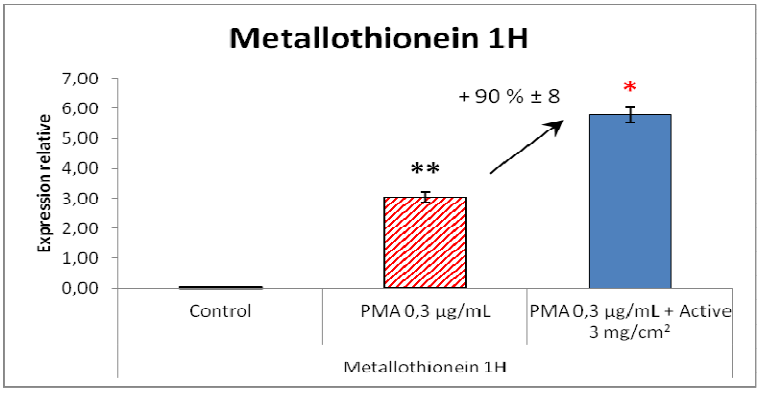


Isoform 1G +58 %



Isoform 1H +90 %



➤ OCEA DEFENCE® increases the gene expression of several isoforms of the metallothionein -1.

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Gene encoded the extracellular superoxide dismutase (EC-SOD – SOD3)

Superoxide dismutases (SODs) are known to play a crucial role in protecting cells against the oxidative stress. They catalyse the dismutation of two superoxide radicals into hydrogen peroxide and oxygen.

There are three forms of SOD: cytosolic Cu-Zn SOD, mitochondrial Mn SOD and extracellular SOD (EC SOD also named SOD 3).

The extracellular SOD (SOD 3) is located in the extracellular matrix and on the cell surface (Kim SH *et al.*, 2005 – Oncol. Res. 15 (7-8) 333-41). It participates in the majority of antioxidant systems in the skin and plays a protective role against various diseases and injuries mediated by oxidative stress (Zou Y. *et al.*, 2009 – Genesis 47 (3):142-154). It functions as a superoxide anion scavenger protecting against oxidative fragmentation of the extracellular matrix components. It has been shown to bind to the extracellular matrix through its positively charged C-terminal, thereby preventing oxidative fragmentation of the ECM components (Folz R.J. & J.D. Crapo, 1994 – Genomics 22: 162-171; Kliment C.R. *et al.*, 2008 – Antioxid Redox Signal 10: 261-268).

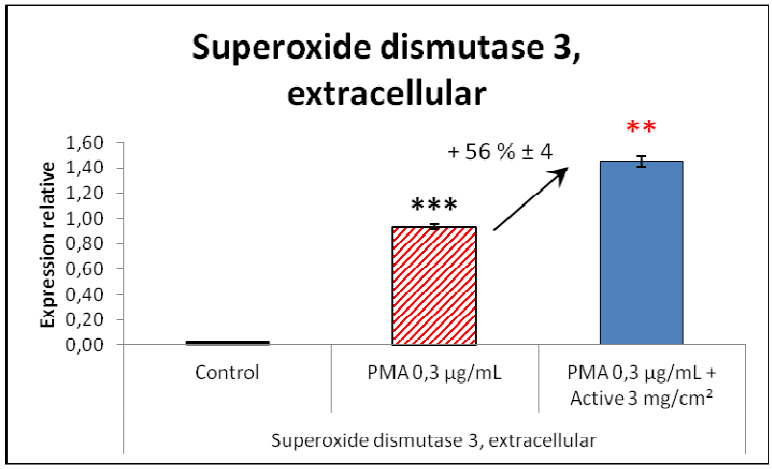
A recent study found that SOD 3 suppresses hyaluronic acid fragments-mediated skin inflammation and could be an effective strategy for the treatment of such skin inflammation (Kwon M.J. *et al.*, 2012 – Antioxid Redox Signal 16 (4) :297-313).

Other scientific studies have demonstrated the major role of the SOD 3 in:

- the angiogenesis and inflammation in chronic inflammatory skin disorders such as psoriasis (Kim Y *et al.*, 2011 – Free Radic. Biol. Med. 51 (11):1985-95).

- the prevention of skin carcinogenesis (Kim SH *et al.* 2005 – Oncol. Res. 15: 333-341),

However, the defensive role of SOD 3 against UV-injury of the skin would be different than the roles of Cu-Zn SOD and Mn SOD (Choung BY *et al.,* 2004 – Exp. Dermatol. 13 (11) 691-9).



➤ OCEA DEFENCE® increases the gene expression of the extracellular superoxide dismutase by 56%.

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OCEA DEFENCE® down-regulates genes involved in the degradation of the basal membrane & the extracellular matrix

Skin turnover and regeneration are largely dependent on extracellular matrix metabolism which is under the control of different systems, specially the plasminogen activator system and various matrix metalloproteinases (MMPs).

OCEA DEFENCE® is able to down regulate the gene expression of different genes involving in the degradation of the extracellular matrix.

Gene linked to the plasminogen activator urokinase PLAU

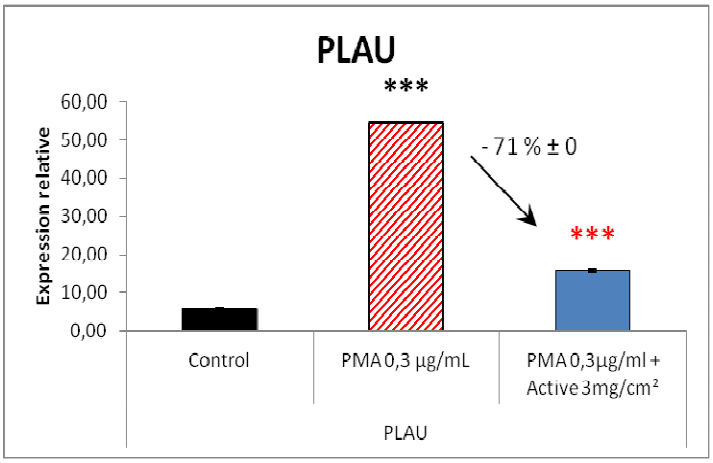
The plasminogen activator system includes two forms: tissue-type (tPA) and urokinase-type (uPA). It is associated with many processes.

Urokinase-type plasminogen activator (uPA) interacts with its receptor on inflammatory and migrating cells to regulate extracellular matrix degradation, cell adhesion and inflammatory cell activation.

It is involved in remodeling of the extracellular matrix by converting extracellular plasminogen into plasmin, a major active fibrinolytic protease via PLAU gene.

Plasmin directly degrades fibrinogen, laminin and fibronectin. It activates several MMPs that degrade extracellular matrix proteins and components of the basal membrane such as collagen, fibrinogen and laminin 5 (Ogura Y. *et al.,* 2008- Br J. Dermatol. 159: 49-60).

The PLAU gene is also in close relationship with thermal injury (Bo Xi *et al.*, 2012- African J. Pharmacy & Pharmacology, 6 (7): 448-453).
Moreover, it has been proved that an activity change of plasminogen activator is closely related to a formation of various types of diseased skin accompanied with rough skin and abnormal cornification (see Patent Shiseido EP 1 112 744 A1).



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OCEA DEFENCE® is able to decrease the gene expression of PLAU by 71% and so could prevent the degradation of matrix and the basal membrane and improve rough skin.

Gene linked to metalloproteinases MMP-1 - MMP-3 - MMP-9

MMPs are a family of ubiquitous enzymes playing a role in many different physiological and pathological processes in the skin.

They have been categorized into different groups, three being predominant:

- collagenases (MMP-1 , -8 , -13 and -18) that cleave interstitial (structural) collagens , MMP-1 being the predominant one cleaving collagens type I , II and III with a high affinity for type III .
- gelatinases (MMP-2 and MMP-9 degrade basement membrane collagens and degrade denatured structural collagens
- stromelysines (MMP-3 , -10, -11 & -19) degrade basement membrane collagens as well as proteoglycans and matrix glycoproteins.

MMPs are responsible for the aging skin condition. Their expression increases with aging and is further enhanced by UV irradiation.

MMPs play an important role in wrinkle formation and loss of elasticity and firmness of human skin.

➤ OCEA DEFENCE® is able to down-modulate the gene expression of three major MMPs (MMP-1 - MMP-3 -MMP-9) that are able to cleave important substrates, especially those of the extracellular matrix that gives tissue its structural integrity.

MMPs	Common name	Substrates
MMP-1	Collagenase -1	Collagen types I,II,III,V,VII,VIII & X
MMP-3	Stromelysin-1	Collagen types II,IV, IX & XI, proteoglycan, elastin
MMP-9	Gelatinase B	Collagen type IV, gelatin, laminin,

The extracellular matrix degradation leads to structural and functional impairment which deleteriously impacts the health and appearance of skin.

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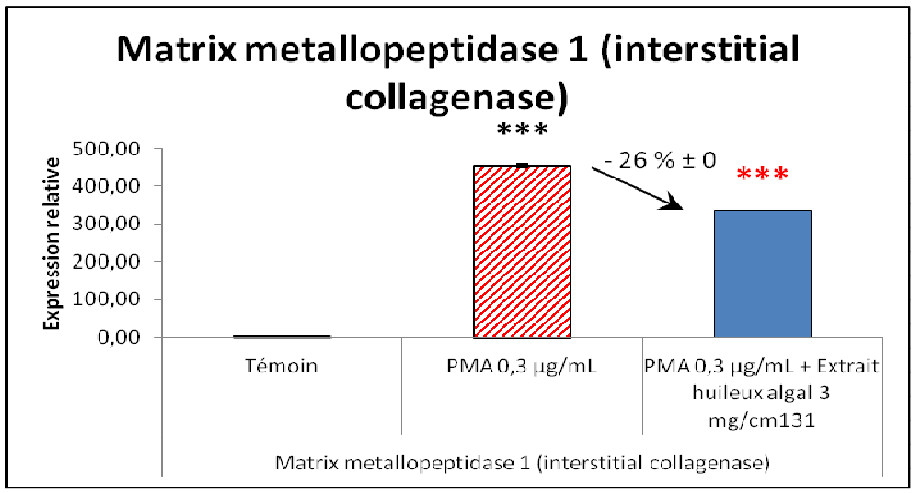
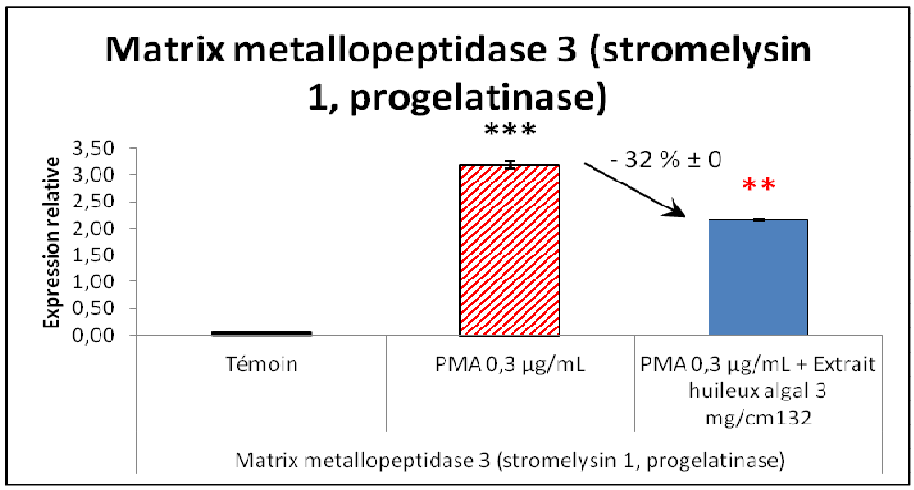
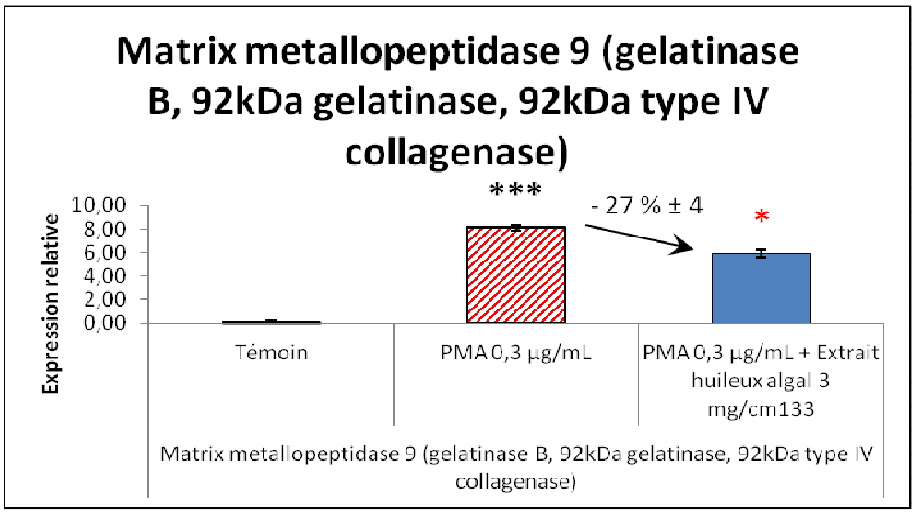
MMP-1 -26 %



MMP-3 -32 %



MMP-9 -27 %



➤ OCEA DEFENCE® may contribute to prevent skin aging by down regulating the major matrix metalloproteinases and therefore by modulating the degradation of dermal extracellular matrix and the catabolism of basement membrane.

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OCEA DEFENCE® down-regulates genes involved in the pro-inflammatory reactions

Inflammation is a beneficial host response to tissue injury that leads ultimately to the restoration of tissue injury and function.

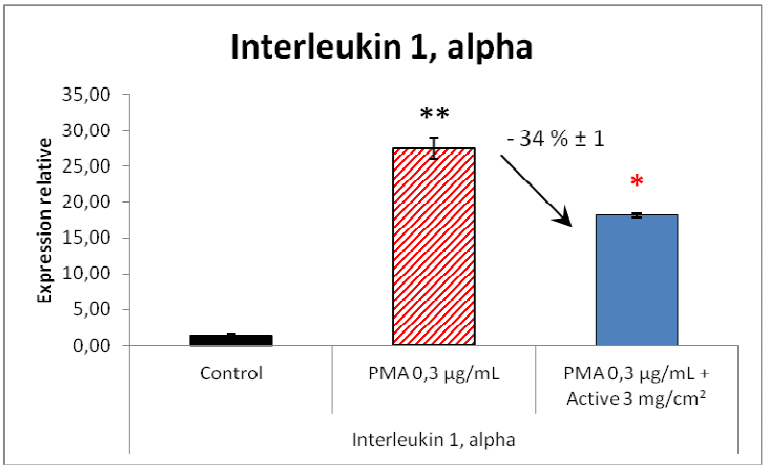
Pro-inflammatory cytokines play a central role in mediating cellular and physiological responses such as autoimmune diseases, chronic inflammatory diseases.

Innate immune cells are the major source of Il-1 and IL-6.

Gene encoded the IL 1 & IL 6

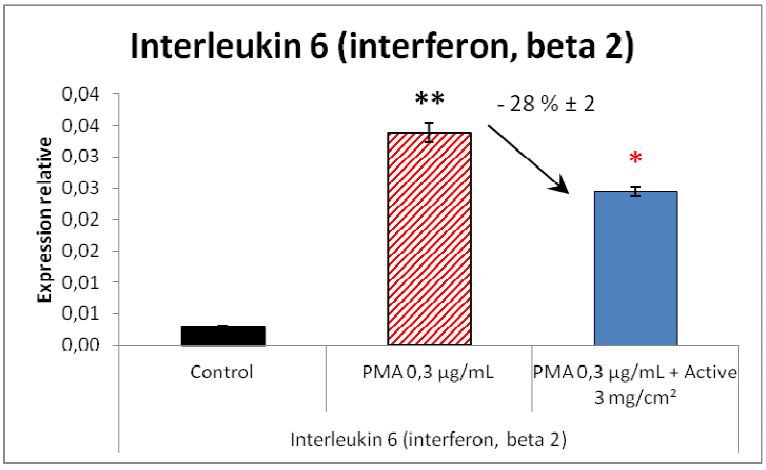
IL-1 lies at the center of the inflammatory response.

It also breaks down collagen and connective tissues.



IL-6 is a pleiotropic cytokine involved in the regulation of the immune responses and the acute-phase reaction.

It also possesses anti-inflammatory properties.



➤ OCEA DEFENCE® is able to decrease the gene expression of IL1a by -34% and IL6 by -28%, thus could prevent inflammation processes.

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II- *In vivo study*

Collaboration : Abich Cosmetic Lab. – Italy.

OCEA DEFENCE® soothes & attenuates the effects of UV-induced erythema

The study has been executed with an open observational modality. It was aimed to evaluate the soothing effect of the product under examination on 10 volunteers with stressed skin irradiated by Solar Simulator. It has been performed at the Abich Cosmetic Lab. in Via Bruno Buozzi, 4 – 20090 – Vimodrone (Milan-Italy).

Method

The phototype was previously evaluated for each volunteer. Then the volunteers have been exposed (T0) to a fixed dose of UVA+UVB radiation calculated as MED x1,5 in two areas of the back (DAY 0); the chosen sites did not present skin damage or naevi or any other abnormalities which could prevent regular testing. After a period of about 26 ± 4 hours (T1), on the two irradiated areas the degree of erythema was measured as index of erythema with Mexameter®. Immediately after measurement, one area was treated with the test substance and a second area was not treated (control).

The same parameter (index of erythema) was measured after 30 minutes (T1+30min), after 60 minutes (T1+60 min), after 120 minutes (T1+120min) and after 240 minutes (T1+240min), after product application, respectively in both areas.

Soothing effects were determined by comparing these parameters at the treated vs control sites.

T0	irradiation;	
T1	first measurement of erythema, application of product;	
T1	+30min	second measurement;
T1	+60min	third measurement;
T1	+120min	fourth measurement
T1	+240min	fifth measurement.

Several extrapolations were made from acquired data, including comparisons of treated with control areas (inter-group analysis), pre- versus post-treatment comparisons (intra-group analysis) and modulation of the differences observed between measurements performed in treated versus control areas over time. These thorough approach allowed to take into account possible variability due to individual and local differences of measured parameters and to achieve solid arguments for the proper interpretation of results.

The study was carried out under standard environmental conditions for each reading time, monitoring and maintaining constant temperature and humidity. Test substance was applied onto the back of each volunteer.

As control area a median zone of the back was identified, where no product was applied. Two skin sites on the back of each volunteer were irradiated at T0; every site was exposed to the same radiations with three beams of light of Solar Simulator having an incremental UV doses second a geometric progression of 1.12 to 1.25.

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Index of erythema was measured on the two sites about 26±4 hours after the irradiation of the DAY 0 (T0).

The product was used in non occlusive epicutaneos application.

Erythema value, assessed by hemoglobin content of the skin, was measured on the DAY 1 at T1 (26 ± 4 hours after irradiation) before product application, after 30 minutes (T1+30 min), after 60 minutes (T1+60 min), after 120 minutes (T1+120 min) and after 240 minutes (T1+240 min), after products application.. The value was assessed at the same times of observation in areas irradiated and untreated. The measurements were performed in a room protected from light in order to avoid that light radiation in the environment could affect the measurements.

Since the solar simulator has three optical waveguides emitting UV radiation at different power, the erythema was assessed at each measurement time in each of the two irradiated areas and the average value was extrapolated. It was also calculated the differential between the erythema induced in controlateral corresponding areas on each subject (check on) to decrease the effect attributable to individual variability in the interpretation of results.

The data obtained using Mexameter ® MX18 probe were expressed as "E"values on a scale from 0 to 999.
Measurements performed at different experimental times were rigorously repeated on the same areas of the skin.

Photographs were taken at T1, T1+60 min, T1+120 min and T1+240 min.

The distribution of the values obtained during the measurements at the different experimental times for the area treated with the product and the area not treated were compared with an inter-group analysis using Student's t test. Also intra-group analysis was performed, to compare measurements at the various experimental times in both treated and control areas. Finally, the differential values of measurements in treated versus control areas at each single experimental time were also analyzed, to take into account the possible effect of individual differences in the extent of induced erythema.

Values of $p < 0.05$ were considered significant.

Results

Under the applied experimental conditions, OCEA DEFENCE® (2% in a basic Carbopol gel) under study has demonstrated an efficacy to reduce erythema value (parameter E) of the UVA+UVB inducted irritation.

In particular the average erythema values E was reduced by a value equal to 6,07% after 30 minutes from the test product application, by a value equal to 8,06% after 60 minutes, by a value equal to 7.84% after 120 minutes and by a value equal to 10,01% after 240 minutes.

The E reduction in the test product treated area was statistically significant vs T1 ($p < 0.05$ - Student test) both after 30 minutes (T1+30 min), after 60 minutes (T1+60 min), after 120 minutes (T1+120 min) and after 240 minutes (T1+240 min).

In the untreated area is also observed a decrease in the parameter E, that resulted reduced by a value equal to 0.82%, 3.55%, 6,11% and 5,72% respectively after 30 min

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The tables below report the means to the Erythema values of the panel of 10 volunteers (table 1) and the p values of Student's t test (table 2), respectively.

Table 1
Mean values of the Erythema value

Time	product	untreated
T1	392.6	384.2
T1+30min	368.8	381.0
T1+60min	361.0	370.5
T1+120min	361.8	360.7
T1+240min	353.3	362.2

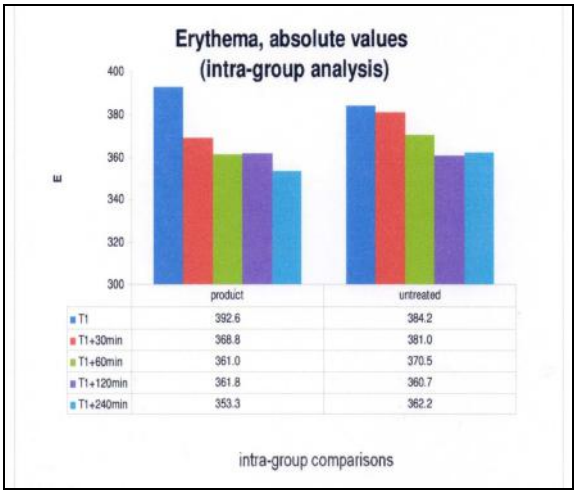
Table 2
p value of Student's t-test for Erythema value

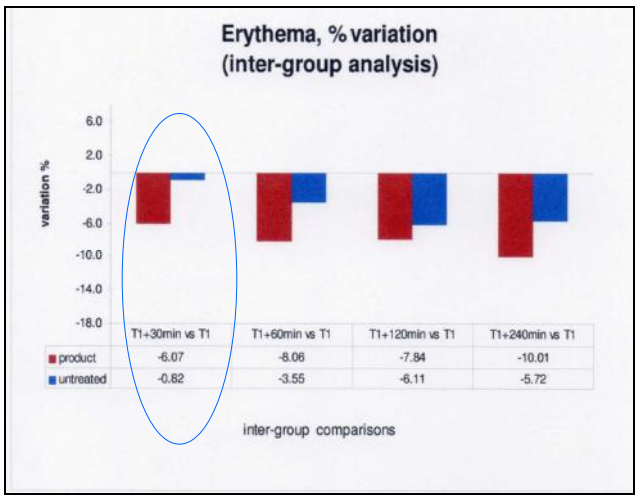
t test	product	untreated
T1+30min vs T1	0.0246	0.5279
T1+60min vs T1	0.0223	0.0504
T1+120min vs T1	0.0391	0.2087
T1+240min vs T1	0.0207	0.2045

The figures included hereafter represent respectively the results obtained:

- in terms of absolute values of erythema, at each experimental time (T1, T1+30 min, T1+60 min, T1+120 min and T1+240 min) and percent variation of erythema E value, at the considered observation times T1+30 min, T1+60 min, T1+120 min and T1+240 min VS T1.

- as inter-group comparisons.





➤ OCEA DEFENCE® (2% in a basic gel) has demonstrated a short term efficacy (after 30 minutes from product application) to reduce the redness at the level of the treated zone compared to measurement at T1, before product application, and to respective values relative to untreated zones.

➤ The product has demonstrated a soothing effectiveness on skin preliminarily irradiated by a solar simulator (UVA+UVB).

These results are illustrated hereafter on three volunteers.

These macrophotographs illustrate representative images of the irradiated areas of the back, treated with product at different times of measurement.

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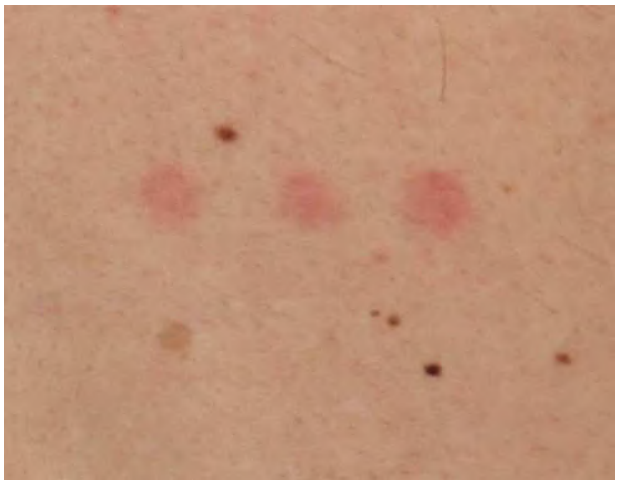
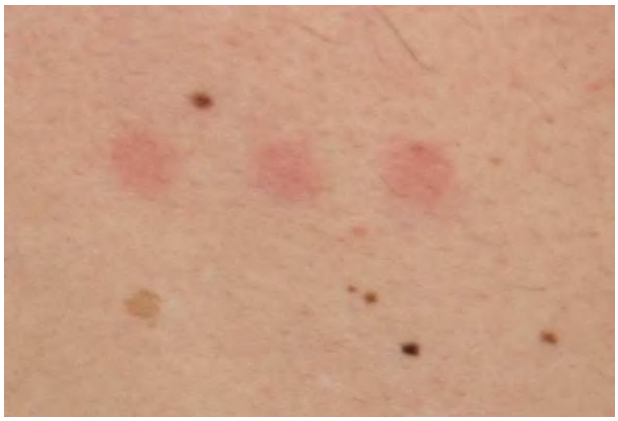
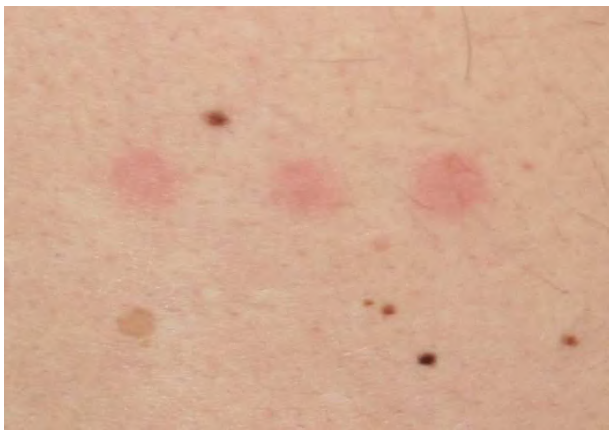
Volunteer
ALBA346
T1



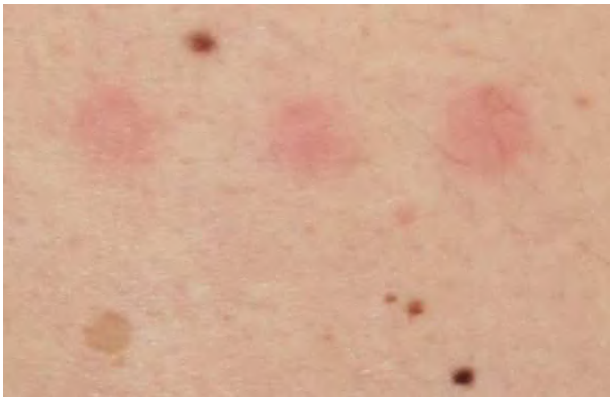
Volunteer
ALBA346
T1+60 min



Volunteer
ALBA346
T1+ 120 min



Volunteer
ALBA346
T1+ 240 min



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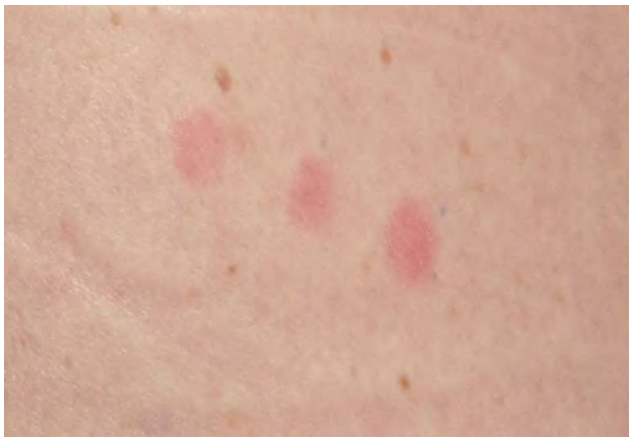
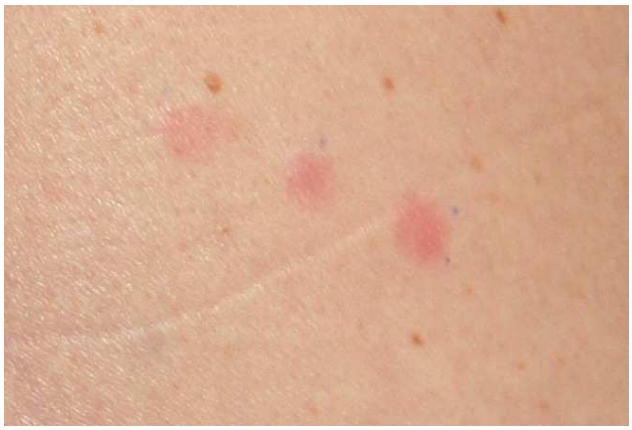
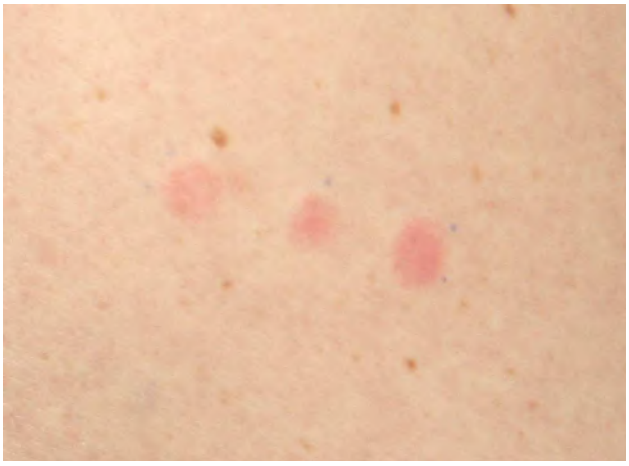
Volunteer
MAPE 215
T1



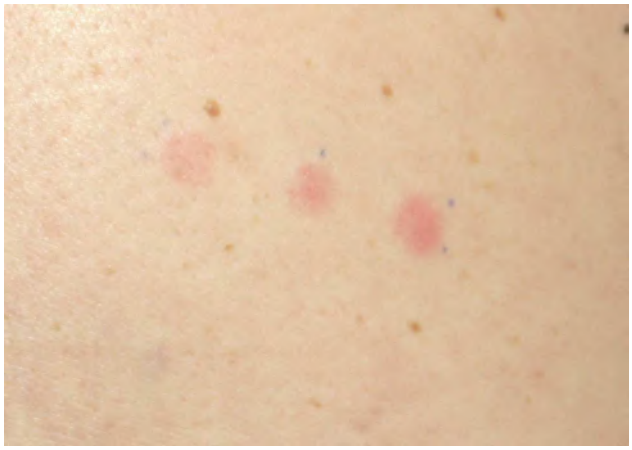
Volunteer
MAPE 215
T1 + 60 min



Volunteer
MAPE 215
T1 + 120 min



Volunteer
MAPE 215
T1 + 240 min



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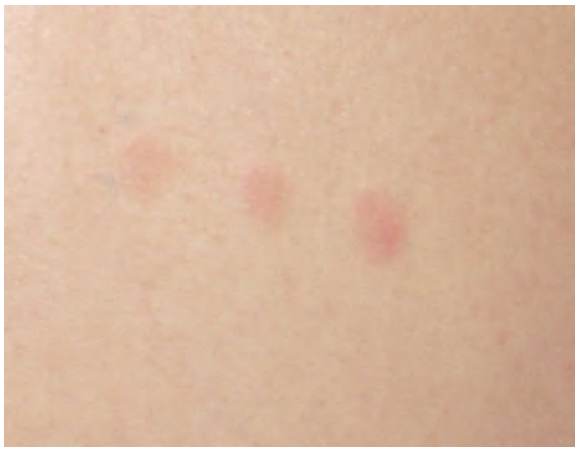
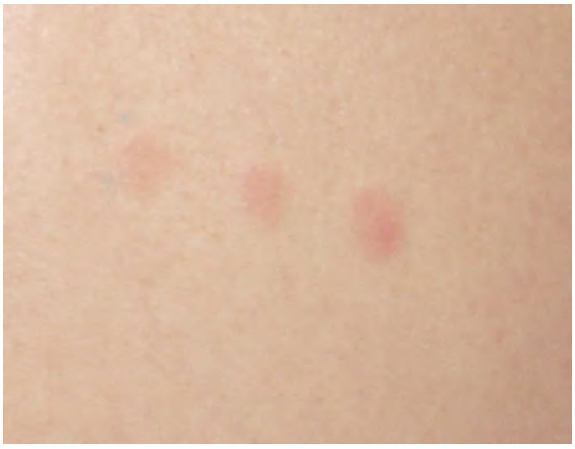
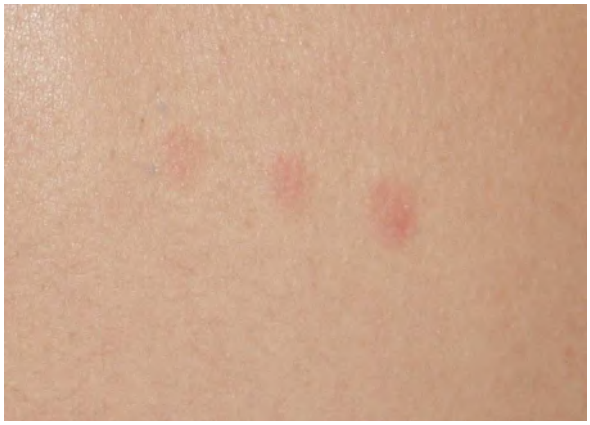
Volunteer
VEPA 284
T1



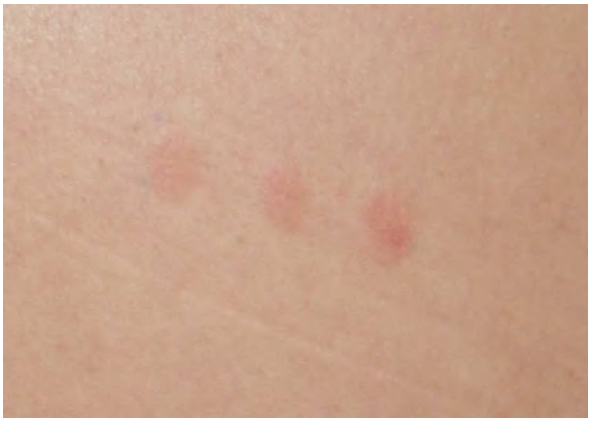
Volunteer
VEPA 284
T1 + 60 min



Volunteer
VEPA 284
T1 + 120 min



Volunteer
VEPA 284
T1 + 240 min



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CONCLUSION & COSMETIC BENEFITS

Skin is the first line of defense against a variety of physical, chemical and microbiological assaults which can lead to structural and biological damage. This cumulative damage to our skin increases with age and can result in chronic inflammation.

OCEA DEFENCE® is an oily active prepared from the brown macroalga *Laminaria ochroleuca*, capable to help the skin react to such aggressions and repair damage by boosting skin's own self defense mechanisms and act as an anti-inflammatory agent.

By reinforcing the cutaneous shield & fighting inflammation, OCEA DEFENCE® boosts skin's own self defence mechanisms. The skin becomes better protected against the risk of infection and irritation.

OCEA DEFENCE® prevents skin redness caused by the sun with a short term efficacy (30 min).

OCEA DEFENCE® is new marine approach to regulate the level of tolerance of sensitive & vulnerable skin, against various cutaneous disorders from the external environment *e.g.* microbial infection, oxidative stress, pollution, UV radiations.

OCEA DEFENCE® is designed for improving cutaneous comfort for sensitive skin and preserving the youthful appearance of fragile and vulnerable skin.

COSMETIC APPLICATIONS

Treatment for sensitive and vulnerable skin
Anti-redness products
Protective care
Sun care & after sun formulations.

Recommended use level: 2%.

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Vérifié par ECOCERT MATIÈRE PREMIÈRE COSMOS APPROVED
CHINA LISTED 2014

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ANNEX

Evaluation of ocular irritation

eurofins | ATS

N° d'étude : 515117F01
Version : 01
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P04.3.DPL.00014.06

SUMMARY

The HET-CAM test is an organotypic method to detect the potential irritancy of compounds applied on the surface of the chorioallantoic membrane (CAM) of a fertilized hen's egg. The CAM is a vascular foetal membrane which represents an in vitro model to analyse the effects induced by chemicals that *in vivo* are observed on the conjunctiva.

The principle of this test is based on a visual observation, by a trained person, of the possible end-points (hyperaemia, haemorrhaging, coagulation / thrombosis) that may appear during the five minutes that follow the application of the product on this membrane.

This method is registered in the Official Journal of French Republic (JORF - Decree of 5 April 1971 modified by the decree of 29 November 1996).

In the performed experimental conditions, the **product OCEA DEFENCE, referenced 12 11 270**, tested by the HET-CAM method at 10 % and according to the JORF classification, is considered as **moderately irritant**.

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Evaluation of cutaneous irritation

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Version N° 1
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STUDY SUMMARY

ASSESSMENT OF SKIN TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER OCCLUDED PATCH DURING 48H ON 10 VOLUNTEERS: *48 hours patch tests*

- **Product tested:** OCEA DEFENCE
- **Promotor:** Liliane PELLEGRINI, GELYMA
- **Objective:** Assessment of the skin local tolerance of the studied product after an epicutaneous test performed in occluded conditions, during 48 hours, on healthy adult volunteers.
- **Investigator:** Doctor Chantal SOULIE-REGNIER, dermatologist
- **Place of the study:** EUROFINS ATS
  Pôle d'activité Aix-Les-Milles - ACTIMART
  3 allée des Ingénieurs
  1140 rue André Ampère
  13851 AIX EN PROVENCE cedex 3
- **Dates of study:** from 12/5/2012 to 12/7/2012
- **Method:**

✓ ***Application:***

Area: on the back
Quantity of product: 0.02 mL
Frequency and duration: only one application during 48 hours
Conditions of application: product applied diluted at 10% under occluded patch

✓ ***Assessment method:***

A dermatologist performs the clinical observation, after the removal of the patches. The quantification of the skin irritation is given through a numeric scale (erythema, oedema, dryness/desquamation, vesicle). The average irritant score of the product to be tested is calculated from the average of the quotations obtained for each volunteer, allowing to rank the product from "non irritant to very irritant" The assessment is always made by comparison with the "negative" control.

- **Panel:** 10 healthy adult volunteers.
- **Result:** The average irritant score of the product is 0.00.
- **Conclusion:**

According to the experimental conditions of the study, **the OCEA DEFENCE product, referenced BATCH 12 11 270**, can be considered as **non irritant regarding its primary skin tolerance.**

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