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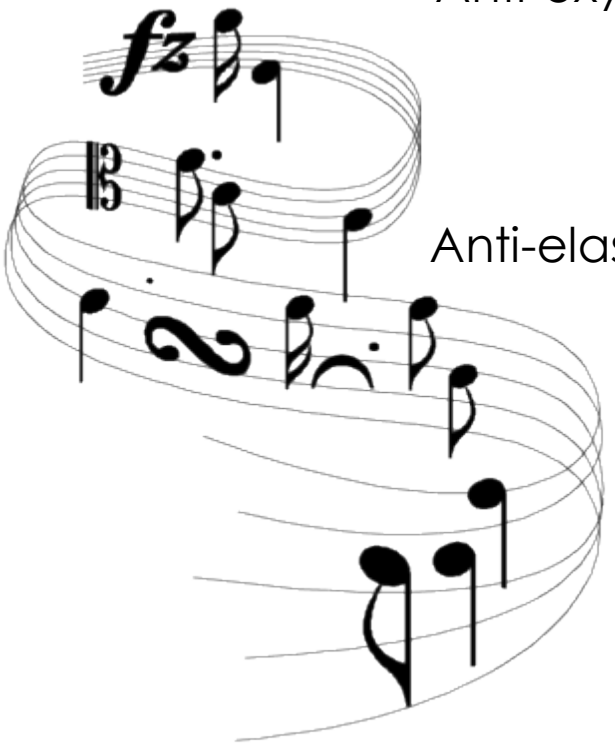
REVERTIME



Anti-oxydant activity

Anti-elastase activity

Stimulation of collagen synthesis

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Aging skin, some reminders ...

- The epidermis, the first line of defense of the skin against external aggressions, is continually subjected to the action of free radicals which constitute one of the main causes of aging skin. UV radiation and other stresses like pollution, for example, generate free radicals that affect the membranes of the skin cells (for review, see Trouba et al., 2002) and degrade the structural proteins of the epidermis and dermis. These radical attacks will also be able to interact with the cellular genetic material, resulting in an impoverishment of the quality of neo-formed cells within the skin tissue during its renewal.

- The dermis is also the site of profound changes during aging. In particular, it is possible to observe a significant decrease in the cell population of the dermis (Kurban and Bhawan, 1990) associated with a reduction in the size of the fibroblasts (Kligman and Lavker, 1988) and qualitative alterations of the organelles involved in the synthesis And secretion of proteins.

- The most noticeable changes in the dermal extracellular matrix occur in the papillary dermis (Pieraggi et al., 1984) where collagen fibrils have a granular appearance and fibrils become more compact due to the loss of proteoglycans.

- Elastic fibers, another important actor in the tone and elasticity of the cutaneous tissue, also undergo numerous alterations (Waller and Maibach, 2006).

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A relevant anti-aging agent must therefore be able to

1. Effectively protect the surface of the skin tissue from the radical attacks caused by the stresses of everyday life

2. To avoid the dermal extracellular matrix (ECM) which ensures tone and elasticity to the skin, undergo too much action of the enzymes able to induce its degradation (elastase, MMPs)

3. To ensure the recovery of collagen neosynthesis, one of the major constituents of ECM

Through in vitro and in vivo tests, Ephyla has demonstrated the relevance of REVERTIME® in the fight against skin aging.

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In vitro : free radical scavenging activity

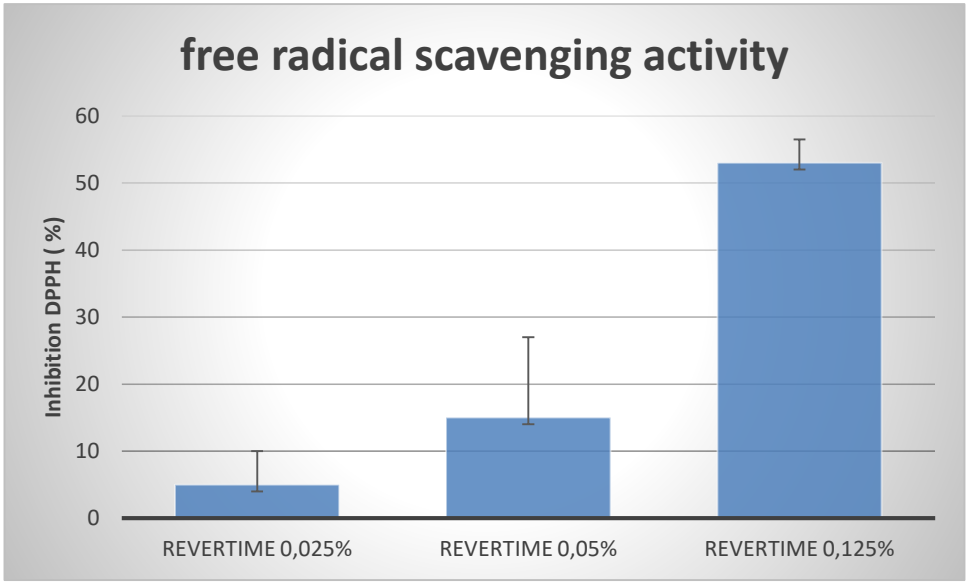
MATERIALS AND METHOD:

Model: Acellular in vitro assay using 1,1-diphenyl 2-picrylhydrazyl (DPPH)

Protocol: DPPH solutions were incubated for 30 minutes in absence (control) or in the presence of increasing concentrations of REVERTIME® (0.025, 0.05 and 0.125%, w / v)).

At the end of the incubation period, the absorbance of the reaction media at 520 nm was measured and the free radical scavenging activity was calculated.

Each experimental condition was performed (at least) in triplicate (n = 3).



Average significantly different from Control's one (p<0,05).

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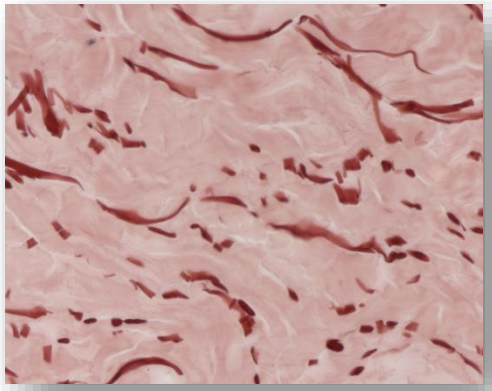
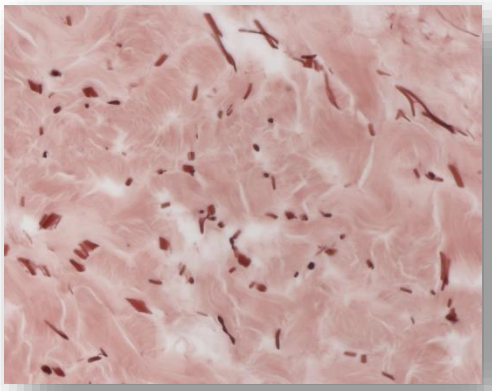
In vitro : Inhibition of elastin degradation

Cryo-sections of human skin are used as reaction substrate. Human skin cryo-sections are incubated at 37 °C during 30 minutes, with or without the active ingredient. After that, human skin cryo-sections are incubated at 37 °C during 3 hours in the same conditions, with or without an elastase.

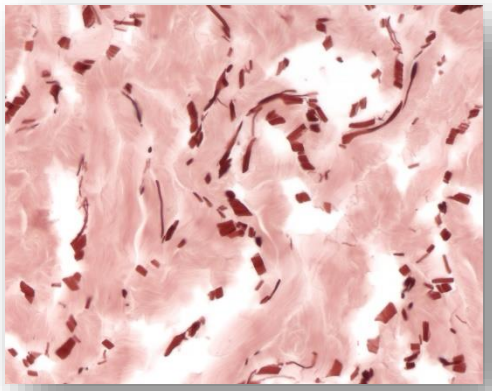
For each experimental condition retained, the elastase activity is measured on the section skins which are colored with orcein, by quantification of the occupied area by elastic fibers.



Normal elastin rate

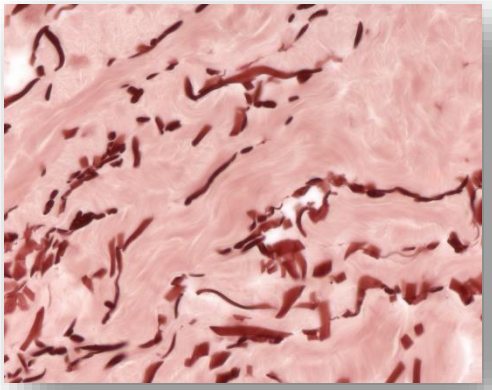


Maximal elastin degradation



Revertime à 0,01%

Degradation inhibition - 37%



Revertime à 0,1%

Dégradation inhibition -100%

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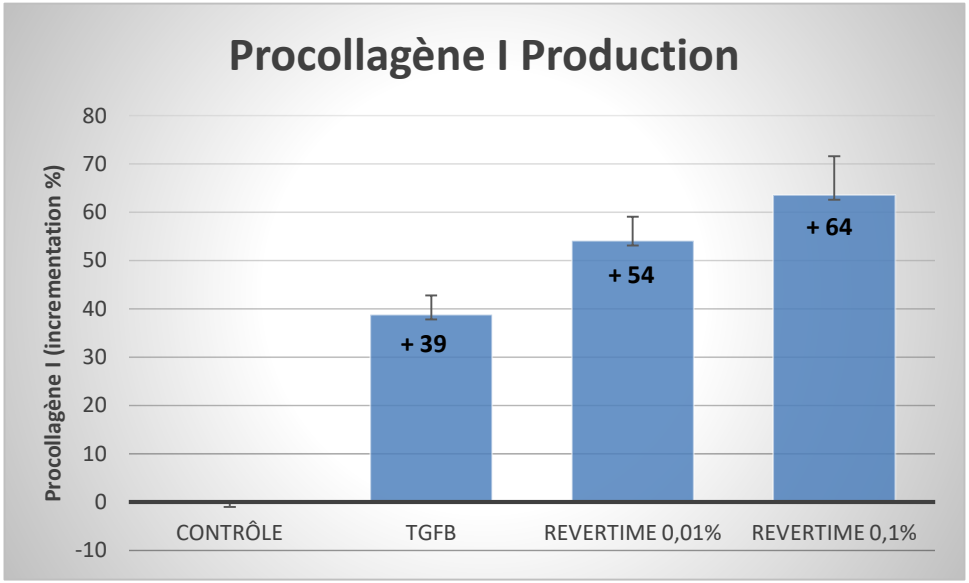
IN VITRO: Stimulation of Procollagen I synthesis

MATERIALS & METHODS :

On a model of a normal dermal monolayers human fibroblasts.

Fibroblasts are incubated at 37°C during 48 hours, under humid atmosphere and 5 % of CO2, with or without the reference product (TGF β 50 ng/ml) or active ingredient at different concentrations.

At the end of the incubation period, procollagen, in the culture mediums, is quantified with a specific and sensitive ELISA kit. Tests evaluated each experimental condition in triplicate.



Average significantly different from Control's one (p<0,05).

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In Vivo: Reduction of crow's feet wrinkles

MATERIAL AND METHOD :

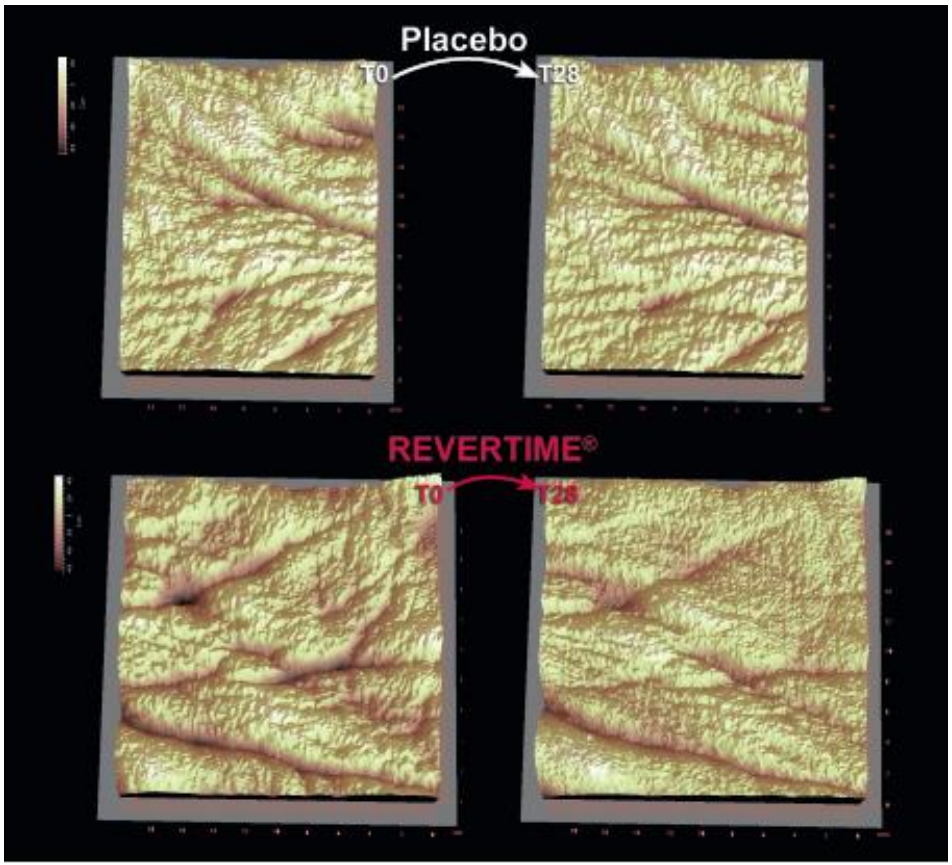
Morphological analysis of crow's feet wrinkles on 15 volunteers for 28 days (analysis are carried out using a fringe projection device). 0.1% Revertime dosage in topical application

Results:

1) Decrease in surface area and volume of wrinkles :

- **12,3 % and 13,2 % respectively**

- 2) Decreased amplitude and average roughness of skin tissue :
  - **5,5% et 2,6% respectively**



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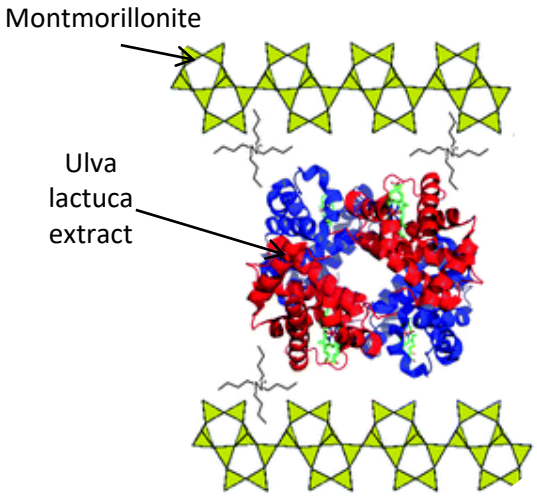
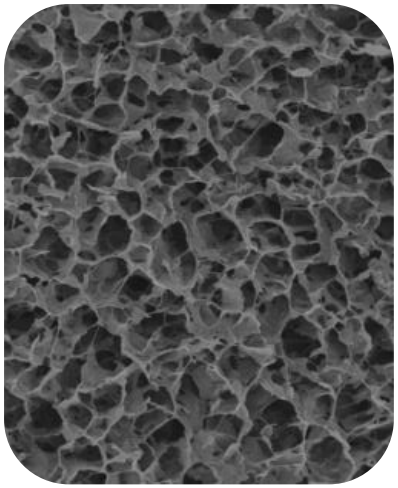
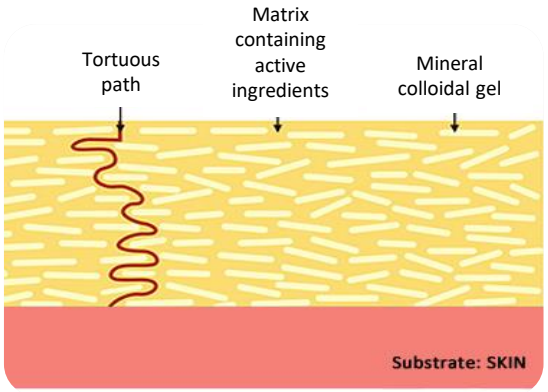
PROTECTION and DRUG RELEASE mechanism

Protection and stability of active ingredients





Drug release mechanism



Active ingredients as raw material in powder from:

Biological active ingredients are integrated in bentonite sheets as a dehydrated form without preservatives.

Active ingredient as finished products:

In aqueous environment, betonite creates a 3D structure as « honeycomb » able to protect biological active ingredients.

Once on the skin:

Creation of a scaffolding structure type « second skin » able to release biological active ingredients in a controled way.

Revertime, technical specifications

- INCI: Montmorillonite & Ulva lactuca extract
- CAS: 1318-93-0 & 97281-59-9
- EINECS: 215-288-5 & 306-651-0
- COSMOS certified; China Compliant
- APPARENCE, beige powder, preservative free
- FORMULATION: dispersible en phase aqueuse
- STORE CONDITIONS: 18 months in a ventilated area
- DOSAGE: 0,1 %
- TOLERANCE:
  - Cutaneous: non-irritant
  - Ocular: moderately irritant
  - Genotoxicity: non-mutagenic
  - Cutaneous skin sensitization test: non-irritant and non-sensitizing
- **Certified estrogen-like endocrine disruptors free**

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Conclusion

1. Revertime helps to fight the radical attacks daily suffered by the skin tissue. The surface of the skin is effectively protected
    - Anti-free radical action: + 53 %
2. Revertime provides effective protection of intradermal collagen, reinforcing its anti-aging action through the preservation of a major actor of tonus and skin elasticity:
    - Elastine protection: + 100 %
3. Revertime allows stimulation of the intracellular mechanisms involved in the production of type I procollagen
    - Stimulation of Type 1 Collagen Synthesis: +64%
4. REVERTIME® is thus positioned as an "anti-wrinkle" active that is also capable of restructuring the skin tissue in depth in order to give it a more youthful appearance:
    - Decrease in surface area and wrinkle volume: 12.3% and 13.2% respectively

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