DERMOCEA®

For a plumper and firmer skin

*

Wards off skin aging

Restructures the cutaneous framework

Gelyma

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SUMMARY

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INTRODUCTION

Our skin is the largest organ of the human body. It consists of two layers namely the epidermis and the dermis.

The epidermis is the uppermost and the thinnest layer of the skin. Keratinocytes constitute the main cell type, approximately 80% of the epidermal cells. About 85% of the total protein content of keratinocytes is keratins that constitute the corneal layer of the epidermis. This corneal layer protects the skin against harmful external factors such as heat, cold and dehydration. The epidermis gives the skin its impermeability and resistance. It is constantly renewed throughout life.

The epidermis is connected to the dermis through a zone named the dermal-epidermal junction (DEJ) or epidermal basal membrane.

The dermis is the inner layer of skin, responsible for both basic cohesion and nutrition functions. It is a conjunctive tissue including cells (fibroblasts) dispersed in a complex medium named the extracellular matrix. This matrix is made of collagen and elastin fibres, glycoproteins (fibronectin and laminin) and proteoglycans (central protein + glycosaminoglycans (GAGs).

- Collagen provides skin firmness.
- Elastin supplies skin elasticity.
- Glycoproteins and proteoglycans keep the skin hydrated.

Skin ageing is a complex biological process which is mediated by a combination of the effects of time (intrinsic ageing) and environmental factors (extrinsic ageing or photoageing) on cellular and extracellular infrastructure.

As a result of skin ageing, important changes affect epidermis and dermis levels.

The epidermis is the first affected. The ability of keratinocytes to divide in the basal layer decreases. The renewal time for the upper corneal layer becomes longer. Cell maturation is imperfect. The basal layer appears more regular and homogeneous. The epidermis becomes less thick, more dry and rougher in appearance.

At the same time, all components of the dermis are affected by important changes, especially glycosaminoglycans and collagen. The synthesis of GAGs decreases affecting moisture level in the dermis. The skin becomes thinner and less supple. The collagen fibers are constantly renewed but this renewal decreases with age. The reduced amount of collagen explains the reduced skin thickness and the loss of dermal firmness.

Moreover, GAGS as well as collagen fibers are susceptible to certain enzymes. Hyaluronidases degrade GAGs such as hyaluronan. Metalloproteinases, especially collagenases deteriorate collagen.

The result of all this is that the skin appears drier, saggy and wrinkled.

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In order to minimize such signs of ageing, GELYMA offers DERMOCEA® prepared from two innovative red seaweeds:

- *Meristotheca dakarensis* (: *M. senegalensis*)
- *Jania rubens,* a crustose alga known as coral moss.

DERMOCEA® acts on several levels synergistically, both in the epidermis and dermis.

In the epidermis, DERMOCEA® increases significantly the expression of the keratin 14 that plays a pivotal role in the maintenance of epidermal cell shape. K14 is also known as a skin biomarker indicative of effects on epidermal keratinocyte differentiation.

Due to this epidermal restructuring activity, DERMOCEA® improves skin cohesion.

In the dermis, DERMOCEA®

- increases significantly the GAGs synthesis
- boosts collagens synthesis, especially collagen I and collagen III synthesis
- reduces the degradation of matrix macromolecules by inhibiting both the action of
  - hyaluronidases that damage hyaluronan, the major GAGs
  - metalloproteinases, especially MMP-1, a key enzyme in collagen degradation.

By stimulating the synthesis of matrix macromolecules and preventing their enzymatic degradation, DERMOCEA® maintains the hydration of dermal tissues, increases skin firmness and reduces saggy skin.

With its conjugated beneficial effects on both the epidermis and the dermis, DERMOCEA® is an innovative marine approach for the prevention and treatment of the major signs of ageing.

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ALGAL SOURCE

DERMOCEA® is prepared from two red seaweeds:

- *Meristotheca dakarensis (: M. senegalensis)*
- *Jania rubens*

Meristotheca dakarensis

➤ Classification

The species *Meristotheca dakarensis* belongs to:

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Rhodophyta*
Class	*Florideophyceae*
Order	Gigartinales
Family	*Solieriaceae*
Genus	*Meristotheca* J.Agardh,1872
Species	*dakarensis* Faye & Masuda , 2004.

E.J. Faye *et al.* (2004-Cryptogamie, Algologie 25 (3) :241-259) consider that the species referred to as *Meristotheca senegalensis* J. Feldmann by several Phycologists is likely to be equivalent to *M. dakarensis* (*cf* page 256). Gene analyses prove that this species is different than *Meristotheca papulosa* found in Japan.

➤ Morphology & Biology

The erect thallus of *Meristotheca dakarensis* is foliose with a blade irregularly dichotomously divide four to twelve times arising from a discoid holdfast (Fig.1).

Marginal proliferations may be present. They are simple or dichotomously divided. They bear reproductive structures.

The thallus can reach 10-30 cm high. It is deeply rose-red or deeply reddish brown in color.

Blades are multiaxial and consist of a pseudoparenchymatous cortex and a filamentous medulla.

Fig.1 – Morphology of *Meristotheca dakarensis*
Photo Gelyma

Sexual thalli are dioecious.

The sexual reproduction appears complex with tetrasporangia zonately divided.

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Young germlings consisting of one to several bladelets are attached to various parts of some female, male and tetrasporangial blades. These germlings are distinguished from marginal proliferations by the presence of the basal attachment disc.

➤ Ecology & Geographical distribution

Meristotheca species occur in warm temperate and tropical areas around the world.

Meristotheca dakarensis is an endemic of Senegal shores (western Africa) where it grows in the upper sublittoral zone between 0 to -6 m.

➤ Chemical composition

Different studies describe the chemical composition of this red seaweed.

Meristotheca dakarensis contains an iota-kappa hybrid carrageenan, the iota part being partially non-sulfated (A.H. Fostier *et al.*, 992 – Botanica marina 35: 351-355). Some amounts of nu-carrageenan may be present too.

The total content of carrageenans can reach 30-36% (dry weight) according the extraction method applied.

Meristotheca dakarensis includes numerous fatty acids, the majors being:

16:0	41.4 % total fatty acids
20:5 ?3	10.2 %
20:4 ?6	3.6%

(J. Miralles *et al.*, 1990 – Phytochemistry 29 (7):2161-2163; M. Aknin *et al.*, 1990 – Comp. Biochem. Physiol., 96B (3): 559-563).

The major sterol is cholesterol: 85.0 % total sterols (cf. M. Aknin *et al.*, 1990 *Ibid*) .

Meristotheca dakarensis contains different triterpenes and oleanolic acid (M.S. Diop & A. Samb., 2000- J. Soc Ouest Africaine de Chimie 6 (10) 25-32).

➤ Utilizations

Meristotheca dakarensis is an economically valuable species for industry and human food.

The polysaccharide serves as a gelling agent in food technology.

The Senegal exports large quantities of this red seaweed to Japan for food.

The figure 2 shows the preparation of this alga in Senegal for carrageenan industry.

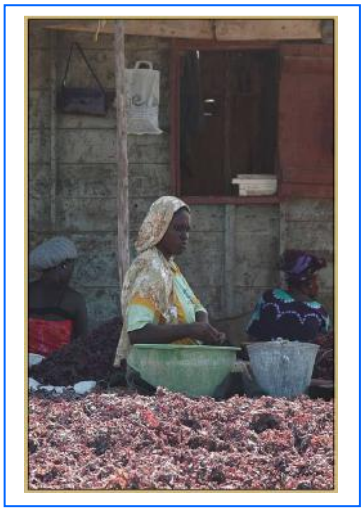

Fig.2

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Jania rubens

➤ Classification

The species *Jania rubens* belongs to:

Empire	*Eukaryota*
Kingdom	*Plantae*
Phylum	*Rhodophyta*
Class	*Florideophyceae*
Order	Corallinales
Family	*Corallinaceae*
Genus	*Jania* J.V. Lamouroux , 1812
Species	*rubens* (Linnaeus) J.V. Lamouroux , 1816.

• Synonyms

Corallina rubens Linnaeus 1758
Jania rubens var. *spermophoros* (Linnaeus) J.V. Lamouroux 1816
Jania spermophorus J.V. Lamourous 1843.

The genus *Jania* has often been regarded as a subgenus of *Corallina.*

Jania named from Janira , one of the Nereides.

• Common name

Coral moss.

➤ Morphology & Biology

Jania rubens shows articulated, branched and calcified fronds fixed by small conical disks to solid substratum. Branching is dichotomous.

The thallus is rose-pink in color and can reach 2-5 cm high.

Fig.3 – Morphology of *Jania rubens* - Photos Gelyma

a : in herbarium b : *in situ*

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The growth is apical.

Jania rubens shows an annual cycle of development, the young fronds arising in autumn, overwintering and becoming fertile in april-may. The reproductive structures are produced in conceptacles.

➤ Ecology & Geographical distribution

Jania rubens is a marine crustose species forming tufts either directly on substratum or epiphytically on brown seaweeds.

This alga is common in tropical, subtropical and warm temperate areas. It is present in Europe, North America, Central America, South America, Africa. It is also quoted in Asia, Australia and New Zealand and along the sea shores of Indian Ocean Islands, Pacific Islands and Caribbean Islands.

➤ Chemical composition

Jania rubens is a calcified alga, so it contains numerous minerals. The total ash content may reach 86.34% DW in samples collected in the Red Sea (A.N. Khalil & B.A.H. El-Tawil – 1982 – Bull. Fac. Sci. K.A.U. Jeddah, 6 : 49-60).

This species shows interesting contents in protein: 25.34% DW and carbohydrates: 68.66% DW.

The main acidic polysaccharides is a corallinan, an agar-like xylogalactan (N. Da Stortz, 2008 – Carbohydrate Res. 13 , 343 (15) : 2613-2622).

The alga contains 0.8% mannitol (F.M. Soliman *et al.,* 1994 - J. Drug Res. Egypt 21: 165-180).

A complete study of the amino acids composition of *Jania rubens* has been performed (G. Balansard *et al.*, 1982 – Ann. pharmaceutiques 40 (6) 527-534).

Jania rubens contains interesting amounts of free amino acids such as:

Proline	38.60 % total amino acids
Alanine	19.23 %
Methionine	15.90 %
Glutamic acid	12.54 %
Glycine	7.15 %
Valine	6.48 %.

Because of this special chemical composition in minerals and amino acids, *Jania rubens* shows has been chosen to prepare DERMOCEA®.

The fatty acids content has also been studied. The total saturated, monoenoic and polyunsaturated fatty acids amounted to 42.1%, 11.8% and 45. 2% respectively (M. Aknin *et al.*, -1990 – Comp. Biochem. Physiol., 96 B: 559-563).

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Cholesterol is reported as the main constituent of the sterol fraction of *Jania* species (J.A. Palermo *et al.*, 1990- Ann. Asoc. Quim. Argent. 78: 157-159).

The lipid fraction (0.35%) of *Jania rubens* contains saturated hydrocarbons (F.M. Soliman *et al*., 1992 – Bull. Fac. Pharm., Cairo Univ. 30: 283-296).

Vitamins are also present, especially ascorbic acid (vitamin C: 19 mg% w/w of DW) and thiamine (vitamin B1: 0.5mg % w/w) (*cf.* Soliman *et al.,* 1992 – *Ibid*.).

Jania rubens contains volatile compounds that consists in *n*-docosane (6.35%) *n*-eicosane (5.77%) and *n*-tetratriacontane (5.58%) as major components (N. Ulku Karabay-Yavasoglu *et al.*, 2006- Phytotherapy Research 21 (2): 153-156).

The presence of brominated diterpenes has also been quoted and identified as isoparguenol, isoparguenol-16-acetate, deoxyparguenol …. (Nagwa E. Awad – 2004- Phytotherapy Resaerch 18 (4) : 275-279).

➤ Bioactivities & Utilizations

Jania rubens shows hypoglycemic, fibrinolytic and lipolytic activities (H.A. Hoppe, 1979 – in Marine Algae in Pharmaceutical Sciences, 215-219, Walter de Gruyter, Berlin).

It has also been used in treatment of intestinal ulceration and in radioisotope intoxication (M.H. Baslow , 1969 – Marine Pharmacology , 296 pages , Baltimore , USA).

The methanol and chloroform extracts of *Jania rubens* shows potent antimicrobial activity due to the presence of volatile compounds previously described.

The isolated diterpenes isolated from *Jania rubens* show a marked antitumor activity and anthelmintic activities on earthworms (F.M. Soliman *et al.*, 1994- J. Drug. Res. Egypt, 21: 155-164).

Jania rubens is quoted as vermifuge alga (V.J. Chapman & D.J. Chapman -1980 – Seaweeds and their uses 1-334, Chapman & Hall, London & N.Y).

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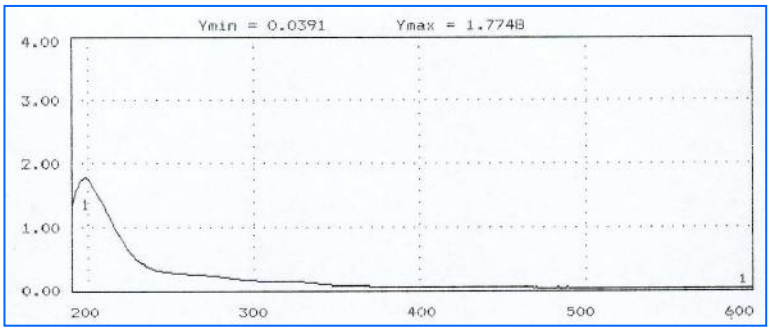
THE ACTIVE INGREDIENT DERMOCEA®

Specifications

on a control batch

- Appearance : liquid limpid
- color : yellow light to dark
- odour : typical
- pH : 6.0 ± 1
- density : 1.20 ± 0.20
- solubility : insoluble in oils

- UV visible spectrum (5% in water):



- microbiology : bacteria : < 100 germs / ml
  : yeasts, moulds : < 100 germs / ml
  : pathogens : free.

Composition

Ingredients	Amounts %
water	73
saccharose	20
Meristotheca dakarensis extract	4
Jania rubens extract	3

Addition of preservative as required.

INCI names water CAS n° 7732-18-5 EINECS n° 231-791-2
Sucrose CAS n° 57-50-1 EINECS n° 200-334-9
Meristotheca dakarensis extract
Jania rubens extract

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Mineral composition

(on a control batch)

➢ Macrominerals (mg/Kg)

Sodium	: 4922
Calcium	: 1629
Potassium	: 339
Magnesium	: 248

➢ Trace minerals (mg/Kg)

Silicium	: 29
Iron	: 1.3
Manganese	: 1.0
Zinc	: < 0.5
Copper	: < 0.5
Selenium	: < 0.5
Iodine	: 0.98

➢ Heavy metals (mg/Kg)

Arsenic	: 0.11
Cadmium	: < 0.05
Mercury	: < 0.05
Lead	: < 0.05

Sodium and **potassium** works closely to maintain the cells osmotic balance.

Sodium improves the permeability of the skin. After penetrating into the skin, the sodium ions bind the water and generate a feeling of suppleness, ideal for a very dry skin.

Potassium is involved in various metabolic functions including protein synthesis, carbohydrate metabolism and several enzyme activations.

Calcium and **magnesium** are necessary to promote conjunctive cell metabolism.

Calcium is a main ingredient in skin. It is greatly implicated in mineralization process of the skin. It plays an optimal role in cellular regulation. It acts on all skin components: differentiation of epidermal cells the structure of the dermal-epidermal junction, the cohesion and communication between the epidermis and dermis. Keratins development and desmosomes formation are highly dependent on calcium but this requires calcium bioavailability. This calcium bioavailability decreases in time and that is one of the several causes of skin ageing.

Magnesium activates numerous enzymes. It increases cell defence mechanisms. It is involved in collagen synthesis.

Silicium has a stimulating action, skin renovation is faster. It helps restructure conjunctive tissue and regenerates collagen and elastin fibres. So it improves the elasticity of the skin. It may also protect cells against free radicals damage.

Iron is indispensable to oxygen transport towards cells and tissues. It acts on collagen synthesis.

Manganese increases cell defence reactions through its antioxidant properties and its participation in the Mn-SOD structure.

Storage

DERMOCEA® should be stored in the original sealed containers in a clean and dry place, at a temperature between 15°C and 25°C. If stored under the advised conditions, DERMOCEA® remains stable for at least 18 months.

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

Safety

No animal experimentation

Standard safety testing has proved that DERMOCEA® is safe for cosmetic use.

Patch assay on 10 healthy adult volunteers during 48h has shown that at 100% DERMOCEA® can be considered as not irritant regarding its primary skin tolerance.

Het Cam method has shown that at 10% DERMOCEA® can be considered as moderately irritant regarding its ocular primary tolerance.

cf. annex pp.25-26.

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EFFECTIVENESS EVALUATION

DERMOCEA®

Action on the epidermis ..... Epidermal restructuring activity

Improvement of keratinocyte differentiation	→	Overexpression of keratin K14 Immunohistochemical study on human skin biopsies

Action on the dermis ..... Reduction of saggy skin

GAGs	Stimulation of GAGs synthesis	→	Overexpression of GAGs Immunohistochemical study on human skin biopsies
	Protection of hyaluronan from degradation	→	Inhibition of hyaluronidase activity In tubo test
Collagens	Stimulation of collagen I & collagen III synthesis	→	Overexpression of collagen I Immunohistochemical study on human skin biopsies
			Overexpression of collagen III Immunohistochemical study on human skin biopsies
			Increase of the production of procollagen I ELISA testing on human fibroblasts
	Protection of collagen from degradation	→	Inhibition of MMP1 activity ELISA testing on human fibroblasts

An innovative marine approach
for providing a plumper & denser skin

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Action on the epidermis …….. Epidermal restructuring activity

The epidermis decreases in thickness with age (L. Baumann, 2007 –J. Pathol. 211: 241-251).

Epidermal thickness decreases at about 6.4% per decade, decreasing faster in women than in men. Changes are most pronounced in exposed areas such as the face and the neck.

As skin ages, the epidermal turnover rate decreases. Cells of the basal layer become less uniform in size. Keratinocytes that compose approximately 80% of the cells in the epidermis change shape too and become shorter and fatter.

Improvement of keratinocyte differentiation

About 85% of the total protein content of keratinocytes is keratins.

Keratins are a group of structural proteins of epithelial cells that can be divided in two subgroups based on biochemical properties such as molecular weight and isoelectric point.

Keratins polymerize to form intermediate filaments which are abundant in particular in the suprabasal and basal layers of the epidermis.

They connect to desmosomes (X. Cheng & P.J. Koch, 2004 – J. Dermatol. 31: 171-187) at cell-cell junctions and, to hemidesmosomes (M.G. Nievers *et al.*, 1999- Matrix Biol., 18: 5-17) at the interface of epidermis and dermis. They constitute a three-dimensional network of fibrous proteins that crosses the epidermis.

Keratins are indispensable for the normal development and maintenance of stratified epithelia. They have a major function in providing stability to epithelial cells under conditions of mechanical stress.

It exist different kinds of keratins (K1-K23) selectively expressed in the different layers of the epidermis. The network is formed from several combinations of keratins.

The keratin 14 (K14) is a low molecular weight keratin, specifically expressed by keratinocytes throughout the differentiating suprabasal layers of the epidermis (*cf.* in D.G. Allen & N.A. Monteiro-Rivière, 1999 – Pharmaceutical Research, 16:1487).

The keratin 14 is known as a marker of the cell renewal.

More recent studies suggest that the keratin 14 may also play additional role in epithelial differentiation, especially in the assembly of the keratin intermediate filament network.

Without the K14, keratin intermediate filaments are unable to assemble into a strong, stable network.
A disruption of this network makes keratinocytes fragile and prone to rupture and consequently can cause skin fragility disorders or abnormalities.

It has also been proved that in humans, mutations of the K14 can lead to important disorders (A.D. Irvine & McLean W.H., 1999- Br. J. Dermatol. 140: 815-828).

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Overexpression of keratin K 14

All *ex vivo* studies included in this report have been performed at Laboratory BIO-EC (Longjumeau – France).

24 skin explants were obtained from an abdominal plastic surgery of a healthy Caucasian woman (53 years old). They are cultured in a specific survival explants medium: BEM (BIO-EC's Explants Medium).

2 mg of a formulation (Carbopol gel) containing 2% of DERMOCEA® were applied on the skin stripes at the following times: Day O, Day 2, Day 5 and Day 7. The results are compared with untreated explants.

Explants were taken off at D0 and D9. Each explant was cut into two parts : one half was fixed in ordinary Bouin solution for previous morphological analysis while the other half was frozen at -75°C for immunostaining.

Optical observations have been performed by using a microscope Leica type DMLB equipped with a camera Olympus DP 72.

Methods

The immunohistochemical study relative to the expression of K14 was carried out on frozen skin sections.

Specific immunolabelling of keratin K14 was performed thanks to monoclonal antibodies Anti-keratin 14 (PROGEN ref 10003) applied for 1 hour at room temperature and revealed by FITC. Cells nuclei were stained with propodium iodine.

Keratin 14 can then be observed in the DJE area due to fluorescent marking.

The analysis of epidermis thickness filled by basal K14 has been performed by using the software LEICA QWIN.

Results

None labelling has been observed when the saline buffer solution PBS can be used in substitute for the antibody.

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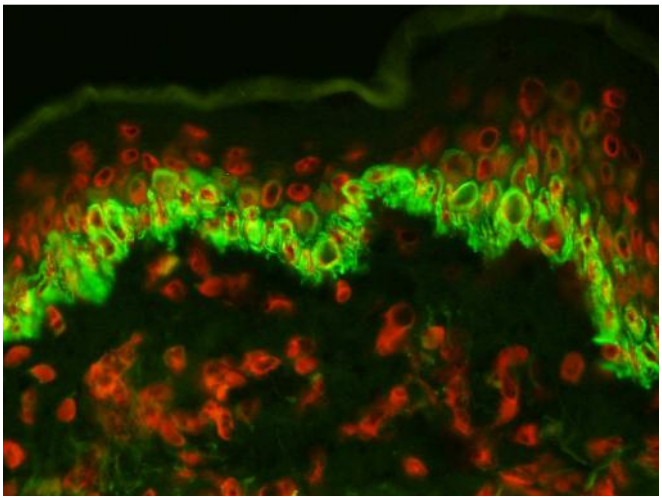
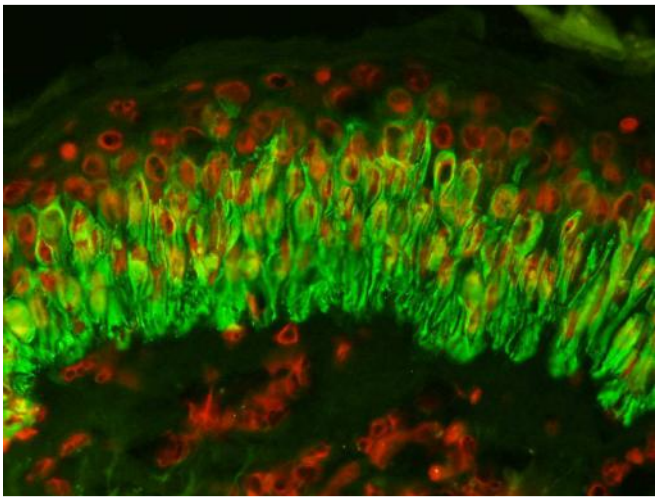
Control explants
without treatment

The labelling appears clearly and regular on 1-2 basal cells layers .

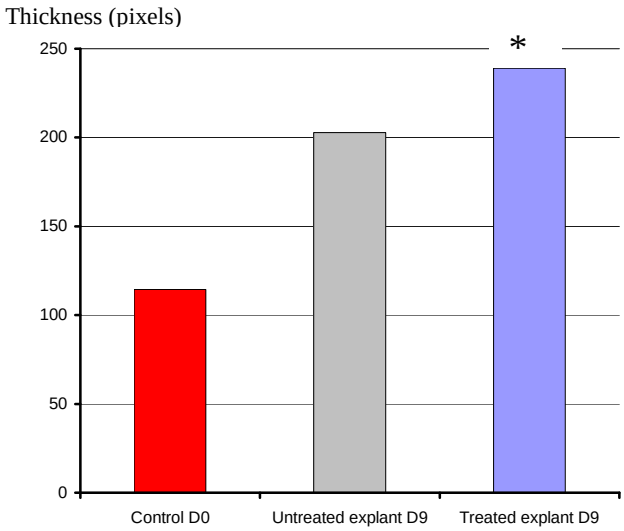


Treated explants
with 2% DERMOCEA®

The marking is very clearly apparent on the 4-5 basal cells layer.



The application of a gel with 2% DERMOCEA® shows a significant increase of the marking thickness of basal keratin of 18% compared to the untreated explants.



* significant compared to untreated explant D9

- At 2% , DERMOCEA® increases the expression of the keratin K14 by the keratinocytes in a significant way.

- DERMOCEA® improves the keratinocyte differentiation and helps to maintain an efficient skin epithelial integrity.

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Action on the dermis…….. Reduction of saggy skin

The extracellular matrix (ECM) is the largest component of the dermal skin layer. It gives the skin its unique mechanical properties, including firmness, strength, suppleness and elasticity.

To a large degree, the signs of ageing reflect the condition of the skin matrix.

The skin matrix is both produced and consumed.

- On one hand, skin matrix is continuously synthesized by fibroblasts.

- On the other hand, skin matrix is broken down by different enzymes, especially hyaluronidases and matrix metalloproteinases (MMPs).

In a healthy, youthful skin, the synthesis and degradation of the matrix are in balance: damaged matrix is degraded while the deficit is replenishes by the ongoing synthesis.

Unfortunately, when we age, this balance gets disrupted: too little of the matrix is synthesized and too much is degraded. The skin begins to loose firmness and appears saggy.

The degradation of the skin matrix plays an important role in the development of wrinkles and other signs of skin ageing. The thinning of the dermis occurs due to a decrease of both glycosaminoglycans and collagen synthesis.

So to fight against this imbalance, and therefore to prevent saggy skin, two solutions:

- either boosting synthesis of the macromolecules of the matrix and
  ⇒ consequently maintaining and rebuilding skin matrix

- or inhibiting the breakdown or the matrix degradation and
  ⇒ consequently counteracting damage.

DERMOCEA® is able both to

- stimulate the matrix synthesis, by boosting the synthesis of
  - glycosaminoglycans and
  - collagens, particularly collagens I & III,
- reduce the matrix degradation, especially by inhibiting
  - hyaluronidase that damage hyaluronan, the major GAGs
  - MMP-1 (type 1 collagenase), a key enzyme in collagen degradation.

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Stimulation of glycosaminoglycans (GAGs) synthesis

Proteoglycans and glycosaminoglycans (GAGs) play in the skin an important role in organizing structurally water at a molecular level in the dermis that means a significant contribution to

- the structure of the extracellular matrix

- skin moisturizing and elasticity.

Indeed, in the dermis, proteoglycans / GAGs molecules interact with collagen fibers, that allows an optimal orientation, the good stability of the dermis structure and skin firmness.

The structure of GAGs consists of long polysaccharide chains that confer negative charges on the proteoglycans allowing them to "capture" ions, water and various metabolites. They are extremely effective natural moisturizers and contribute to skin moisture and resistance to pressure and stretch forces.

Therefore, GAGs help the skin stay plump and fresh.

With age, the synthesis of GAGs decreases, the major result is the loss of moisture. The decrease of GAGs content may also impair the skin's ability to repair itself and possible affects the synthesis of other skin matrix components. Consequently the biomechanical properties of skin become altered. The skin turns out thinner, drier and less supple.

Overexpression of glycosaminoglycans

This *ex vivo* study has been performed at Laboratory BIO-EC (Longjumeau – France).

Method

Specific immunostaining of GAGs was performed by Mowry staining method (Alcian blue stain).
It is enables to visualize the GAGs present in the papillary dermis and along the dermal-epidermal junction due to pink-violet staining.

The analysis of epidermis thickness filled by basal K14 has been performed by using the software LEICA QWIN.

Results

Results are illustrated next page.

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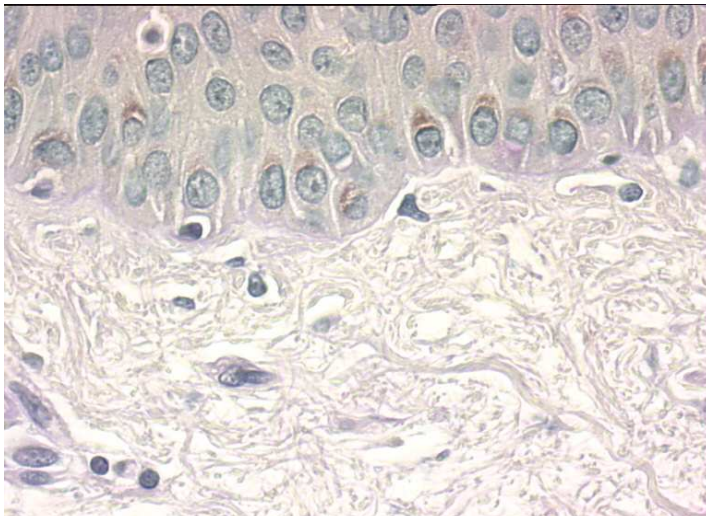
Control explants
without treatment

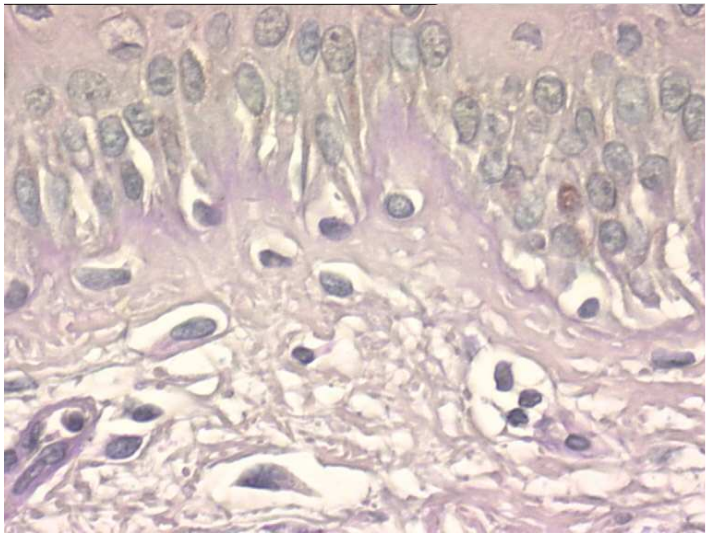
GAGs form a pink-violet band, irregular and thin along the DEJ. The marking is weak in the papillary dermis.



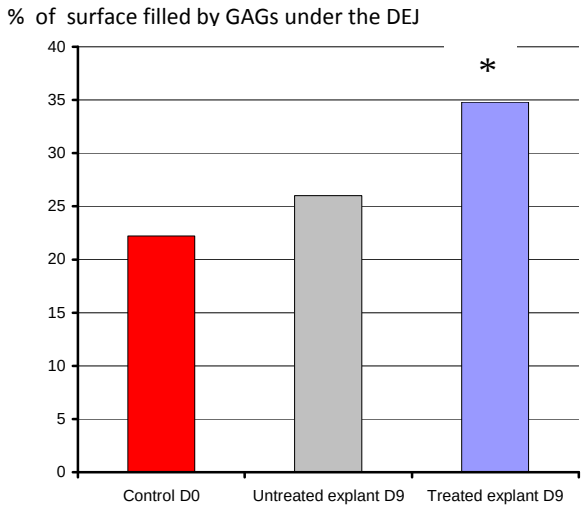
Treated explants
with 2% DERMOCEA®

The application of a gel with 2% DERMOCEA® after 9 days treatment induces a clear overexpression of GAGs along the DEJ.

The pink-violet staining is regular and variously important along the DEJ. It is also present in the remaining papillary dermis but more slight.



The percentage of the surface occupied by GAGs for the explants treated DERMOCEA® after 9 days treatment reaches significantly more than 34% compared to untreated explants at Day 9.



* significant compared to untreated explants D9

➤ DERMOCEA® is able to stimulate significantly the biosynthesis of glycosaminoglycans (GAGs) in the dermis

➤ Thus DERMOCEA® helps to enhance skin moisturizing and restore the skin volume.

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Protection of hyaluronan from degradation

Hyaluronan or hyaluronic acid (HA) is the major GAGs. It is present in dermis as well as in epidermis. However the HA content in the dermis is far greater than that in the epidermis. In the dermis, concentrations of about 0.5mg/g wet tissue and in the epidermis about 0.1 mg/g wet tissue have been observed.

The metabolism of HA appears very dynamic, HA being the predominant mechanism for skin moisture.

Hyaluronan undergoes a rapid turnover with a half-life in skin of 1-2 days. However dermal cells synthesize more hyaluronan than they catabolize. This catabolic activity is primarily the result of hyaluronidases. Hyaluronidases break down hyaluronan and lead to skin ageing.

To counteract the ageing process, one way to do is to inhibit the HA-degrading enzyme hyaluronidase.

Inhibition of hyaluronidase

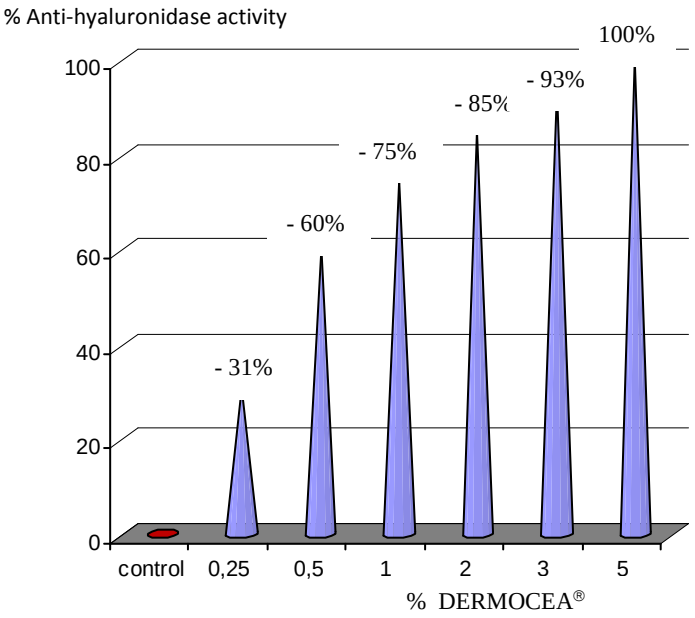
Method

Hyaluronidase activity was determined spectrophotometrically according to the method of Reissig *et al.*, (1955- J. Biol. Chem., 217:959-969).

Results

Results represent the average of three experiments for control and each concentration tested ($\alpha = 0.05$).

They are expressed in % of anti-hyaluronidase activity in presence of 6 concentrations in DERMOCEA® (0.25 -0.5- 1 – 2 – 3 and 5%) compared to control without algal extract.



➤ DERMOCEA® shows a dose-dependant anti-hyaluronidase activity.

With 1% active, the inhibition reaches - 75%.

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Stimulation of collagen I & collagen III synthesis

Collagen is a major structural protein in the skin. It gives the skin its strength and durability. It is responsible in a large part for the smooth plump appearance of young and healthy skin.

Two different collagens: Type I and Type III (collagen I and collagen III) are found in significant amounts in the dermis:

-the Type I (60- 80%) is found in all dermal layers, but it predominates in the deeper layers.

-the Type III (15-20%) is located mainly in the upper dermis.
(*cf.* L.C.V. Junqueira *et al*., 1983 – Histochemistry 79: 397-402).

Collagen synthesis occurs continuously throughout our lives to repair and replace damaged collagen tissue or build new cellular structures.

With age, the secretion of both type collagens decreases. Intrinsic ageing has a dramatic effect on the network of collagen fibers of human skin. Solar ultraviolet irradiation also damages skin collagen.

It has been proved that the biosynthesis of collagen slows a steady decline up about the third or fourth decade of life. This decrease occurs linearly with a 29% loss in secretion ability between 19 to 68 years (M. Dumas *et al*., 1994- Mech. Ageing Dev. 73 (3):179-187). After that, collagen biosynthesis remains at a level that is too low to allow mature skin to repair and replace the collagen that has been lost.

Damage is manifested primarily as the disorganization of collagen fibrils and accumulation of abnormal, amorphous, elastin-containing material. These changes cause premature ageing characterized by skin thickening, rough texture and coarse wrinkles.

So, it is important to promote and protect collagen synthesis, because collagen gives the skin its strength, durability and smooth, plump appearance.

Overexpression of collagen I

Collagen I undergoes in skin continuous turnover which is required for optimal connective-tissue function. This turnover is regulated by both its rate of synthesis and its rate of breakdown.

Imbalance in collagen synthesis and degradation can result in thickened, hardened skin due to collagen build up, or thin, fragile skin due to collagen deficiency.

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Method

This *ex vivo* study has been performed at Laboratory BIO-EC (Longjumeau – France).

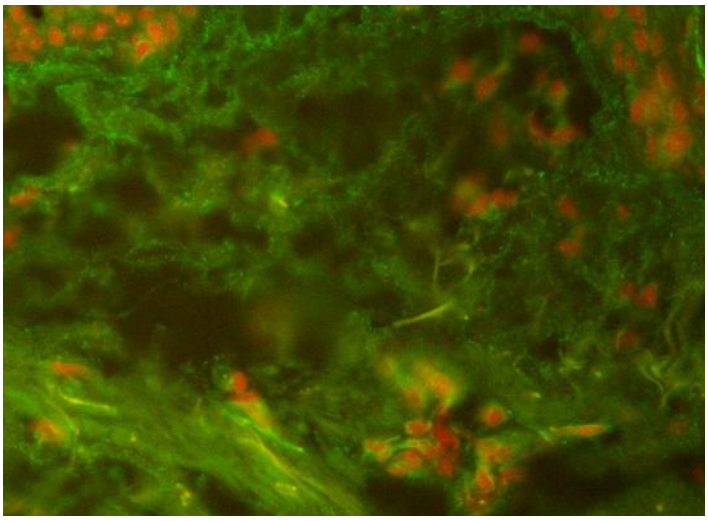
Specific immunolabelling of Collagen I was performed thanks to monoclonal antibodies Anti-collagen I (MONOSAN ref. PS 047) applied for 1 hour at room temperature and revealed by FITC. Cells nuclei were stained with propodium iodine.

Results

None labelling has been observed when the saline buffer solution PBS can be used in substitute for the antibody.

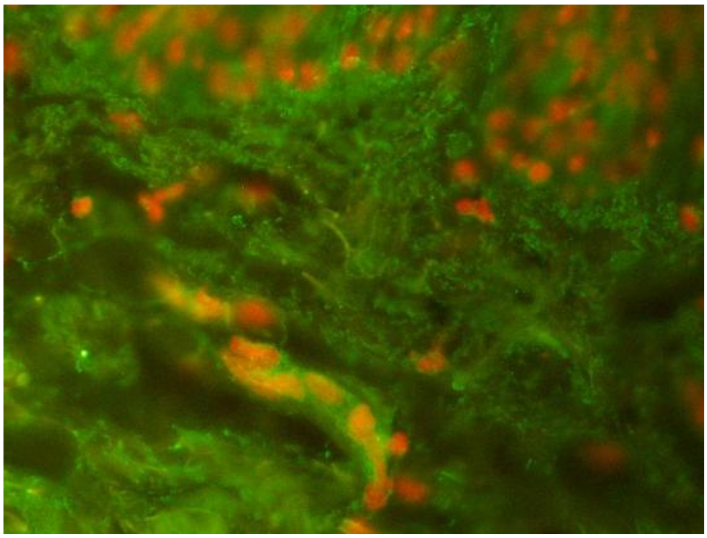
Control explants without treatment

The marking is very weak and less dense in the papillary dermis.



Treated explants with 2% DERMOCEA®

The marking is amplified but remains relatively dense and enough regular in the papillary dermis.



➤ DERMOCEA® included at 2% in a formulation, increases the expression of the collagen I.

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Overexpression of collagen III

Collagen III is predominant in young skin and during wound healing process. It is known as a “restructuring” collagen.

During ageing, the collagen synthesis decreases. Both collagens I and III give its strength, resistance and firmness to the dermal tissue. The ratio of collagen III / collagen I changes and reduces dramatically that induces relative thinning of the extracellular matrix and consequently the formation of facial lines and wrinkles.

An increase of these two components will have a direct impact on the mechanical properties of the skin and therefore improving the skin appearance.

Method

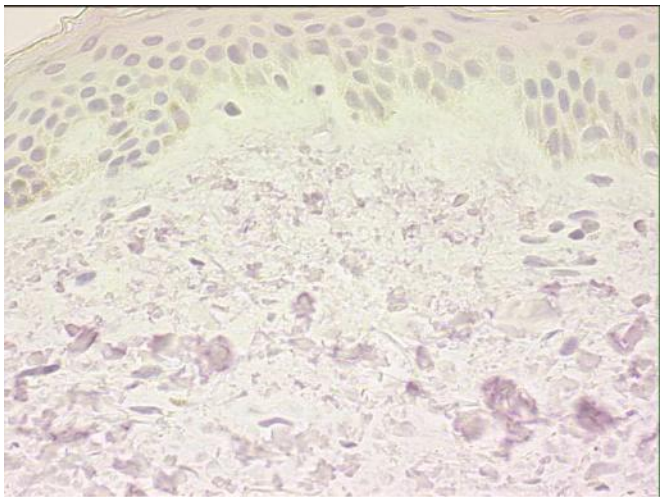
This *ex vivo* study has been performed at Laboratory BIO-EC (Longjumeau – France).

Specific immunolabelling of Collagen III was performed thanks to monoclonal antibodies Anti-collagen III (SBA ref 1330-01) applied for 2 hours at room temperature and revealed by VIP with nuclei stained by Masson hemalun.

Results

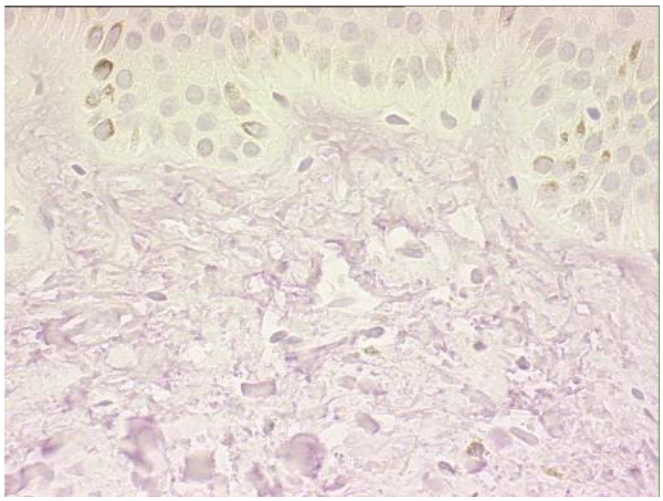
Control explants without treatment

The marking is very feeble, more or less regular and less dense in the papillary dermis.



Treated explants with 2% DERMOCEA®

The marking is moderately dense but appears regular in the papillary dermis.



➤ DERMOCEA® increases the expression of the collagen III.

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Increase of the production of pro-collagen 1

Fibroblasts have a major role in the synthesis of extracellular matrix.

They synthesize and secrete type I pro-collagen (procollagen I).

Pro-collagen I will form mature collagen which spontaneously assembles into thin collagen fibrils.

Because pro-collagen I is a precursor molecule of mature collagen, its level reflects the level of collagen biosynthesis.

Method

This *in vitro* study has been performed at the testing Company SEPhRA (Puteaux –France).

The synthesis of procollagen I has been performed on the supernatant of the cultures of normal human fibroblasts after 48 H cultivation in the presence of 2% DERMOCEA® by using the kit "Procollagen type 1C-peptide (PIP) EIA Kit " ref. MK101 –TANAKA.

The standard was vitamin C 50 µg/ml. The protein amount was evaluated according the bicinchoninic acid method.

Results

Results are quoted below.

	PIP ng/ml	ng/ml/µg prot.	% stimulation
DERMOCEA 2%	347.3 ± 35.0	90.7 ± 18.3	26.3 *
Vitamin C	487.7 ± 50.1	155.2 ± 19.1	116 **

* $p < 0.05$ test of Student
** $p < 0.01$

- DERMOCEA® stimulates significantly the synthesis of pro-collagen I that is the precursor of collagen I
- With 2% DERMOCEA®, the increase of pro-collagen I synthesis is over 26%.

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Protection of collagen from degradation

Mature collagen I in skin undergoes continuous turnover. This turnover is regulated by both its rate of synthesis and its rate of breakdown. The breakdown of collagen fibrils is dependent on the action of collagenases.

Collagenases form part of a family of enzymes called metalloproteinases (MMPs) which are members of a family of proteolytic enzymes (endopeptidases).

These enzymes are present but poorly expressed in normal physiological phenomena such as the renewal of tissues.
However their overexpression and activation are linked to many processes, sometimes pathological, which involve the destruction and remodelling of the matrix due to the degradation of several components (collagen fibers, glycosaminoglycans, fibronectin). This results in either an uncontrolled resorption of extracellular matrix or, conversely the installation of a state of fibrosis.

It exists several well defined groups of MMPs based on their resemblance in terms of structure and substrate specificity (see J.F. Woesser – 1991 – Faseb J. 5: 2145).

The major enzyme responsible for collagen I digestion is the matrix metalloproteinase-1 (MMP-1: interstitial collagenase, matrix collagenase). It degrades collagens I, II, III, VII and X.

This enzyme also plays a significant role in photoageing wrinkle formation and loss of firmness and elasticity (see G. J. Fisher *et al*., 1996 – Nature 379: 335-339; G.J. Fisher & J.J. Voorhees – 1998 – J. Invest. Dermatol. Symp. Proc. 3 : 61-68).

So, the best way to maintain a youthful looking appearance of skin is to prevent future collagen degradation, moreover to protect current collagen levels in order thus to combat skin ageing.

Inhibition of MMP-1

This *in vitro* study has been performed at the testing Company SEPhRA (Puteaux –France).

Method

The ability of DERMOCEA® to inhibit MMP-1 has been evaluated in human fibroblasts treated with 0.5 % and 1 % of active after PMA stimulation (Phorbol Myristate Acetate 10ng/ml) for 24 hours.

Then, the medium below each cultures has been collected and analyzed for secreted MMP-1 by the ELISA kit “Matrix Metalloproteinase-1 MMP-1, human, Biotrak ELISA System”,
Amersham. Each well is in duplicate.

The reference product is the inhibitor GM 1489 (Calbiochem).

The protein amount was evaluated according the bicinchoninic acid method.

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Results

The results of such experiments are included below.

	MMP-1 ng/ml		MMP-1 ng/ml/µg prot.		% inhibition
	NS.	S	NS	S	
Control	5.5 ± 0.9	43.2 ± 0.5	2.1 ± 0.9	16.6 ± 1.7	
DERMOCEA® 0.5%	8.1 ± 1.7	39.2 ± 2.6	1.2 ± 0.3	12.9 ± 2.4	22 *
DERMOCEA® 1%	4.7 ± 1.2	37.0 ± 3.6	1.4 ± 0.7	10.5 ± 1.9	37 **

	MMP-1 ng/ml		MMP-1 ng/ml/µg prot.		% inhibition
	NS.	S	NS	S	
Control	2.6 ± 0.3	26.6 ± 3.6	2.2 ± 0	32.6 ± 1.6	
GM 1489 2µM	1.6 ± 0	21.6 ± 2.8			19 *

* $p < 0.05$ test of Student
** $p < 0.01$
NS no stimulation
S stimulation PMA 10 ng/ml.

➤ DERMOCEA® reduces significantly the amount of MMP-1 that is the major enzyme responsible for collagen I degradation.

➤ At 1%, DERMOCEA® is able to inhibit significantly the expression of MMP-1 by 37%.

By inhibiting the action of MMP-1, DERMOCEA® is an efficient tool to support the dermal matrix consisting mainly of collagen 1.

As a result, the skin keeps its firmness.

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CONCLUSION & COSMETIC BENEFITS

DERMOCEA® is prepared from two innovative red seaweeds:

- *Meristotheca dakarensis* (: *M. senegalensis*), endemic from Senegal
- *Jania rubens,* a crustose species known as coral moss.

DERMOCEA® is a real marine innovation to treat skin ageing more deeply. It targets both in the epidermis and dermis for improving the skin status.

In the epidermis, DERMOCEA® increases significantly the expression of the keratin 14 that plays a key role in the maintenance of epidermal cell shape. K14 is also known as a skin biomarker indicative of effects on epidermal keratinocyte differentiation.

By promoting cell differentiation, DERMOCEA® reinforces the skin epithelial integrity and shows an epidermal restructuring activity.

In the dermis, DERMOCEA®

- ➢ increases GAGs synthesis,
- ➢ boosts collagens synthesis, especially collagen I and III synthesis
- ➢ reduces the degradation of matrix macromolecules by inhibiting both the action of
  - hyaluronidases that damage hyaluronan, the major GAGs
  - metalloproteinases, especially MMP-1, a key enzyme in collagen degradation.

By stimulating the synthesis of matrix macromolecules and preventing their enzymatic degradation, DERMOCEA® promotes restructuring of the dermis in a complete way by enhancing skin moisturizing, restoring skin volume and increasing skin firmness.

With its conjugated effects on both the epidermis and the dermis, DERMOCEA is an innovative marine approach for the prevention and treatment of the major signs of ageing.

As results the skin is plumped up and recovers its volume and firmness.

COSMETIC APPLICATIONS

Anti-ageing skin care products - Firming face and body care - Skin renewal formulations.

Recommended use level: 0.5% - 2%.

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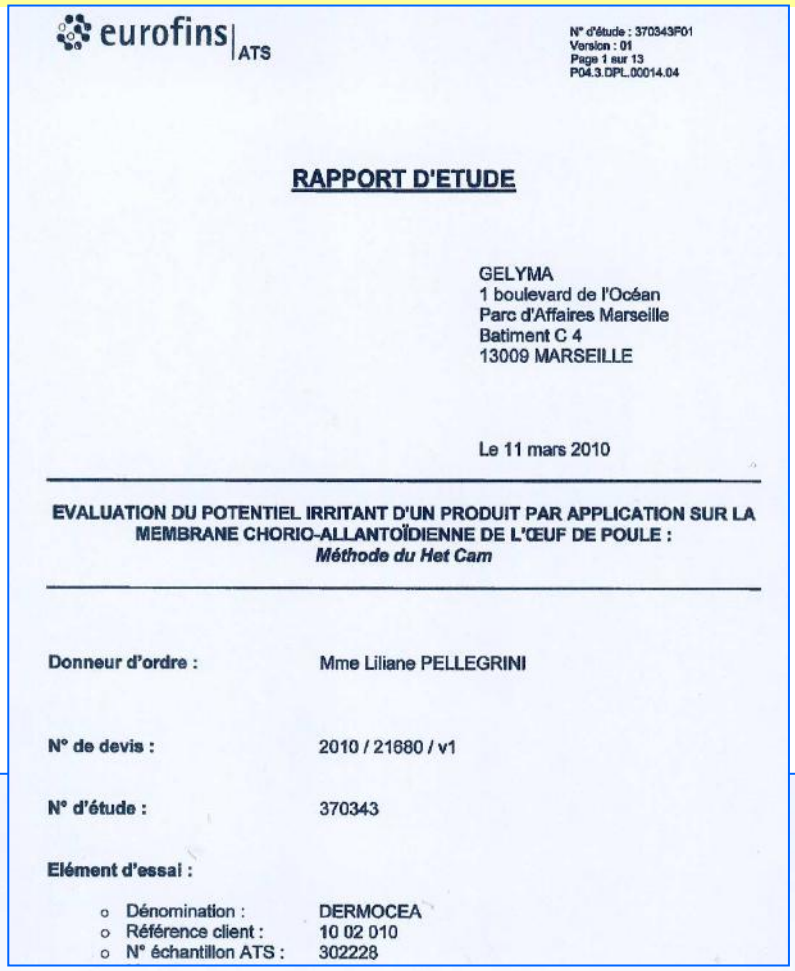
ANNEX

Evaluation of ocular irritation



eurofins | ATS

N° d'étude : 370343P01
Version : 01
Page 1 sur 13
P04.3.DPL.00014.04

RAPPORT D'ETUDE

GELYMA
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Parc d'Affaires Marseille
Batiment C 4
13009 MARSEILLE

Le 11 mars 2010

EVALUATION DU POTENTIEL IRRITANT D'UN PRODUIT PAR APPLICATION SUR LA MEMBRANE CHORIO-ALLANTOÏDIENNE DE L'ŒUF DE POULE :
Méthode du Het Cam

Donneur d'ordre : Mme Liliane PELLEGRINI

N° de devis : 2010 / 21680 / v1

N° d'étude : 370343

Elément d'essai :

- Dénomination : DERMOCEA
- Référence client : 10 02 010
- N° échantillon ATS : 302228

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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 DERMOCEA, referenced 10 02 010**, 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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N° Etude : 370344F01
Version : N° 1
Page: 13/15 + annexe 2
P05.0.DOC.00017.03

STUDY SUMMARY

EVALUATION OF SKIN TOLERANCE OF A COSMETIC PRODUCT AFTER A SINGLE APPLICATION UNDER OCCLUDED PATCH DURING 48H ON 11 VOLUNTEERS:
48 hours occluded patch tests

- **Product tested:** DERMOCEA
- **Promotor:** MADAM 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.
- **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
- **Investigator:** Doctor MARY CREST, DERMATOLOGIST
- **Dates of study:** from 02/03/2010 to 04/03/2010
- **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 pure 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:** 11 healthy adult volunteers.
- **Result:** The average irritant score of the product is 0.00.
- **Conclusion:**

According to the experimental conditions of the study, **the DERMOCEA product, referenced LOT N° 10 02 010**, can be considered as **non irritant regarding its primary skin tolerance.**

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N° SIRET : 33761796300083 – Code APE :7120B

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