

FUCOREPAIR

The anti-ageing for sensitive skin



FUCOREPAIR is composed of fucoses designed for anti-ageing skin care products and particularly suitable for sensitive skins.

Fucose plays a major role in the skin physiology as it is a constituent of the cellular receptors. It allows regulation of inflammation, protection of the extracellular matrix and its regeneration. A clinical trial on FUCOREPAIR, randomized doubleblind against placebo, concerning 42 subjects, highlighted a significant reduction of wrinkles depth. The difference between the active group and the placebo group was on average 9,1 % and up to 18,9 % in the active group (student test $P=0,018$). Moreover, the clinical trial confirmed a better skin tolerance of the product in the active group compared with the placebo.

1- The unique composition of FUCOREPAIR

What are fucoidans?

Fucose is an essential sugar only present in nature, in some mushrooms and brown algae cell walls.

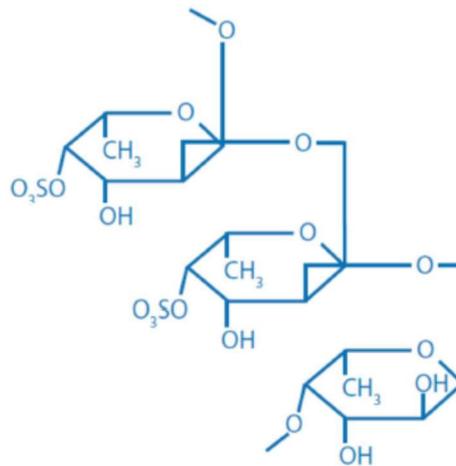
Fucoidans are polymers composed of sulfated fucose. They are insoluble, and are high molecular weight molecules: 50 to 1000 kDalton. They are known to be involved in a wide range of biological activities, including stimulation of cellular metabolism and immunomodulation.

Fucoidans are a class of non-gelling sulphated polysaccharides, found only in brown macro algae. Their sugar backbone composition, structure and sulphation patterns, differ according to their origin and the extraction techniques used. The common feature is a high content of « fucose »-like backbone.

Fucoidans have diverse bioactivities including heparin-like anticoagulant activity, inhibition of selectins and scavenger receptors, inhibition of viral entry to cells and immunomodulation. Fucoidans also inhibit UVB-induced MMP (Matrix Metallo Proteinase) expression in vitro, known to be involved in extracellular matrix degradation. (Moon HJ., 2009 Yang JH).

Fucoidans largely differs according to their molecular structure and their bioactivity depending on the algae used as well as the extraction method.

Fucoidans from laminaria are rich in fucoses with high degree of sulfatation.



Fucoidan chemical compounds

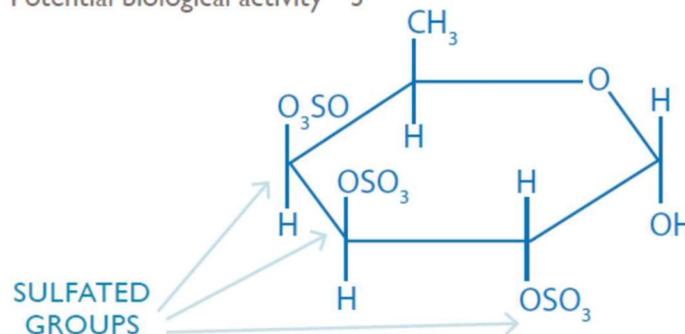
The unique composition of FUCOREPAIR

Using a unique technology, the fucoidans can be hydrolysed until an extremely low molecular weight is obtained. FUCOREPAIR is a combination of fucose mono, di- and trimers. The molecular weight is below 2 kDa. The second interest of this technology is to saturate the fucose molecules in sulphated groups.

FUCOREPAIR : Saturated fucoses in sulfated groups

Fixing ability * 3

Potential biological activity * 3



Consequences on biological activity

The Biological activity of fucoidans derivatives is in inverse proportion to the molecular weight of these molecules. Thus the fucoidan derivative is more biologically active when the saccharide chain is short.

The biological activity is also in direct correlation to the fucoidan derivative's degree of sulfation. In effect it is these sulfated derivatives which, by binding themselves to biological receptors, lead to cosmetic activity (Robert C., 2005).

For these reasons, FUCOREPAIR is the most biologically active form of fucoidan.

Skin absorption

By using hydrolysis technology, it is possible to obtain highly ionized water-soluble oligofucoidans with an extremely low molecular weight. These 3 criterias define the skin absorption capacity of a cosmetic agent. This is why FUCOREPAIR is so easily and so rapidly absorbed across the skin's barrier.

FUCOREPAIR : High skin absorption



2- Fucose : an essential glyconutrient for the skin

2.1- Fucose: one of the 8 essential sugars

Sugars are vital for the well-being of human. There is a wide variety of sugars: simple sugars sources of energy and also more complex molecules involved in vital functions of human body. Sugars molecules which play a key-role in cellular communications and cellular functions are called essential sugars.

The rare sugar fucose is one of the 8 essential sugars. Supplying fucose is vital. However only glucose and galactose among these essential sugars are supplied in sufficient amount by food. Our body can synthesize fucose from galactose but by a complex cycle requiring a big quantity of energy. 15 different enzymes are needed to convert galactose in fucose.

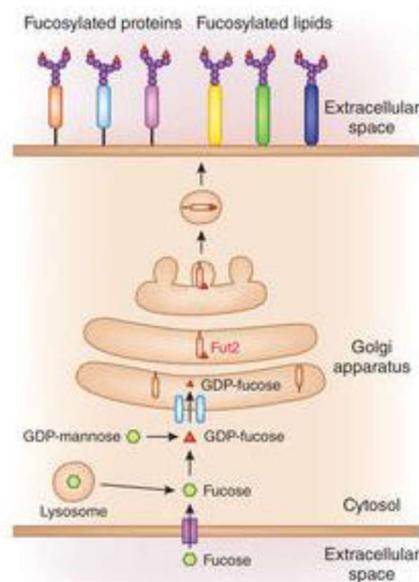
The available fucoses directly bring a better bioavailability. (Gardiner, T., 2000).



2.2- Fucosylation

These essential sugars form, in association with proteins and lipids, glycoconjugates which are present on cells membranes surface. These unique structures play a vital role in cellular recognition and communication between cells. This process of “saccharification” from fucose is called fucosylation.

Fucose has important effects in immunoresponse when it is associated with proteins. Patients affected by a lack of glycoprotein are not capable of Leucocytes recruitment. In this genetic disease, cells are not capable of binding the fucose to proteins. Thus patients are not able to protect themselves against infections. (Vanhooren, P. T. et al., 1999).



Fucosylation on fibroblast receptors

2.3- Presence of fucose in the skin

Fucose can be found both on dermis and epidermis. Membranes sugars play a central role in skin cells maturation. Fibroblasts are able to absorb fucose and convert it into glycoproteins. Skin cellular membranes are composed of lectins receptors presenting a strong affinity with fucose. Their binding on keratinocytes and fibroblasts receptors allows communication between cells, and regulation of diverse biological phenomenas. (Robert et al., 2005).

3- The protection and repair of the dermis

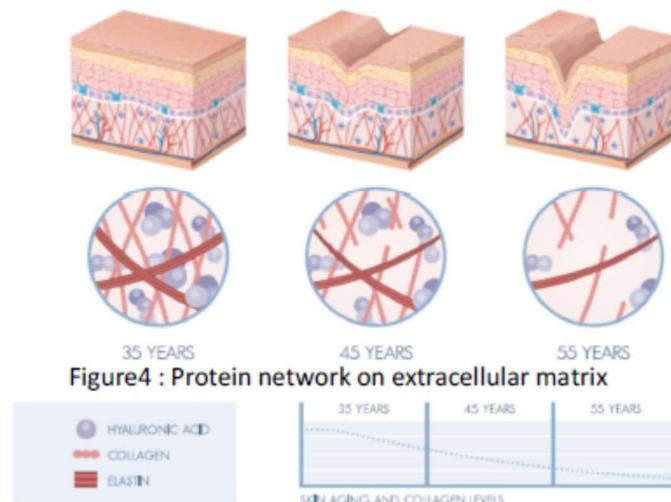
3.1- Role in protection of extracellular matrix

The passing years have negative effects on skin and its properties, deteriorate them. There are biochemical, histological and physiological alterations that include ECM functional impairment, collagen and elastin degradation, and slower metabolism rate. The degenerative changes that come with age can cause loose of skin vibrancy. A major factor in this process is the degradation of extracellular matrix.

The extracellular matrix in dermis is composed of a molecular network (collagen, elastin and glycosaminoglycans) between fibroblasts which ensures the skin's structure and tone. (Robert et al., 2004) When deterioration and repair of this network are imbalanced, wrinkles appear and skin loses its firmness. Fucoidans protect this network by blocking the enzymes responsible of extracellular matrix deterioration and pro-inflammatory cytokines which damage collagen. (Isnard et al., 2002) (Péterszegi et al., 2003).

FUCOREPAIR also acts as a repairing agent by stimulating metabolism of fibroblasts and collagen synthesis. With these 2 synergistic actions, FUCOREPAIR helps to reverse the signs of ageing by bringing back the skin's structure and firmness.

WRINKLES APPEAR WITH DETERIORATION OF THE EXTRACELLULAR MATRIX



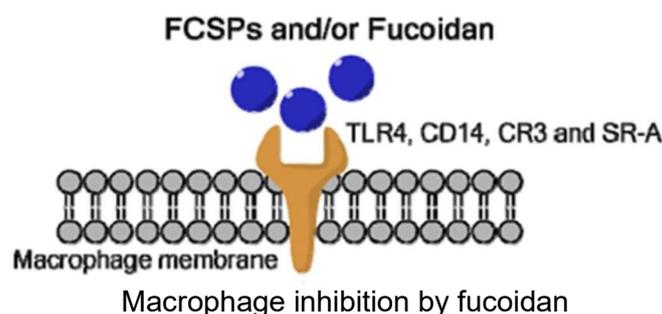
Anti-inflammatory, anti-allergy effect

The fucose glycoconjugates (glycoproteins and glycolipids) are essentials to invert inflammatory processes and play an essential role in the immune response. In inflammatory diseases, fucose is able to inhibiting skin allergy as dermatitis. Fucose also plays an essential role in psoriasis. The distribution of fucose on skin of patients affected by psoriasis is different than in a normal skin.

Fucose plays a key-role in the modulation of cutaneous sensibility. This can be explained by its capability to regulate cellular messages by settling in membranes receptors, but not only. It also plays a direct role in the activation of macrophages, during inflammatory and allergic response. (MK Nemanic, 1983) (Hasegawa et al., 1980)

The M.I.F (Macrophage Migration Inhibiting Factor) is one of the factors produced by lymphocytes T, responsible of cutaneous hypersensitivity. It is an activator of macrophages to hold them on the site of the irritation. It fixes in macrophages through rich specific fucose receptors.

A topical supply of fucose, could play the role of delusion for the M.I.F, which would not settle them anymore on the macrophages membrane receptors and would not be activated anymore. The fucose would have a modulator effect on inflammatory response by competition. (Umehara K, 1989) (Gordon et al., 1987)

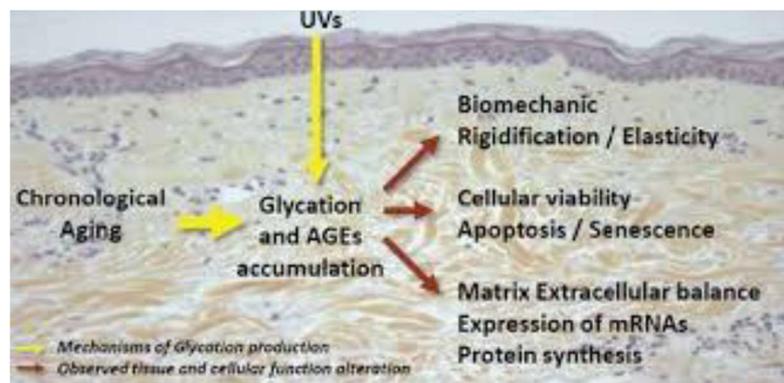


Protection against the glycation

Glycation is a non-enzymatic reaction between an amino acid and a hydroxyl group of sugars (Maillard reaction).

When this reaction acts in the dermis, extracellular matrix rigidify inducing a loss of skin flexibility. The glycation is a natural process arising with age. External factors as pollution, ultraviolet rays or cigarette smoke accelerate this process.

It is well documented by several studies that fucoidanes allow to inhibit that glycation. (Robert L, 2009)



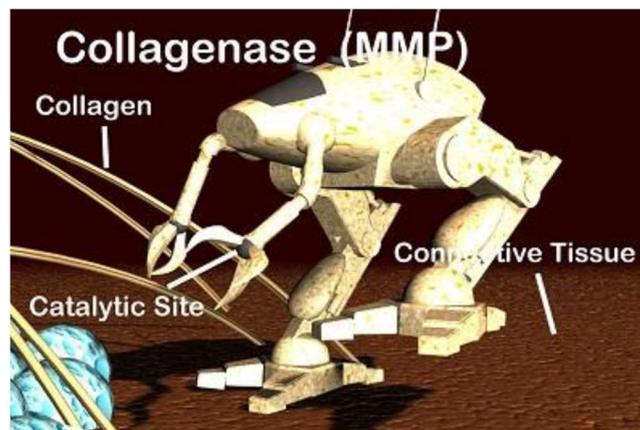
Glycation mechanisms

Inhibition of enzymes involved in degradation of the extracellular matrix

Elastases, collagenases and metalloproteinases are enzymes of the extracellular matrix that degrade respectively elastin, collagen and other proteoglycans.

The integrity of extracellular matrix depends on the balance between these natural processes of degradation and regeneration. With age, the processes of degradation are more important than regeneration causing the extra-cellular matrix deterioration.

The inhibition of these enzymes is an effective way to slow down the effects of aging and to stimulate tissues building. (Isnard et al., 2002) (Moon HJ, 2009)



Collagen destroyed by collagenase

3.2- Role in the regeneration of the dermis

FUCOREPAIR stimulates processes of cellular regeneration. Fibroblasts, main cells of dermis proliferate and at the same time the metabolism increases as illustrates the study concerning synthesis of the collagen (Péterszegi et al., 2008) (Ronan O'Leary, 2004)

3.2.1- In vitro tests: Skin densifying efficacy of FUCOREPAIR

Study aim:

This study evaluates by in vitro experimental system (on human fibroblasts cell culture) the efficacy of FUCOREPAIR to positively modulate fibroblast proliferation.

Experimental models:

Carried out study was performed by using human skin fibroblasts (ATCC-CRL-2703), cultured in DMEM in which Foetal Bovine Serum 10% and L-Glutamine 2 mM were added.

FUCOREPAIR was tested at 2 concentrations. The tested concentrations were: 0,15% and 1,5%.

Cell treatment were performed for 48 hours. Untreated cells were used as negative control.

Statistical analysis: obtained data were subjected to statistical analysis by t-test.

Culture medium containing FUCOREPAIR was added to the cells containing cells in the G0 phase of cell cycle. Cells were exposed to each solution for 48 hours.

At the end of incubation period, MTT test (a colorimetric assay for assessing cell metabolic activity) was performed in order to evaluate cell viability and the increasing proliferating rate compared to untreated control cell culture.

Results:

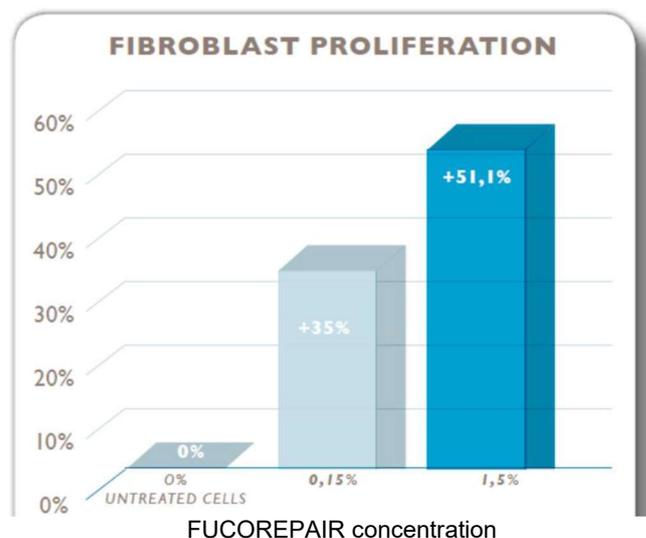
The data are reported as the percentage increases of cell proliferation and collagen synthesis after cells exposure to the test item in the different experimental conditions of the study respect untreated cells.

Fibroblast proliferation increases (% mean +/- st.dev) in cell culture treated with FUCOREPAIR at 0,15% and 1,5% compared to untreated cells.

FUCOREPAIR 0,15% : 35,5% +/- 3,3%

FUCOREPAIR 1,5% : 51,1% +/- 2,1%

The treatment with FUCOREPAIR has positively modulated cells proliferation in the considered experimental system. All recorded variations are significant vs negative control.



FUCOREPAIR has significantly modulated the fibroblast proliferation

3.2.2- In vitro tests: collagen synthesis

Study aim:

The study evaluated by in vitro experimental system (on human fibroblasts cells culture) the efficacy of FUCOREPAIR to positively modulate the anabolism function (collagen synthesis).

Experimental models:

Carried out study was performed by using human skin fibroblasts (ATCC-CRL-2703), cultured in DMEM in which Foetal Bovine Serum 10% and L-Glutamine 2 mM were added.

FUCOREPAIR was tested at 2 concentrations : 0,15% and 1,5 %.

Cell treatment were performed for 48 hours.

Untreated cells were used as negative control.

Statistical analysis: obtained data were subjected to statistical analysis by t-test.

The determination of collagen synthesis is carried out by quantitative dye-binding method. The chromogen agent used in the assay is Sirius Red (Direct red 80). Sirius red is an anionic dye with sulphonic acid side chain groups. These groups react with the side chain groups of the basic amino acids of collagen. The specific affinity of the dye for collagen, under the assay conditions, is due to the elongated dye molecules becoming aligned parallel to the long rigid structure of native collagen that have intact triple helix organisation dye affinity is much reduced when collagen rigid structure of native collagen that have intact triple helix organisation. Collagen concentration is calculated by means of data interpolation on a standard curve obtained with increasing collagen concentrations.

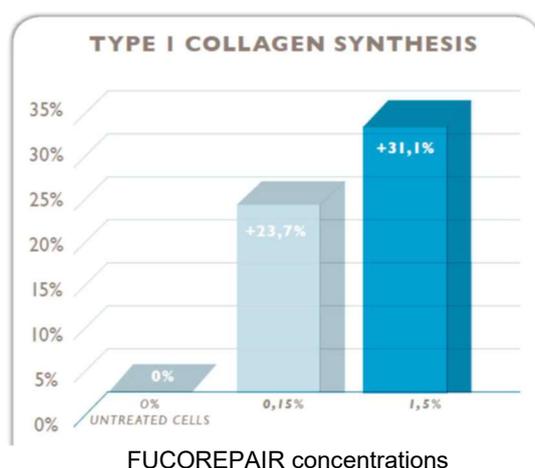
Results

Table: collagen synthesis (mean) in cell culture treated with FUCOREPAIR at 0,15% and 1,5% compared to untreated cell. The % increases respect to untreated cells.

FUCOREPAIR 0,15% : 23,7%

FUCOREPAIR 1,5% : 31,1%

The treatment with FUCOREPAIR has significantly modulated collagen synthesis in the considered experimental system. All recorded variations are significant vs negative control.



FUCOREPAIR has significantly stimulated the collagen synthesis

4- Clinical evaluation

4.1- Protocol

The aim of the study is to evaluate the safety of use and the efficacy of FUCOREPAIR, whether the product works on the wrinkles depth.

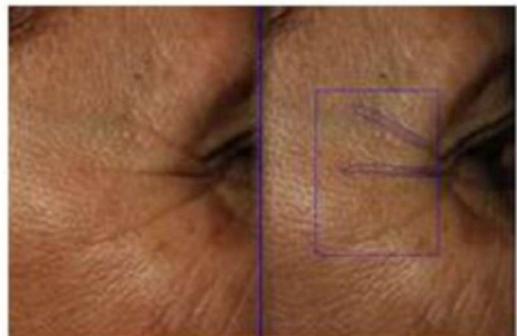
In order to reach this goal, a clinical-instrumental study is carry out in line with the following protocol:

- ✓ 42 volunteers (women showing wrinkles on the crow's foot area).
- ✓ Divided in 2 groups according the randomisation lists : 21 women applied a formulation with 1,5% FUCOREPAIR (active group), while the control group of 21 women applied the same formulation without FUCOREPAIR (placebo).
- Age : 43 – 68 years old
- Application area : skin face and neck
- Twice a day for 28 days
- Measurements were made on Day 0 (D0), before the application, and again on Day 28 (D28).
- Evaluated parameters : Product tolerability by means of dermatological clinical evaluation ; Skin profilometry (intrumental evaluation)

Wrinkles depth: evaluation of skin profilometry

Skin surface is quantitatively assessed by LIFEVIZ Micro (Quantificare). It is a non-contact in vivo skin measurement device based on structured light projection. In conjunction with a comprehensive 3-D measurement and evaluation software, the sensor allows to evaluate skin surface properties (wrinkle depth, volume, etc...). In this study, the wrinkle depth is calculated.

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



Skin profilometry by means of 3D LIFEVIZ Micro™

Dermatological evaluation of skin reactions

During the checks, the following skin adverse reactions are checked: Oedema, erythema, dryness, desquamation, Itching, Burning, Tingling, and Tightness.

Statistical analysis:

Obtained data are submitted to the bilateral Student t test. Variation is considered statistically significant when the test value is $p < 0,05$.

4.2- Results

Wrinkles depth:

Active group:

Used at 1,5%, FUCOREPAIR determines a statistically significant decrease of wrinkles depth at D28 compared to the baseline.

Average a decrease of 6,3% and **up to 18,9%**. T-test vs T0 = 0,018

70% of women observed a reduction of the main wrinkles.

Placebo group :

Placebo determines a statistically non-significant increase of wrinkles depth at D28 compared to baseline. Average an increase of 2,8 %.

Comparaison active group and placebo group:

Used at 1,5%, FUCOREPAIR decreases significantly the wrinkles's depth of 9,1% compared with placebo.

FUCOREPAIR decreases wrinkle depth

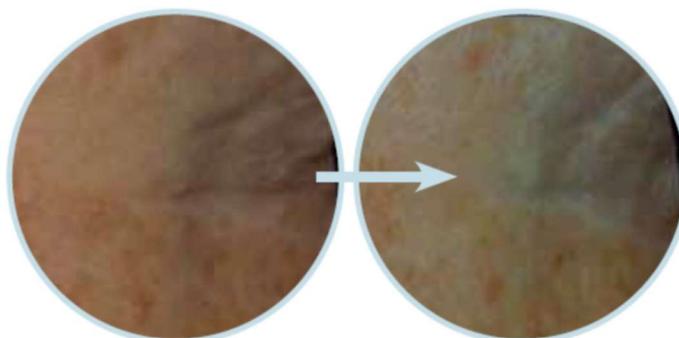
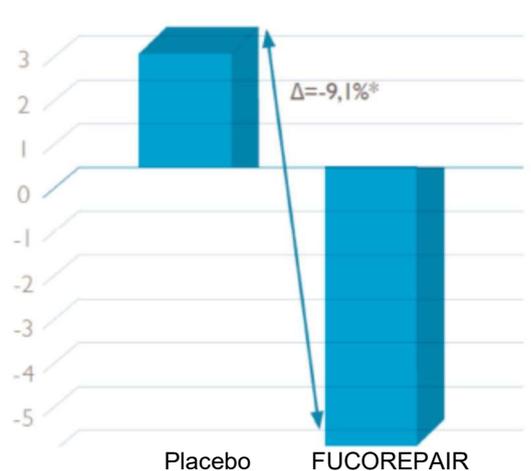


Photo of the cross foot area wrinkles before (left) and after 28 days (right)

Product tolerability:

Active group: the result of tolerance is good.

Placebo group: the result of tolerance is moderate.

Results show that FUCOREPAIR significantly decreases irritations versus placebo. These results suggest the use of FUCOREPAIR to calm sensitive skin.

Conclusion

Thanks to its unique composition, FUCOREPAIR acts by regulating the balance between deterioration and repair of extracellular matrix to decrease the wrinkles and the redness on the skin, thereby reversing the signs of ageing. Wrinkles' depth is significantly reduced compared with a placebo group.

5- Safety

FUCOREPAIR presents a good skin compatibility. It is non-irritant, non phototoxic, non genotoxic and non mutagenic.

FUCOREPAIR was subject to toxicological tests. The conclusions are described hereafter :

✓ Cutaneous irritation :

In accordance with observations performed during the study and the results of cell viability obtained after incubation (96.11%), the product is classified as non-irritant.

✓ Ocular irritation :

According to obtained results, FUCOREPAIR is not irritant.

✓ Phototoxic potential :

Tested product does not show any toxic effect after irradiation on experimental model. It does not show a predictive photo-toxic potential.

✓ Mutagenicity :

In accordance with experimental protocol and the basis of the results from this investigations, the product did not show any evidence of mutagenicity.

6- Formulation

6.1- Guidelines

Use level : 1.5 %

Use pH : We recommend to use of FUCOREPAIR with a pH from 3 to 8.

Solubilities :

Highly soluble in water

Insoluble in mineral oil and vegetable oils

Process :

FUCOREPAIR should be added to the aqueous phase. This active ingredient is easy to formulate in colourless in formulas.

It can be added to all kinds of formulations

6.2- Incompatibilities

FUCOREPAIR is not compatible with alcohols (ethanol, glycols...).

6.3- Odorless and Colorless in formula

Used at 1.5% in emulsion, FUCOREPAIR does not give any odor or color to the formulation.

7- Applications

- Anti-ageing
- Anti-wrinkles
- Sensitive skin
- Eye contour
- Skin perfectors

INCI Name : Hydrolysed fucoidan

Chinese INCI Name : Laminaria digitata extract

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