

THE ART OF NATURE MICROALGAE

September 2014 – P. Goyat



Content

- Microalgae generality
- Microalgae cosmetic interests
- Microalgae production

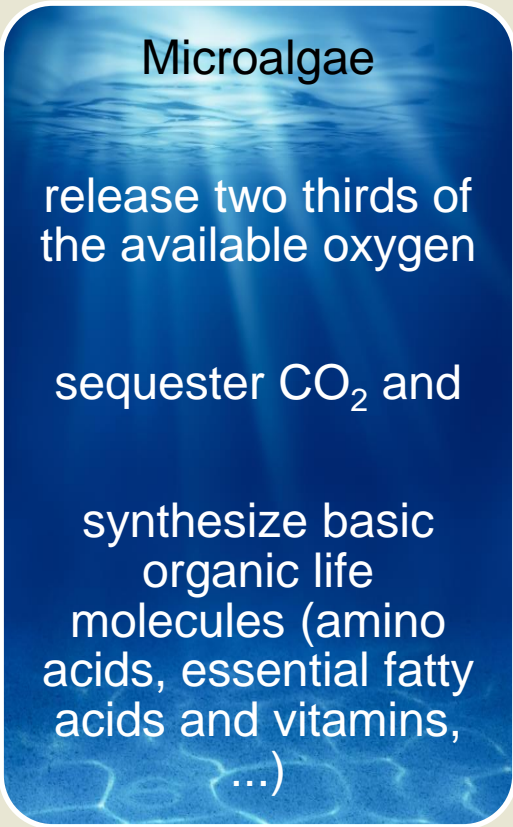
In collaboration with



Microalgae as « origin of life »



Microalgae are
unicellular
organisms



Microalgae
release two thirds of
the available oxygen

sequester CO₂ and

synthesize basic
organic life
molecules (amino
acids, essential fatty
acids and vitamins,
...)



Microalgae are
the first link in the
energy chain of
the oceans.

Consumed by
animals, they
ensure the
functioning of
major biological
cycles and the
maintenance of
biodiversity on
earth.

Microalgae « potential »

An estimated 200,000 to 7 million species are found in the oceans, rivers and lakes of the world.

Each species is producing its own unique and sometimes rare mix of compounds.

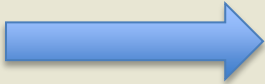


However, only around 10% of all species are currently identified and described by the scientific literature and just a handful is produced at an industrial scale.

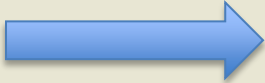
The commercial market for microalgae-based actives will increase as species with valuable compounds are discovered and produced in a cost-effective and sustainable manner.

Microalgae as « cosmetic interests »

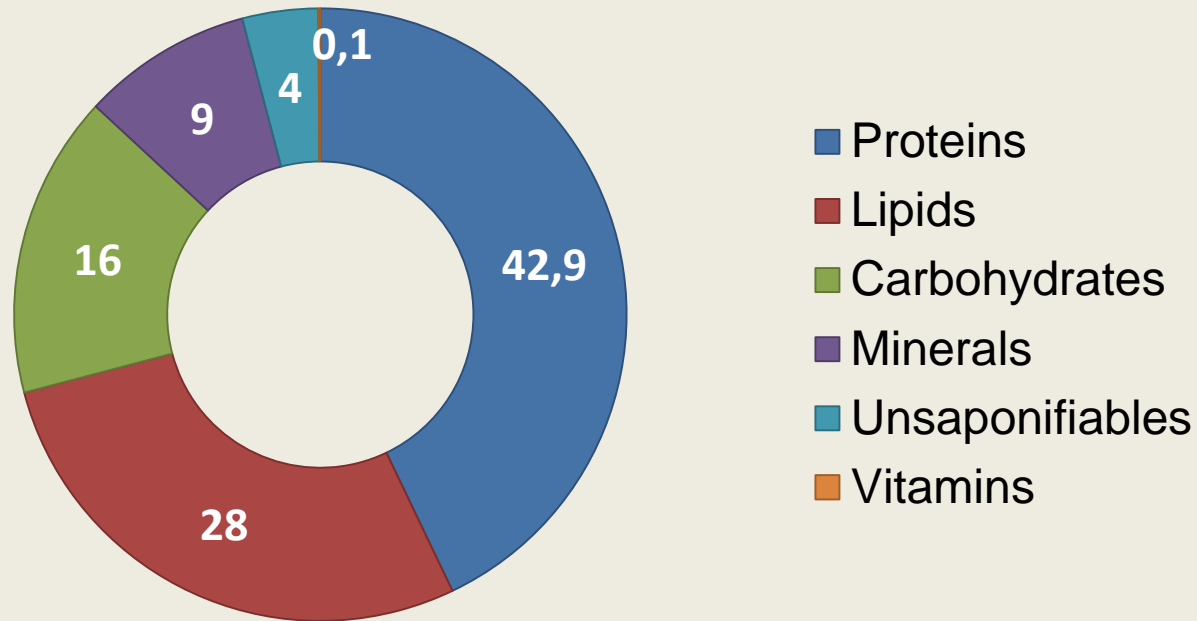
- ❖ Microalgae main property :

 Ability to protect themselves from environmental stresses to which they are subjected.

- ❖ Microalgae produce :

 antioxidants, fatty acids (omega), pigments but also vitamins (A, E, B1, B3 ...) which can be used in cosmetic products to fight against dry skin and free radicals or help cell regeneration. They can have detoxifying properties and can slow cellular ageing.

Microalgae – General composition



Provide top quality oils such as **omega-3** and **omega-6** fatty acids. Some species are known to yield high volumes of omega 3 docosahexaenoic acid C_{22:6} (DHA) and eicosapentaenoic acid C_{20:5} (EPA)

Microalgae – General composition

- Microalgae produce high levels of **carotenoids**, a group of more than 600 molecules, which offer strong antioxidant potential and several benefits for the cosmetic, food and pharma industries.
- Microalgae provides various possible compounds with hydrating, nourishing, regenerating, slimming, draining, detoxifying, soothing, remineralizing or antiseptic properties.

Our technology

- Cultivation in closed photobioreactor
 - High technology
 - Low risk of contamination by other species or external pollution
 - Allows controlling of environmental conditions and stable production
 - Possibility to cultivate a large variety of microalgae strains
- Sustainable manufacturing process from renewable sources

Photosynthetic field

Inside greenhouse

1000 m²

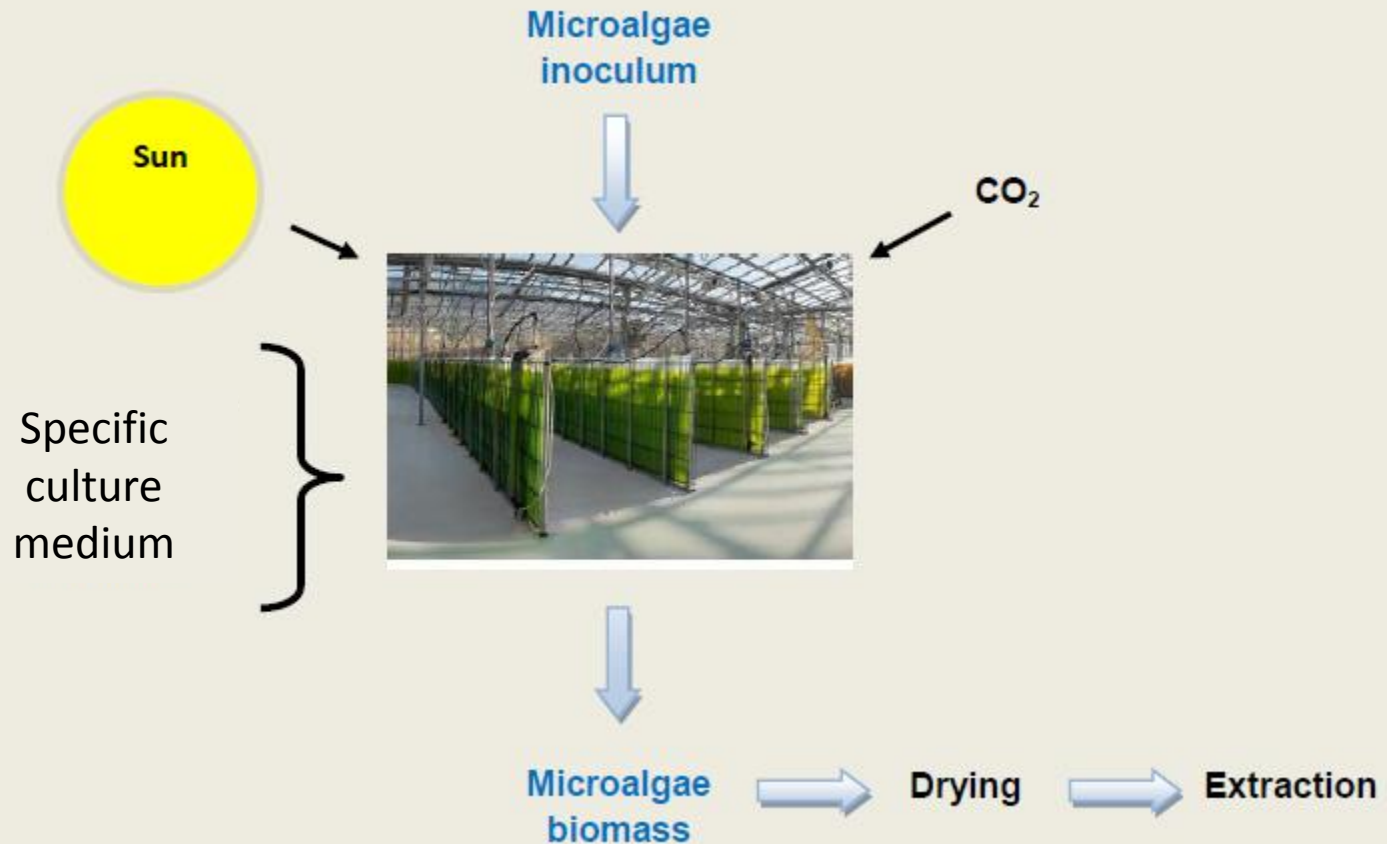
29000 lt

Patented process : ref n°/GE11A21-IT

HACCP conform



Biomass production

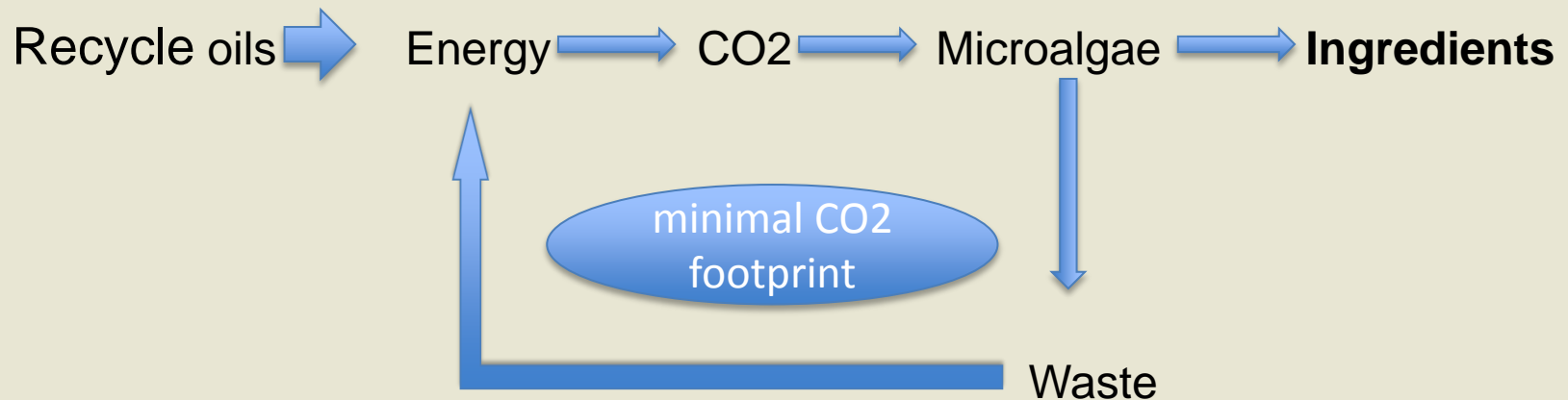


Microalgae production conditions

- The optimal parameters as well as the tolerated ranges are species specific.
- The most important parameters regulating microalgae growth are :
 - nutrient quantity and quality,
 - light,
 - pH,
 - turbulence,
 - salinity,
 - temperature.

Sustainable process

Archimede Ricerche Plant – A&A Parodi Group – Ventimiglia – Italy



NATURA-TEC MARINE BLUEVITAL C

September 2014 – L. Brun, P. Goyat



Natura-Tec Marine BlueVital C

CHARACTERISTICS

- ✓ Advanced and highly effective **anti-wrinkle active**
- ✓ Liquid, yellow to orange color
- ✓ Beneficial ingredients for the skin :
 - Carotenoids : Fucoxanthin and others
 - Essential fatty acids : EPA Eicosapentaenoic acid
C20:5 (omega 3)
 - Sterols
- ✓ Store in a cool dry place, below 10°C, away from light, in original unopened packaging



Microalgae

CYLINDROTHECA FUSIFORMIS

| | |
|----------|---------------------------|
| Division | Bacillariophyta – diatoms |
| Class | Bacillariophyceae |
| Order | Bacillariales Hendey |
| Family | Bacillariaceae Ehrenb |
| Genus | Cylindrotheca Rabenh |
| Species | Cylindrotheca fusiformis |



- ✓ INCI : Caprylic Capric Triglyceride and Cylindrotheca Fusiformis Extract
- ✓ INCI IECIC 2014 : Caprylic Capric Triglyceride and Algae Extract

Natura-Tec Marine BlueVital C

PROPERTIES

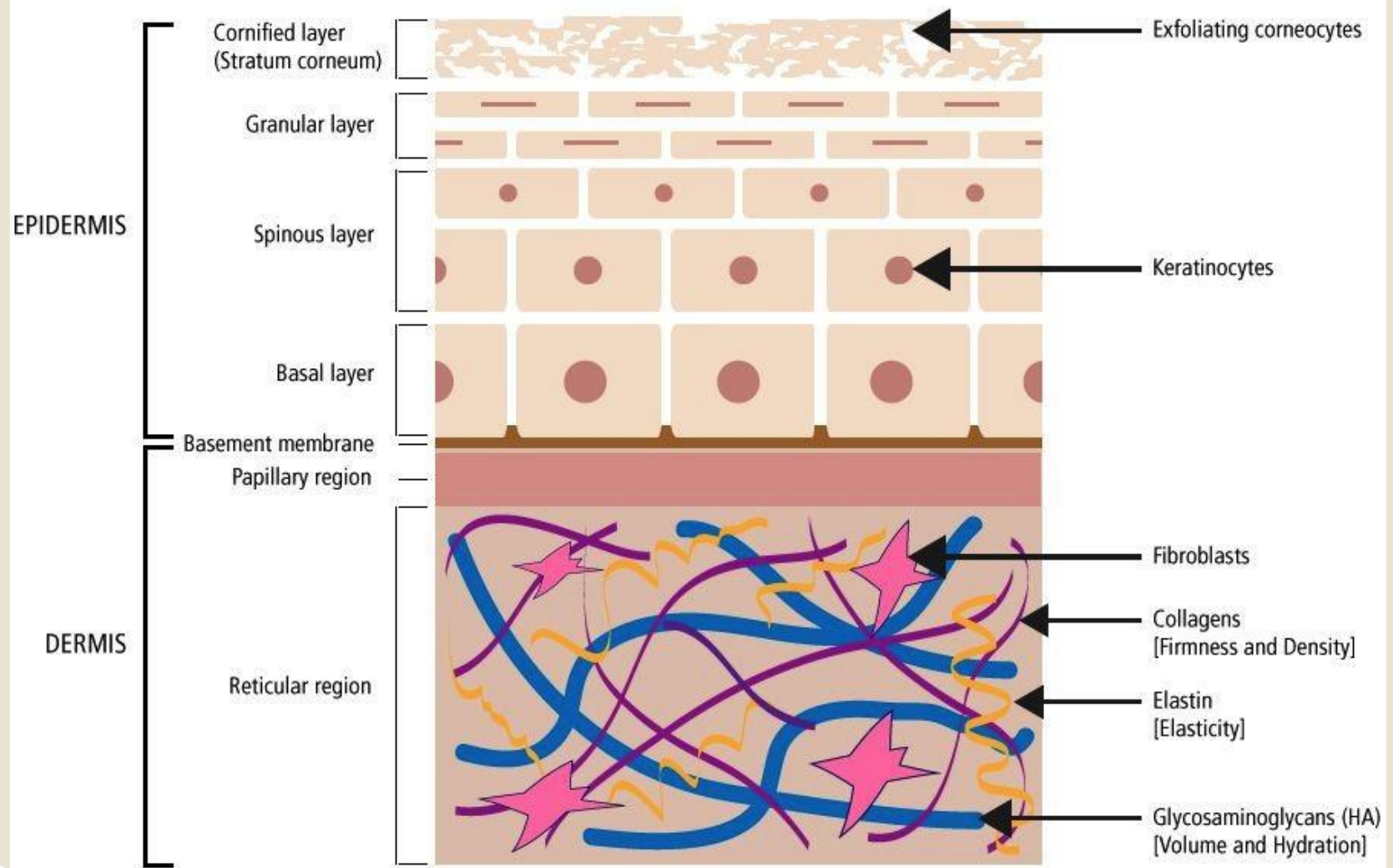
Strongly stimulates production of structural skin proteins

Acts on cellular proliferation and ex-novo synthesis of collagen (In-vitro test on fibroblast)

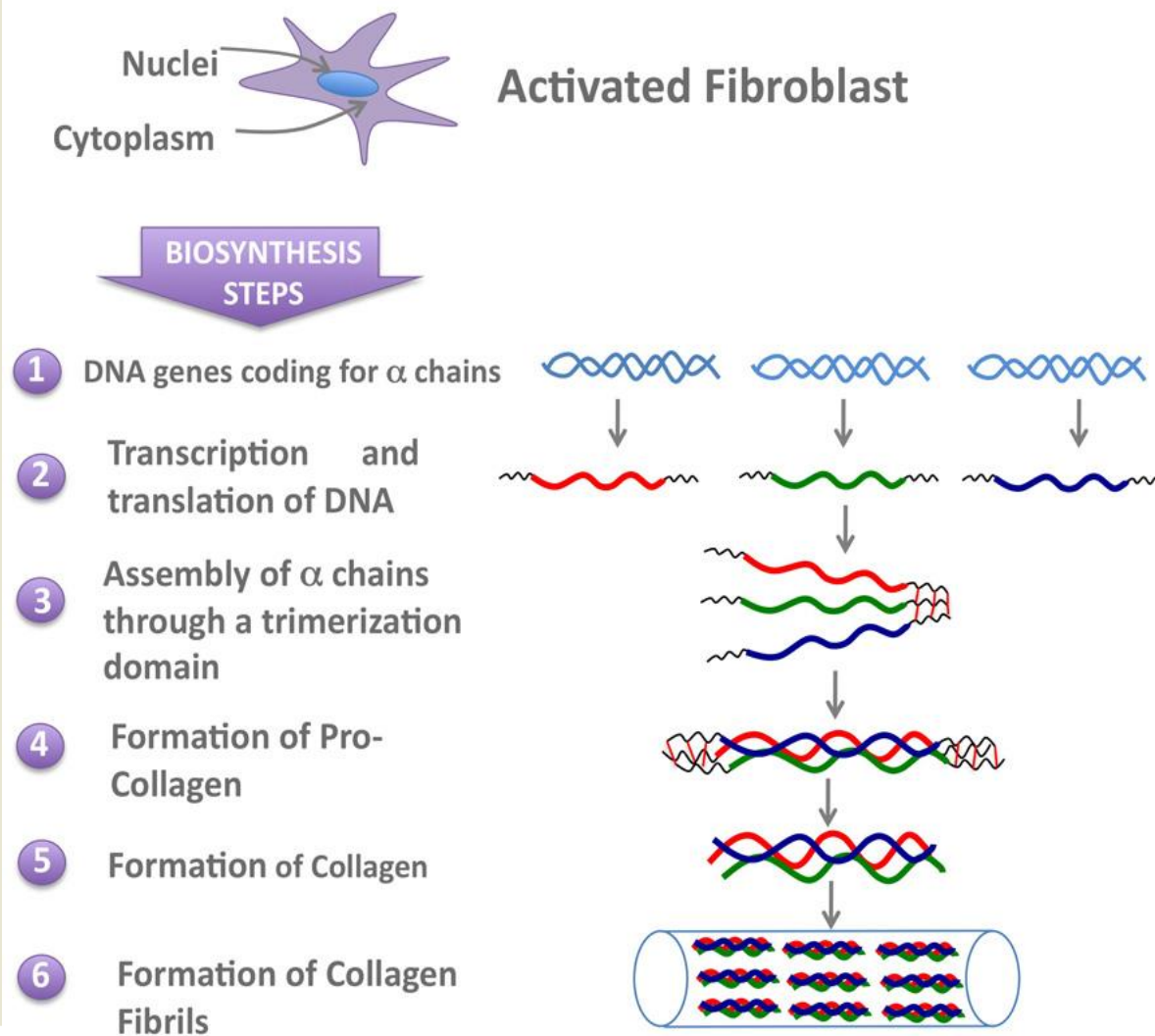
Anti-wrinkle activity demonstrated by an in-vivo test on the pericular eye area

Presents soothing and anti-inflammatory effect and can show photo-protective activity

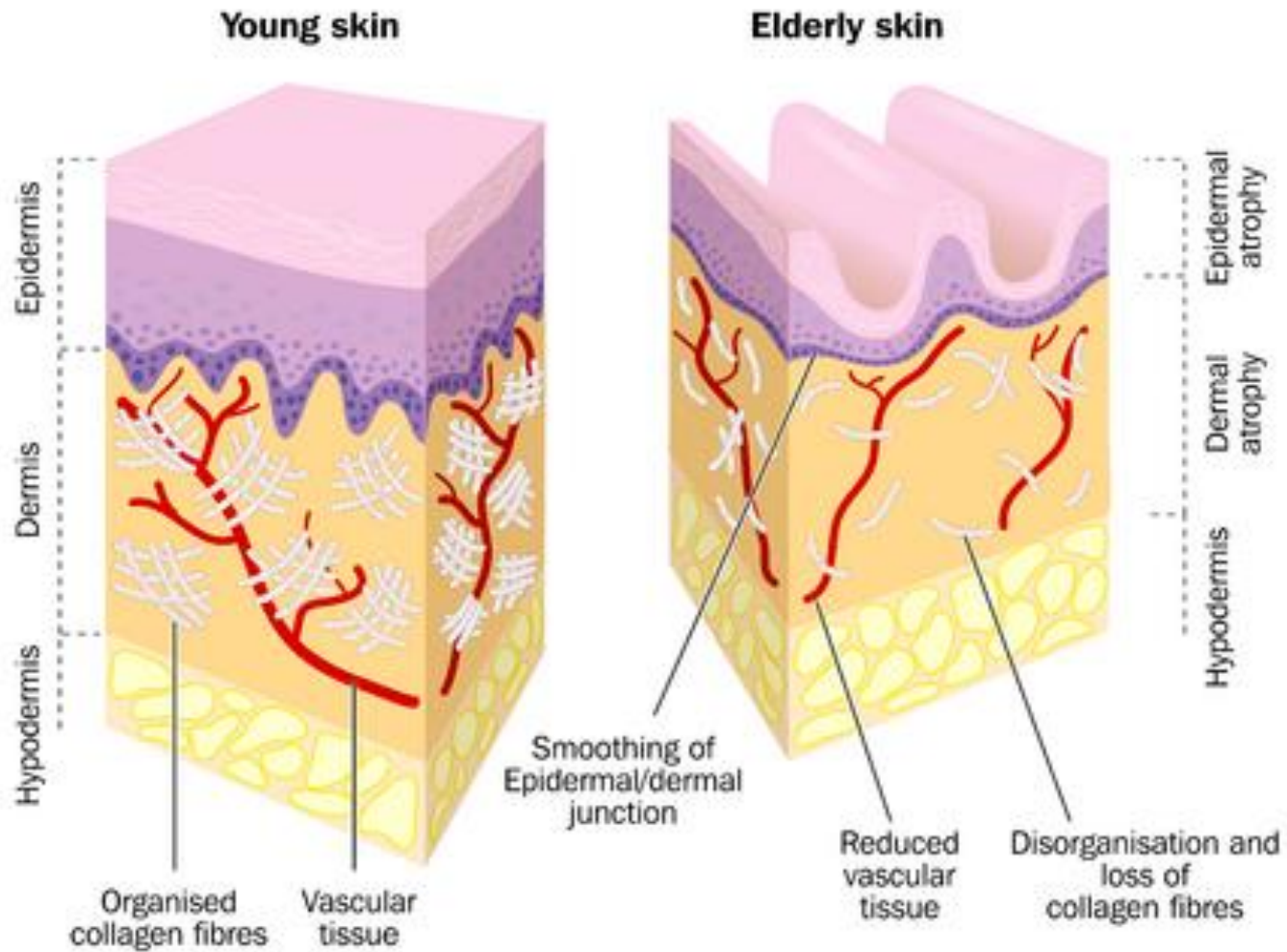
Skin structure



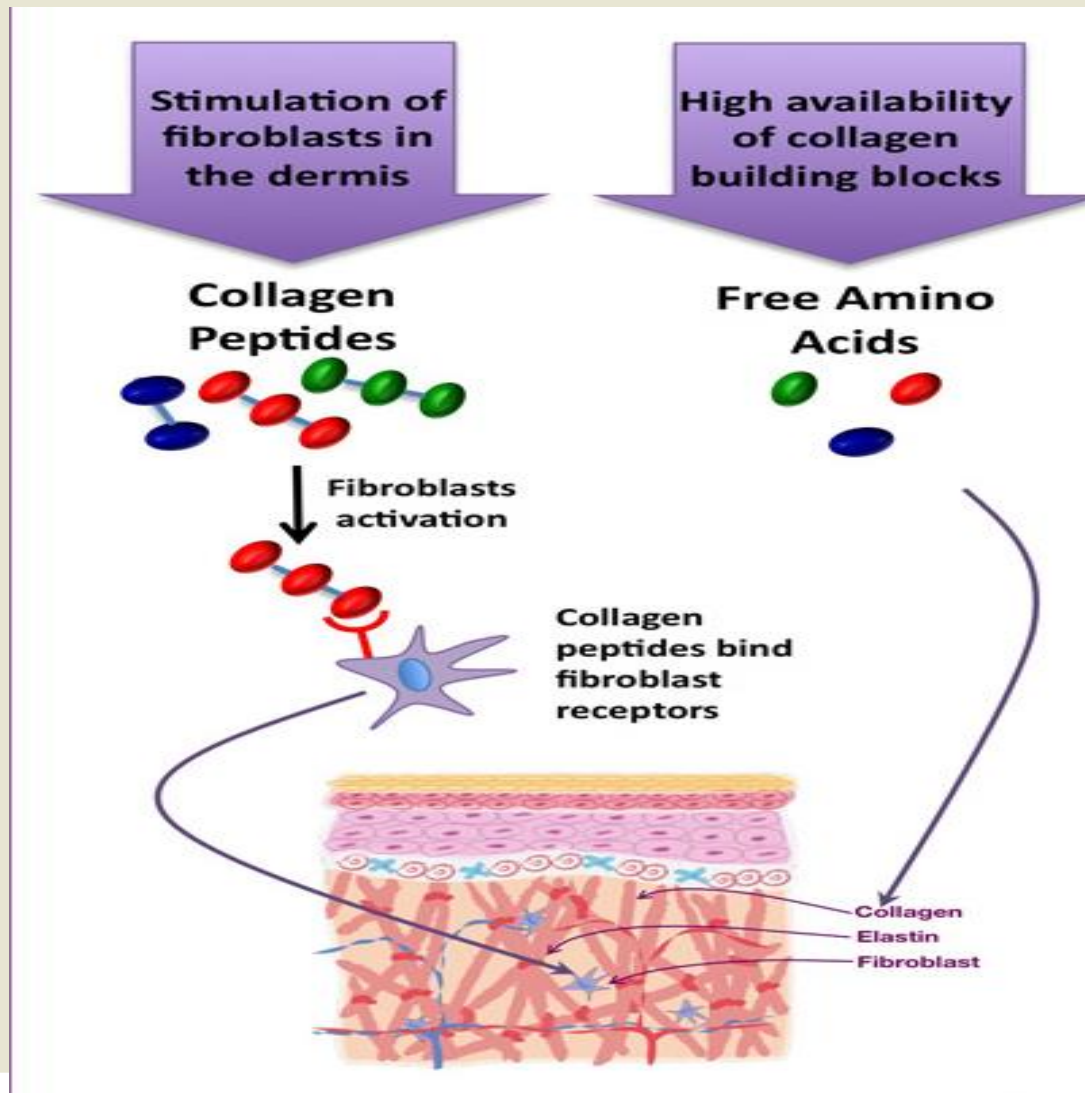
Mechanism of collagen formation



Skin ageing changes



Fibroblast stimulation



Natura-Tec Marine BlueVital C

EFFICACY DATA

- Toxicological information
- Evaluation of stimulating properties for collagen synthesis in cell culture – In-vitro test
- Evaluation of the anti-wrinkle efficacy – in-vivo test
- Auto-evaluation

Toxicological information

HET CAM TEST

The “Hen's Egg Test - chorioallantoic membrane” is an alternative Ocular irritating test.

Aim : observe the irritating effects (hyperemia, hemorrhage, clotting) that may occur within 5 minutes after the deposit of a substance on the CAM chorioallantoic membrane of an embryonic chicken egg.

Natura-Tec Marine BlueVital C has been tested undiluted on the CAM of 6 fertilized eggs in specific conditions.

Results :

Slightly irritant (IS value = $2,67 \pm 1,14$)

Toxicological information

CYTOTOXICITY TEST – MTT TEST

MTT - In vitro evaluation of the cytotoxicity of a cosmetic product with an assay on fibroblasts/keratinocyte cell cultures (UNI/EN ISO 10993-5: 2009 (E))

The MTT assay (colorimetric test) evaluates in vitro the vitality of cells exposed to different concentrations of the investigated cosmetic product in comparison with untreated cells.

Results :

Natura-Tec Marine BlueVital C is non cytotoxic at 0,5%, 1,0%, 2,0% of use.

Collagen synthesis

- **Aim of study**

In vitro evaluation of collagen synthesis in human skin fibroblasts exposed to our **Natura-Tec Marine BlueVital C** at different concentrations. Collagen synthesis is measured by means of colorimetric assays

- **Protocol**

Monolayer culture of human fibroblasts (ATCC-CRL-2703)

Three different concentrations : 0.5%, 1.0% and 2.0%

Three different times : 24h, 48h, 72h

Controls without the active

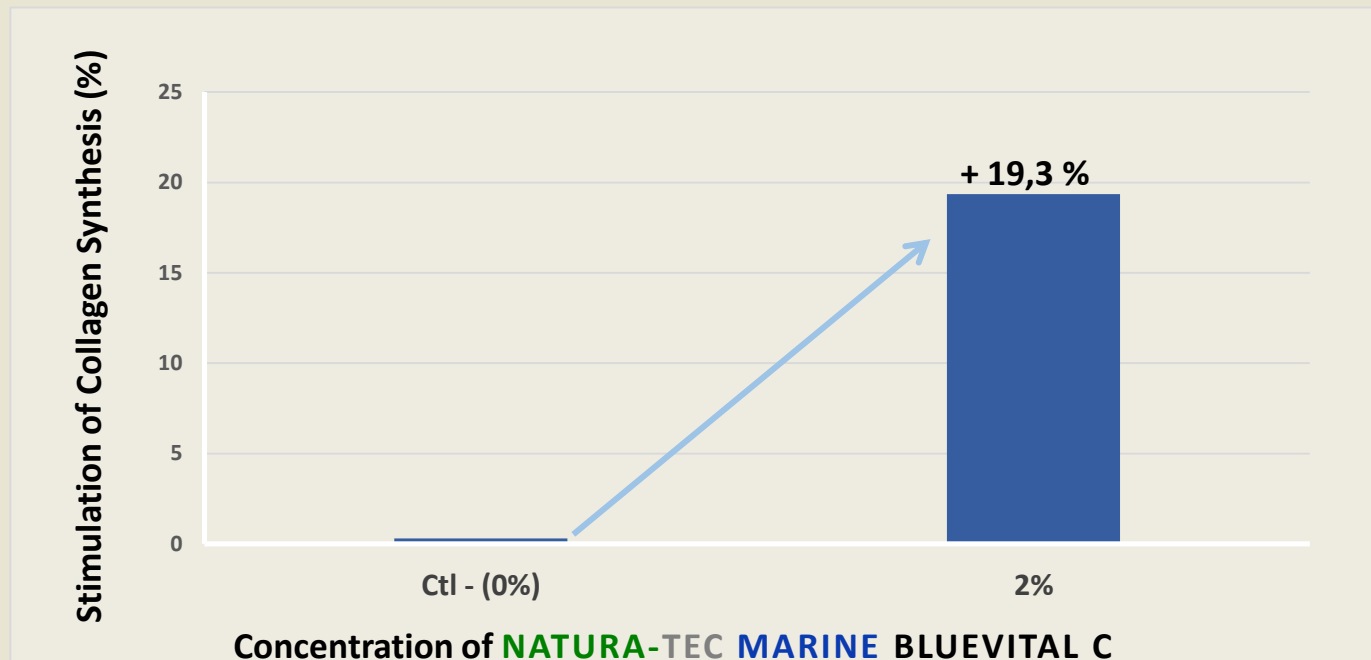
- **Collagen assay** : Quantitative dye-binding method with Sirius Red

- **Results :**

Mesure of mean collagen content (μg) and % variation compared to untreated cell cultures (CTR-)

Collagen synthesis

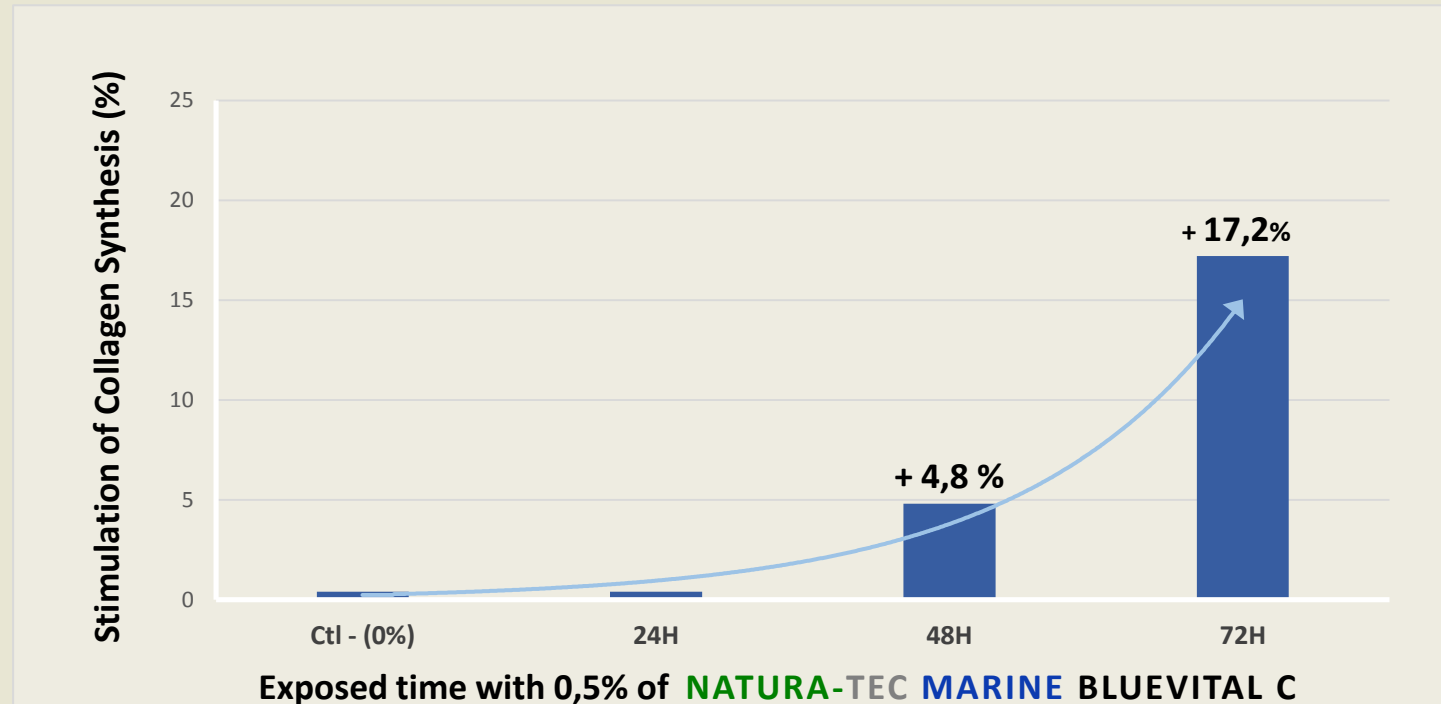
Capacity of **Natura-Tec Marine BlueVital C** to stimulate synthesis of Collagen on fibroblasts in 24H



Used at 2%, increase of collagen synthesis by 19,3% in 24h

Collagen synthesis

Capacity of **Natura-Tec Marine BlueVital C** to stimulate synthesis of Collagen on fibroblasts in 24H, 48H, 72H at 0,5%



Used at 0,5%, gradual increase of collagen synthesis by 17,2% in 72 hours

Collagen synthesis

- **Natura-Tec Marine BlueVital C** has an immediate and excellent efficacy on collagen synthesis at 2%
 - At small dosage (less than 1%), **Natura-Tec Marine BlueVital C** has a time – dependent good efficacy
- Highly effective active : with 0,5% almost same collagen production as with 2% over a slightly longer period
- 2 concentrations of use depending on the age of consumer
- For 50+ years old – use at 2%
 - From 20 to 40 years old – use 1% or less

Evaluation of the anti-wrinkle efficacy

- **Aim of study :**

In vivo evaluation of changes of the wrinkles after a repeated application on healthy human skin of two cosmetic products (with and without our **Natura-Tec Marine BlueVital C**) by fringe projection.

- **Protocol**

21 volunteers, female, 35 – 55 years, T0-T1m-T2m, hemi-facial application of the active cream (1,5% of **Natura-Tec Marine BlueVital C**) and placebo cream twice per day.

Auto-evaluation questionnaire

- **Technique**

Dermatop Blue uses Breuckmann technology based on a fringe projection unit using blue light combined with imaging techniques.

Evaluation of the anti-wrinkle efficacy

Calculations and statistics

Rz (mm): Average Maximum profile height difference : represents the average width of the negative peak

Ra (mm): Linear average profile roughness: represents the arithmetic average of the volume of the micro profile of the skin.

Variation % = $[(v_1 - v_0) / v_0] \times 100$, Student t-test $\alpha = 5\%$

Evaluation of the anti-wrinkle efficacy

Results :

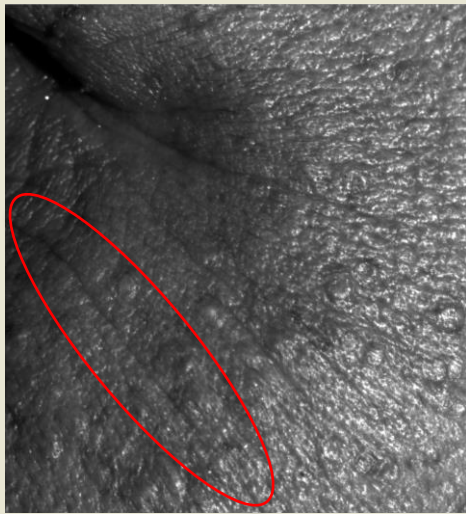
| Placebo | 1 month | | 2 months | |
|----------------------------------|---------------------------------|-------------|----------------------------------|-------------|
| Compared to T0, % | Marine BlueVital C | Placebo | Marine BlueVital C | Placebo |
| Main wrinkle average depth (Rz) | -8,2% $\alpha < 0,01$ | 0,4% ns | -10,4% $\alpha < 0,01$ | 0,5% ns |
| Main wrinkle average volume (Ra) | -8,8% $\alpha < 0,01$ | -0,2% ns | -12,0% $\alpha < 0,01$ | -0,1% ns |

Natura-Tec Marine BlueVital C significantly decreases wrinkle depth and volume at only 1,5% of use and already after 1 month of use

Evaluation of the anti-wrinkle efficacy

Illustrative results :

Photo of wrinkles crow's feet before and after application of a cream with 1.5% **Natura-Tec Marine BlueVital C**



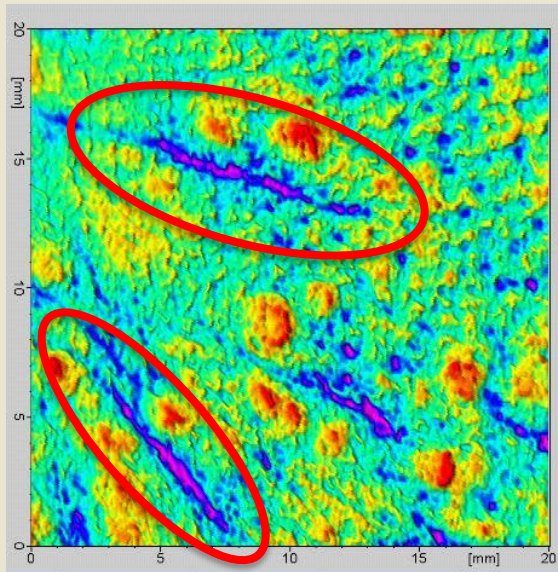
T0



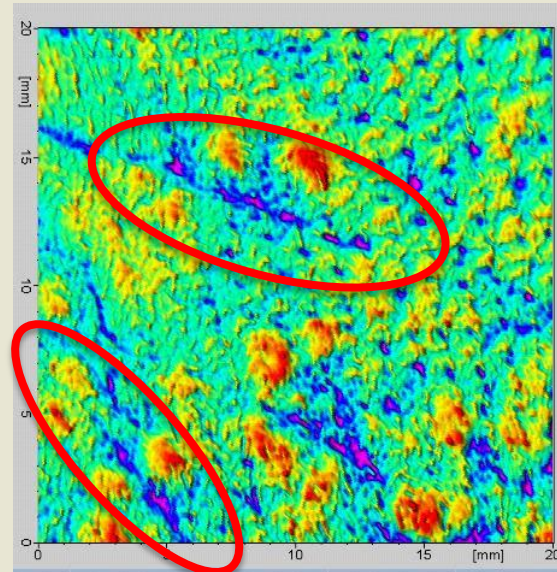
T2 months

Evaluation of the anti-wrinkle efficacy

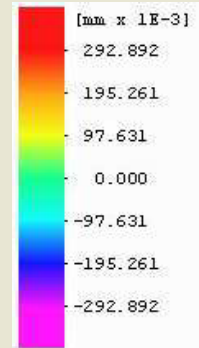
Illustrative results : Skin topography –Dermasurf software



T0



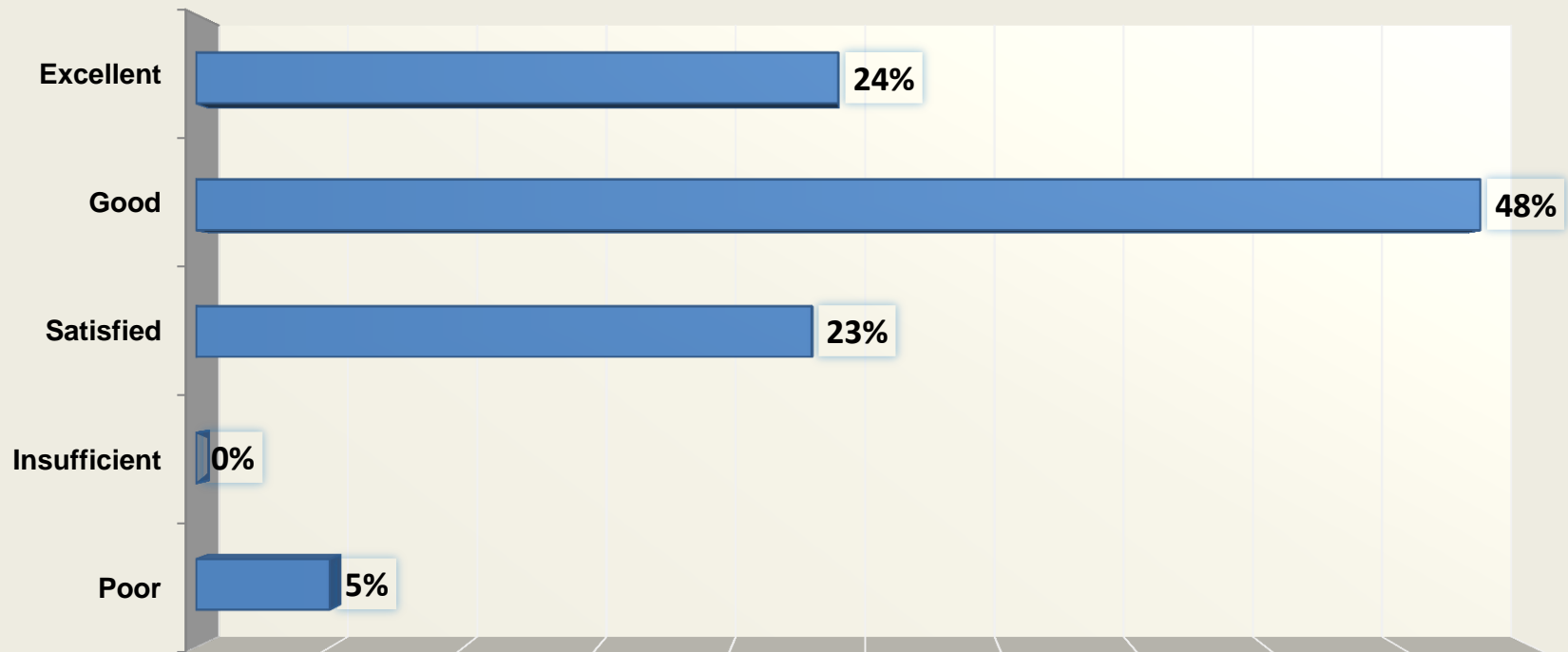
T2 months



Natura-Tec Marine BlueVital C visibly decreases appearance of wrinkles at only 1,5% of use after 2 months of use

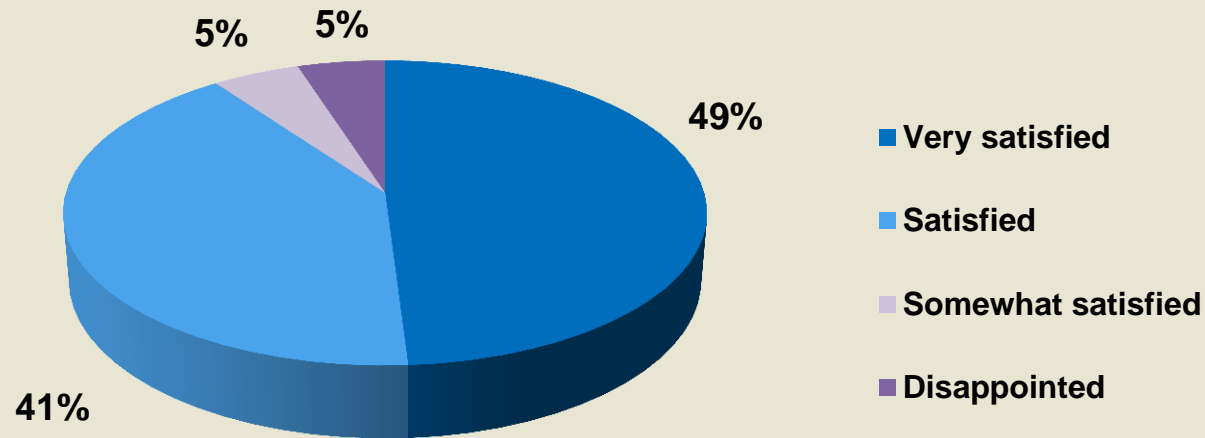
Auto-evaluation

EVALUATION – ANTI-WRINKLE EFFICACY



Auto-evaluation

EVALUATION – SATISFACTION



Conclusion In-vivo test

Instrumental control

The formulation containing 1,5% Natura-Tec Marine Blue vital C shows **significant** anti-wrinkle results when compared to the placebo formulation.

In 2 months, with 2 applications per day, the wrinkle depth decreases by 10,4%, and the wrinkle volume decreases by 12% when compared to T0.

Auto-evaluation study

The panel confirms the anti-wrinkle efficacy of the formulation, 95% as « excellent » « good » and « satisfied ».

Additionally, the volunteers confirm an improvement of the elasticity, hydration and softness of the skin.

Natura-Tec Marine BlueVital C

COSMETIC APPLICATIONS

Formulation of anti-ageing, anti-wrinkle treatments tailored to suit the story of your skin.

It will inspire your lifestyle, your emotions and your environment with an action on the youth and vitality of your skin.

- ❖ Skin care (0,5 – 2,0%) Anti-wrinkle, anti-ageing and moisturising creams, daily creams, eye serums
- ❖ In all kinds of products (sun care, make up ...) for a “Blue” anti-ageing / anti-wrinkle claim.