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Ephylife

Rewinding your epigenetic clock

A road map to everyone true potential: Hallmarks of aging

The evolutionary theory of aging has set the foundations for a comprehensive understanding of aging.

In 2013, , López-Otín and colleagues listed and described the "hallmarks of aging," i.e., cellular and molecular interconnected mechanisms involved in human aging[1].

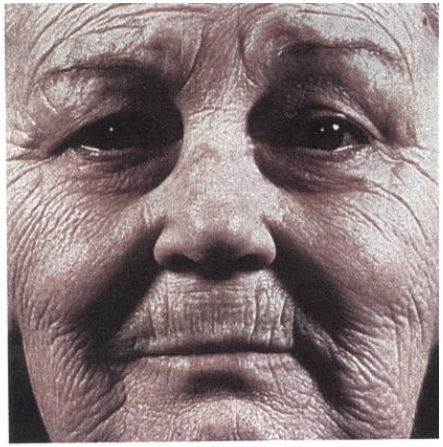
In 2022, during the Copenhagen ageing meeting, a consortium of renowned research institutes completed the list with 6 new hallmarks[2].

Same DNA, Different Epigenetics
TWINS example

Chronological aging

Skin not exposed to UV

Actinic aging



UV-exposed skin

The critical outstanding questions are:

- Can aging processes be slowed down or even restored?
- Can we reverse our epigenetic clock?

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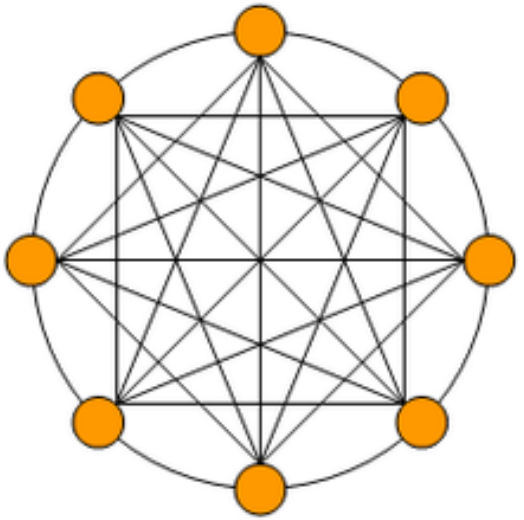
A road map to everyone true potential: Hallmarks of aging

- **Action:** Primary hallmarks of aging (causes of damage)
  - Epigenetic alterations#1
  - Genome instability#2
  - Telomere shortening#3
  - Splicing dysregulation#14
- **Reaction:** Antagonistic hallmarks (responses to damage)
  - Deregulated nutrient sensing#7
  - Mitochondrial dysfunction#10
  - Cellular senescence#5
  - Compromise autophagy#11
  - Inflammation#9
- **Expression:** Integrative hallmarks (impact on the phenotype, visible marks)
  - Stem cell exhaustion#4
  - Loss of proteostasis#6
  - Altered mechanical properties#8
  - Altered intercellular communication#12
  - Microbiome disturbance#13



These hallmarks are interconnected

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Cashew nuts, origin

- The «Anacardium occidentale» is a shrub originating from South America which grows today in all the tropical zones of the planet and in particular, in West Africa.

- In the Organic Cashew Nut, we draw a powerful epigenetic active ingredient that is particularly effective in improving skin hydration and erasing the unsightly stigma of time.

- EphyLIFE is titrated in natural ANACARDIC ACID:
  Minimum Concentration= 2000 mg/Kg.

- **No food or cosmetic allergen according to the regulation CE 1223/2009**

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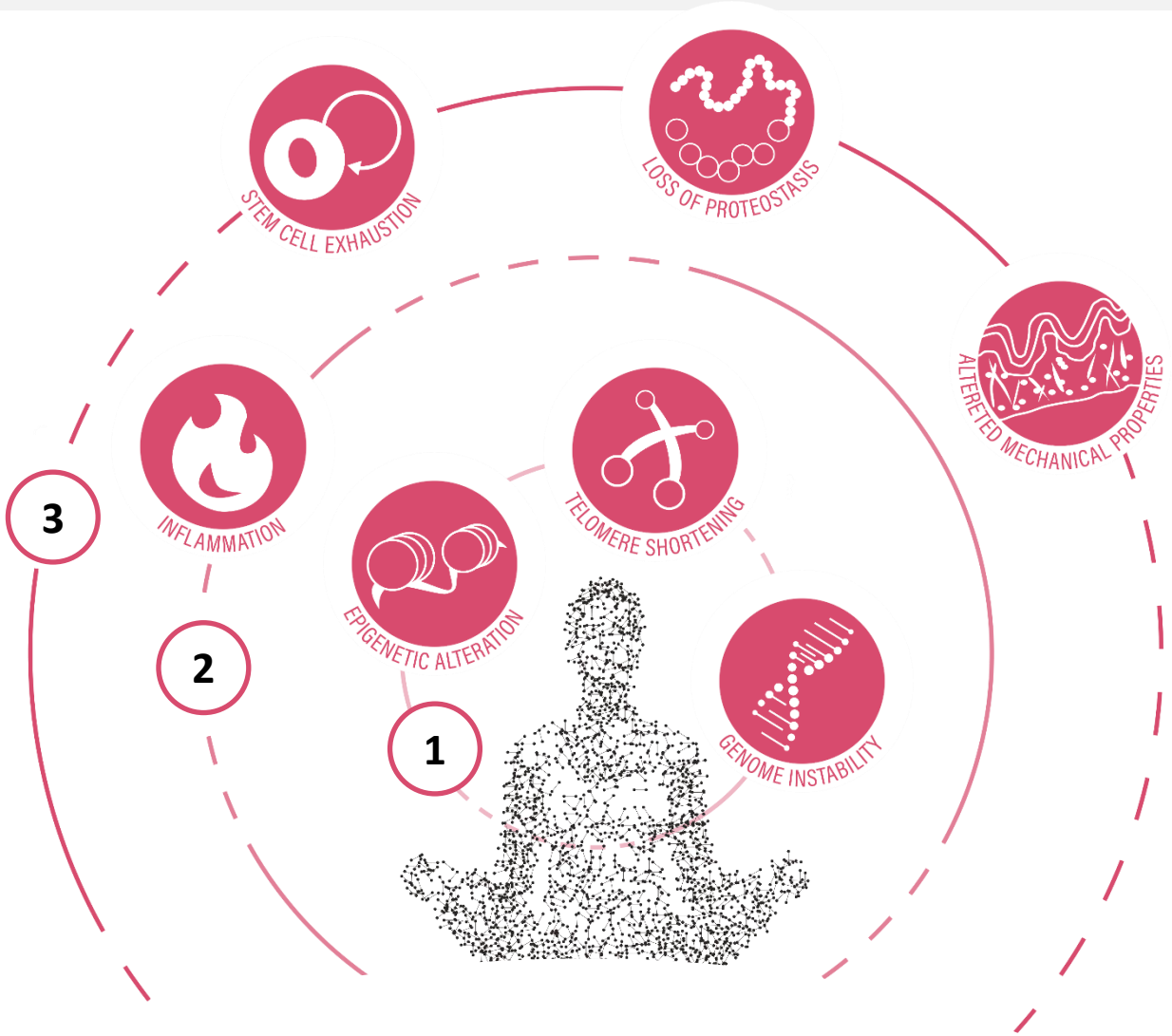
Beyond wellness, Wellbeing: Hallmarks of aging

3) Expression: Integrative hallmarks (impact on the phenotype, visible marks)

2) Reaction: Antagonistic hallmarks (responses to damage)

1) Action: Primar hallmarks of aging (causes of damage)



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Hallmarks of aging #1,2

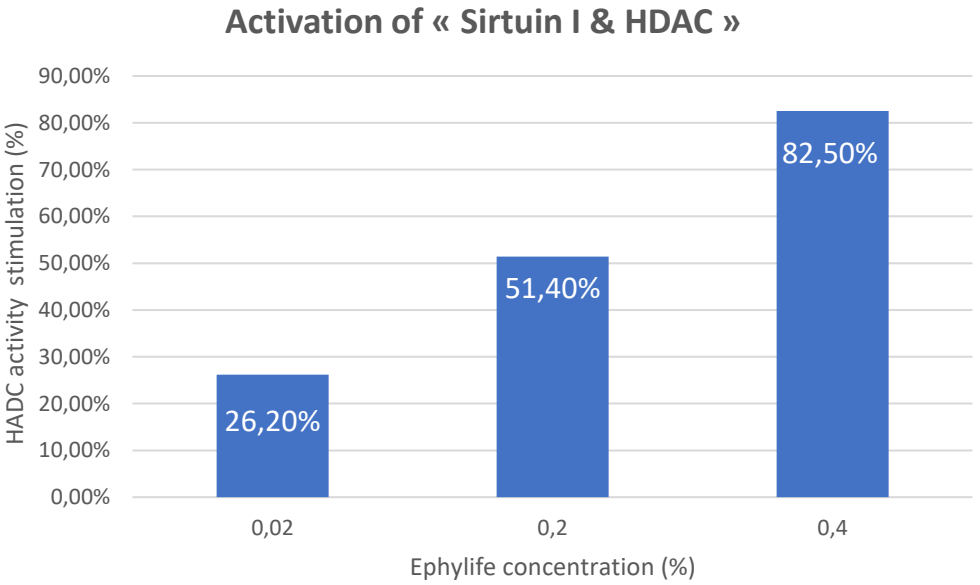
HDAC Activation

EphyLIFE acts at the heart of the cell nucleus, it maintains the flexibility of the chromosomes by stimulating the good winding of the chromatin around the histones (Stimulation of the activity of HDAC and in particular, of Sirtuin I).

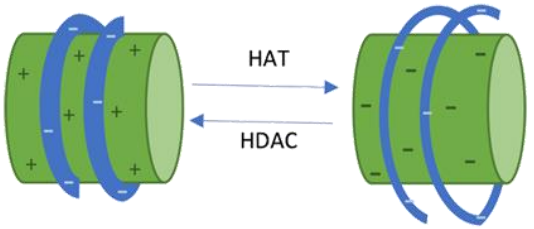
This action preserves the quality of the genetic material when chromatin decodes and opens up by unwinding its DNA to express a gene.

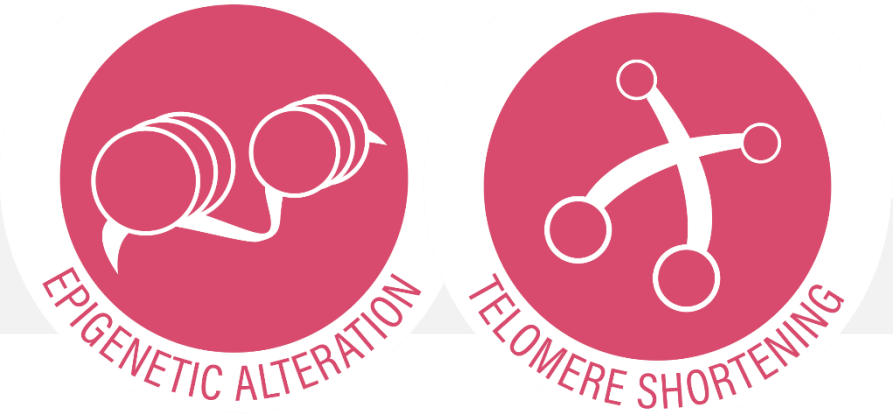
It is then important that the DNA remains exposed to the numerous enzymes and molecules in the cell nucleus for only a short time.

The proper winding of DNA around histones is ensured mainly by HDAC (Histone deacetylases) and in particular, type I Sirtuins. The activity of these enzymes offers a high protection to the genetic material. The higher their activity, the more efficient and faster the chromatin condensation.



Material and method: enzymatic test_*In Tubo* evaluating the increase of the basal activity of the enzymes (basal activity reduced to zero in this graph). Test carried out in triplicate with controls.





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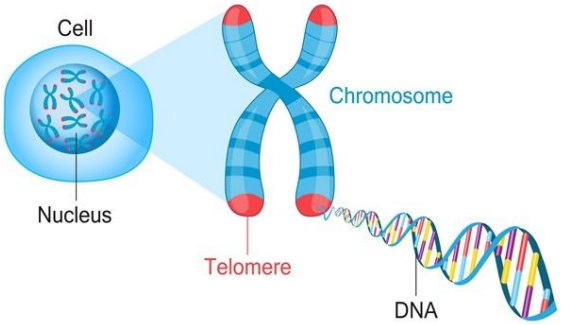
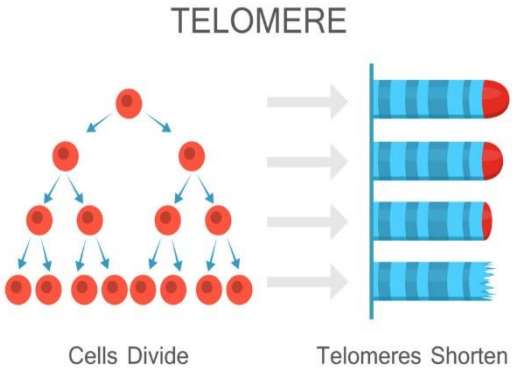
Hallmarks of aging #1&3

Sirtuins & telomere

SIRT1 is a positive regulator of telomere length *in vivo* and attenuates telomere shortening associated with aging, an effect dependent on telomerase activity
Using chromatin immunoprecipitation assays, it has been demonstrated that SIRT1 interacts with telomeric repeats *in Vivo*. In addition, SIRT1 overexpression increases homologous recombination throughout the entire genome, including telomeres, centromeres, and chromosome arms. These findings link SIRT1 to telomere biology and global DNA repair and provide new mechanistic explanations for the known functions of SIRT1 in protection from DNA damage and some age-associated pathologies [3].





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Hallmarks of aging #3

Ephylife slows down the shortening of **telomere**

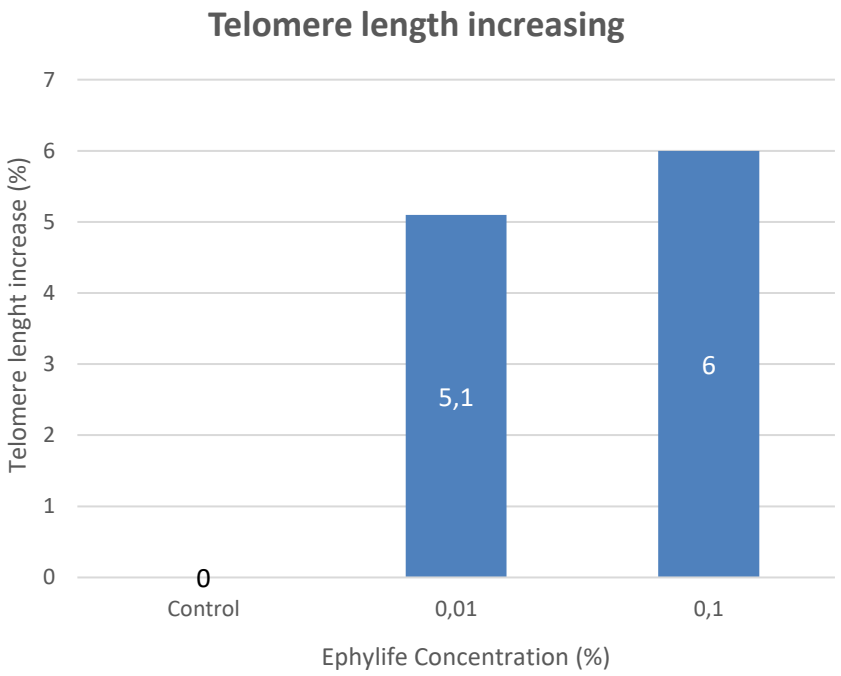
MATERIAL AND METHOD : *In cellulo* test

The aim of this study was to evaluate the effect of Ephylife on telomere shortening in a model composed by human normal fibroblasts in monolayer culture.

Human fibroblast cells were obtained from a 44-years-old donor. Fibroblasts were cultivated during 3 consecutives passages in absence (control) or in presence of increasing concentration of active ingredient

At the end of the incubation, cells were trypsinised. DNA was extracted from cells using a dedicated DNA extraction kit. DNA was quantified by nanodrop.

Telomere length measurement have been performed by using quantitative PCR, and by comparing telomere length between cells at passage 2 and 5



Winding back the biological clock

Hallmarks of aging #4

STEM CELL EXHAUSTION

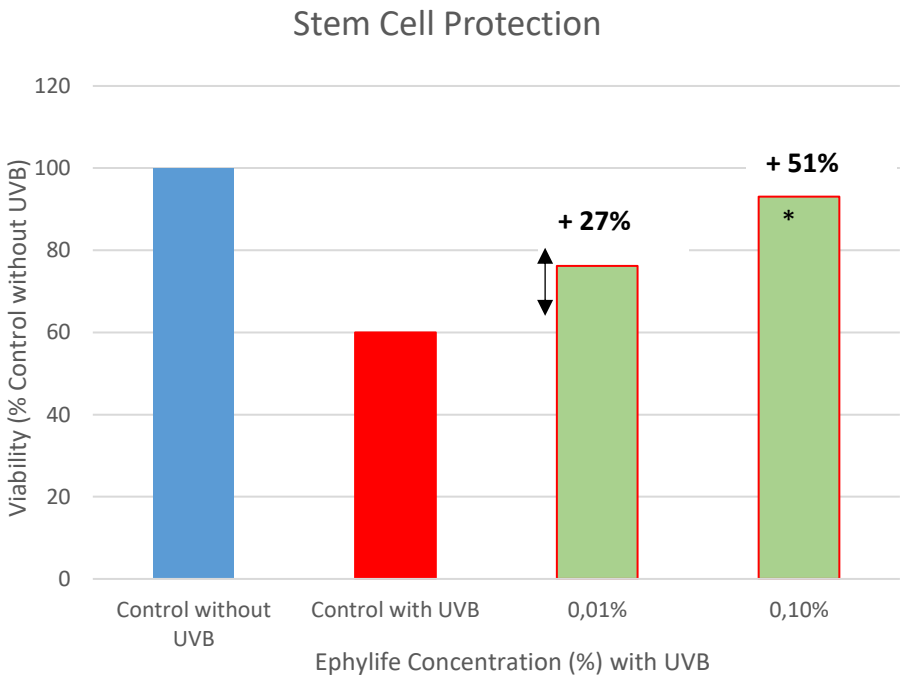
The youthfulness of our skin lies in the pool of stem cells that reside in the basal layer of the epidermis. The genetic freshness and proper cell renewal of our skin depend on this pool of stem cells. In essence, very fragile and extremely sensitive, stem cells are the first to be impacted by stress and cellular constraints.

MATERIAL AND METHOD : *In Cellulo* test

Normal human keratinocytes were obtained from a 76-year-old donor. The cells were grown to approximately 80% confluence. The media were then "enriched" with stem cells using the method of Goodell et al(*). The cells were pre-incubated for 24 hours at 37°C in the absence (control) or presence of the reference product.

At the end of the pre-incubation period, the cells were irradiated with UVB (30 mJ/cm²) and then incubated for 6 days at 37°C in the absence (control) or presence of the active ingredient. The viability of the primary cells is obtained by the Blue Alamar test, performed in triplicate with control.

(*)Hoescht 33342 HSC staining and stem cell purification protocol. (1996) J Exp Med 183, 1797-806



EphyLIFE, through its **epigenetic action** protects the genetic material of differentiated cells, and **maintains** the youthfulness of the skin by **preserving** the stem cells.

Ephylife is able to protect 92% of the stem cells pool under UV stress, avoiding 51% apoptosis.

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Hallmarks of aging #1,6,8

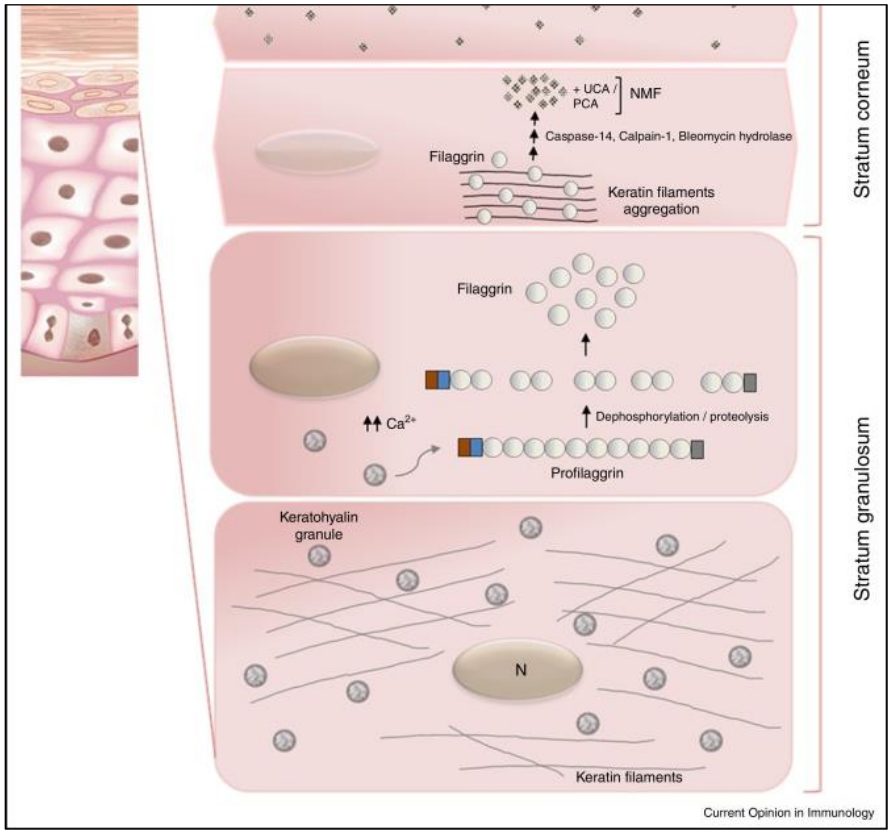
Filaggrin is a component of the cornified cell envelope (corneocytes packing) and the precursor of free amino acids acting as a natural moisturizing factor (NMF) in the stratum corneum. Deimination is critical for the degradation of filaggrin into free amino acids

The ability of filaggrin to aggregate keratin filaments into tight parallel arrays has been demonstrated[4]. This molecular bundling confers mechanical resilience and flexibility to the Stratum corneum

Bleomycin Hydrolase (BH) generates free amino acids from citrullinated peptides in the last step of the filaggrin degradation pathway. This step is crucial in the production of **Natural Moisturizing Factors** (NMF). Bleomycin Hydrolase is the key enzyme in this last proteolytic step.

BH also acts to regulate secretion of proinflammatory chemokines CXCL8 and GROa.

But in skin from atopic dermatitis and psoriasis patients, level of BH is reduced and levels of CXCL8 and GROa increased promoting inflammation and lost of hydration[5].

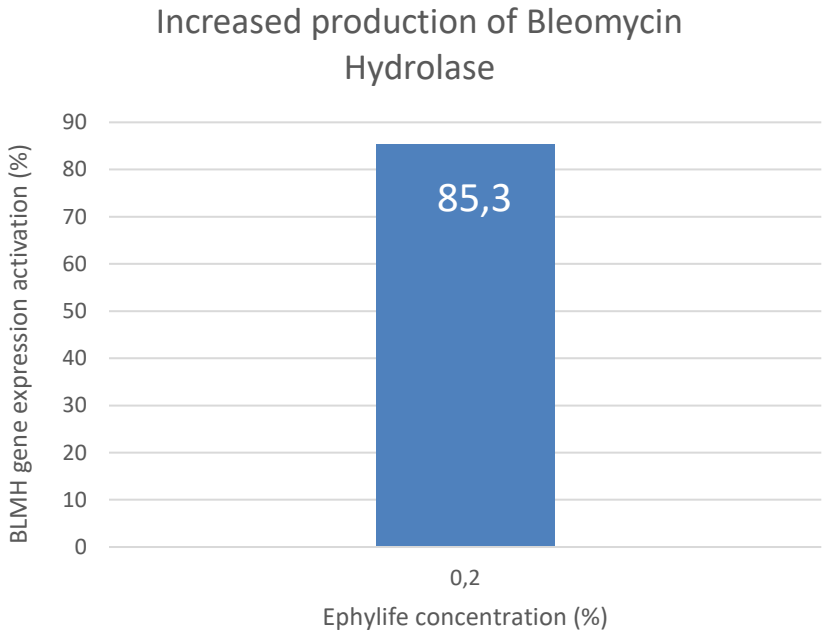


Hallmarks of aging #1,6,8

MATERIAL AND METHOD : In Cellulo test

From a culture of human HeLa cells (9903) incubated at 37°C under humid atmosphere with 5% CO2. At confluence, the culture media are enriched with controls and test products for 48h. At the end of this incubation period.

The cell suspension is centrifuged to recover the cell pellet. The BH assay is then performed using an Elisa kit (Human BLMH Elisa kit). The test is performed in triplicate with controls.



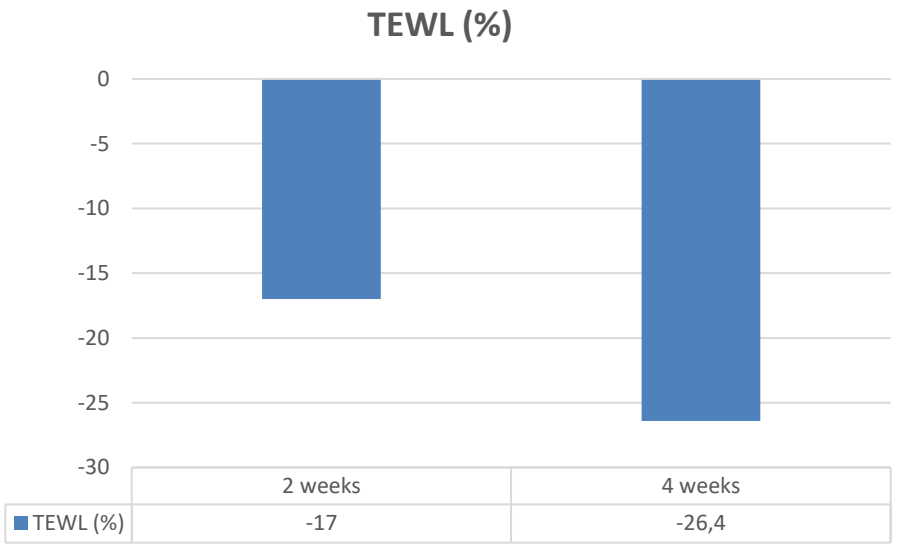
EphyLIFE at a dose of 0.2% is able to increase by more than 85% the gene expression and transcription of Blemomycine Hydrolase (BH)

EphyLIFE has the ability to promote gene expression and transcription of Bleomycin Hydrolase (BH) within skin cells.

Clinical study

Description of volunteers

- Fifteen volunteers
- Age: 37-58
- Women 67%
- Men: 33%
- Application: twice a day
- Ephylife dosage: 1,5%



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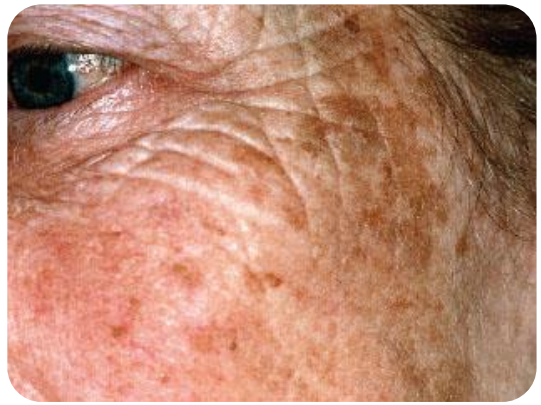
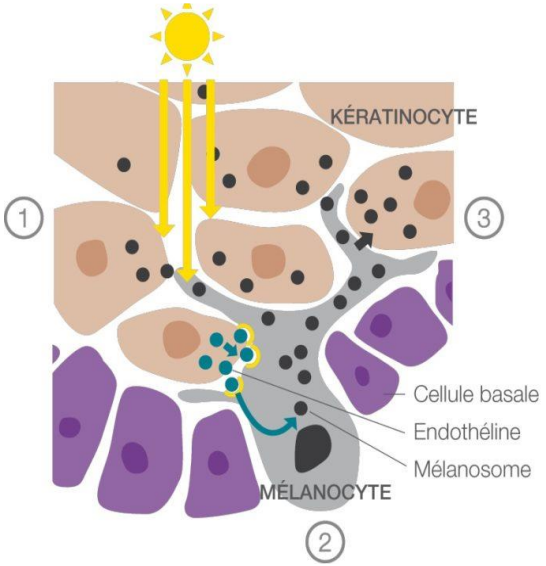
Hallmarks of aging #1,6,8

Endothelin epigenetic silencing

- Recent evidence has suggested that melanocyte functionis regulated by several cytokines that are secreted by surrounding epidermal cells (keratinocytes) in a paracrine fashion. In ultraviolet B (UVB) induced pigmentation, human keratinocytes exposed to UVB are stimulated to produce and secrete vasoconstrictive peptides, termed endothelins (ETs), which trigger the activation of melanocytes and act as potent mitogens and melanogens for human melanocytes 1.
- Lentigo senilis (LS) is the skin condition of common aging spots with accentuated epidermal pigmentation.
- Thus upregulation of the ET cascade, which consists of the production and secretion of ET-1 by keratinocytes and the ET/ETBR binding-mediated signal transduction pathway in melanocytes, plays an important role in the stimulated pigmentation in LS
- Thus, it seems likely that the increased secretion of ET-1 noted in LS epidermis is the major factor responsible for the hyperpigmentation found in this pigmentary disorder[6].





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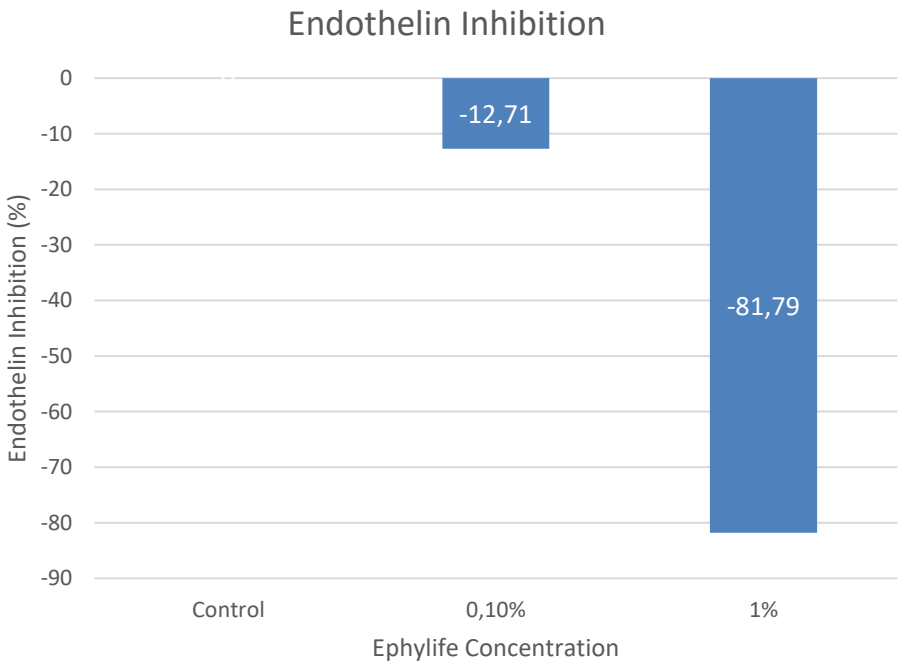
Hallmarks of aging #1,6,8

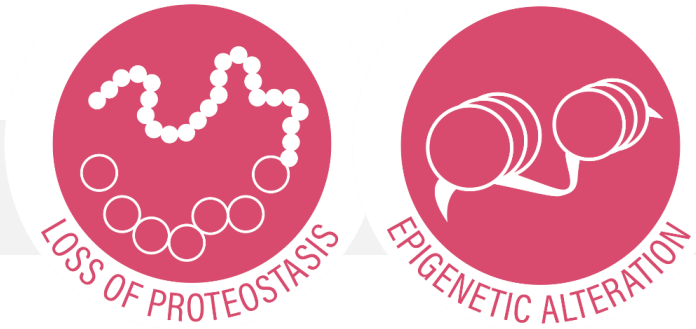
In vitro : Endothelin Inhibition

MATERIAL AND METHOD : *In Cellulo* test

From a culture of human endothelial cells incubated at 36.5°C in a humid atmosphere with 5% CO2. At 80% confluence, the culture media are enriched with controls and test products for 24 hours. At the end of this incubation period, the cell supernatants are removed. The supernatants are then assayed for Endothelin I using an Elisa kit. The test is performed in triplicate with controls.



EphyLIFE inhibits endothelin I in a dose-dependent manner by 82 % at the 1% dose, and provides a further inhibition of 13 % at the 0,1% dose



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Hallmarks of aging #1,6

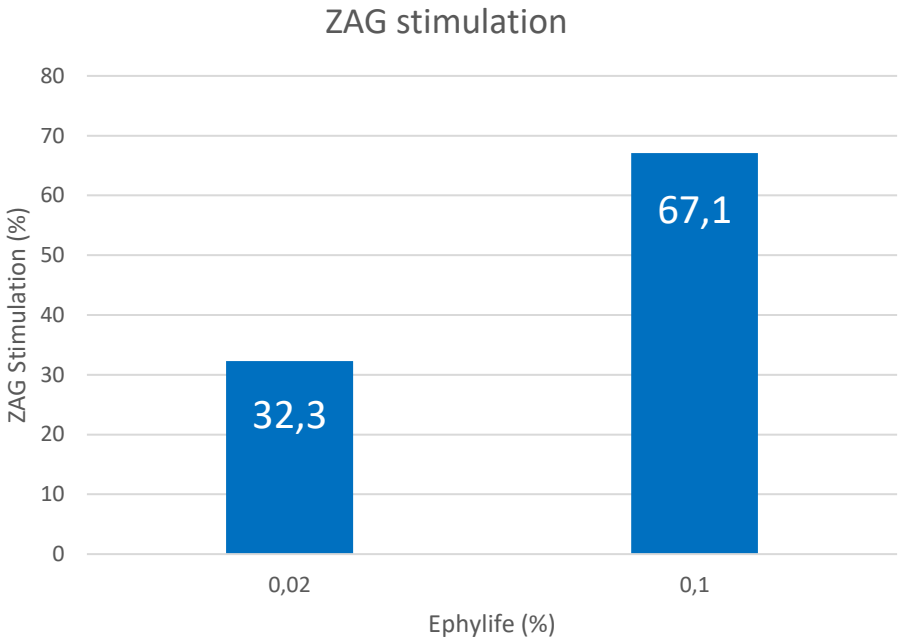
ZAG stimulation

Zinc-a2-glycoprotein (ZAG) is an adipokine; a multidisciplinary protein; which is secreted in various body fluids.

The ZAG plays roles in lipolysis, regulation of metabolism, cell proliferation and differentiation, regulation of melanin synthesis, cell adhesion, immunoregulation, and so forth[7].

Increased ZAG can decrease melanin production by decreasing levels of tyrosinase protein and its activity, which are the key steps for melanin synthesis.

It has also been shown that ZAG is produced by normal epidermal keratinocytes, where its expression improves with cellular differentiation[8,9]. It appears that ZAG as a keratinocyte-derived factor influences melanocyte behavior, including melanocyte proliferation, dendricity, and melanin synthesis[8,10,11].



***In Cellulo* test** From a culture of human keratinocytes incubated at 36.5°C in a humid atmosphere with 5% CO_2. At 80% confluence, the culture media are enriched with controls and test products for 48 hours. At the end of this incubation period, the cell supernatants are removed and then assayed in ZAG using a ELISA Kit.

EphyLIFE promotes ZAG production by 32,3% at the 0,02% dose, and provides a further promotion of 67,1% at the 0,1% dose

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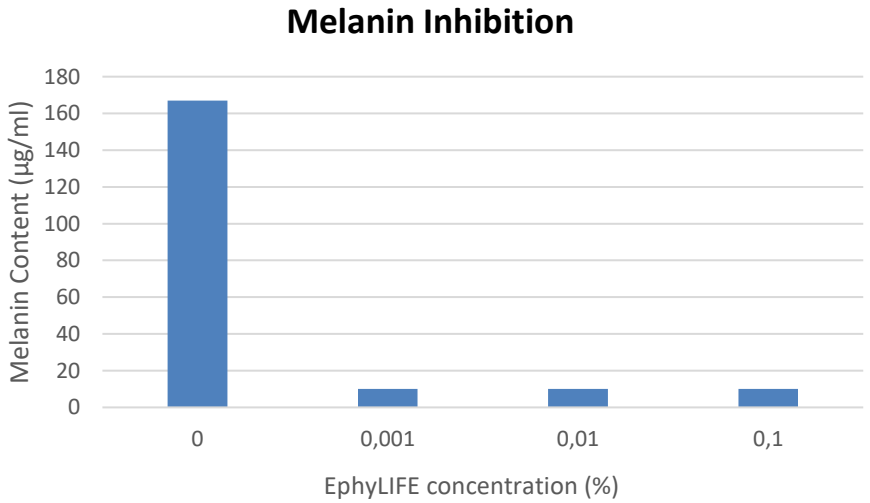
Hallmarks of aging #6

Melanin inhibition

MATERIAL AND METHOD : *In Cellulo* test

From a culture of human melanocytes incubated at 37°C in a humid atmosphere with 5% CO_2. At confluence, the culture media are enriched with controls and test products for 48h. This modulation is evaluated by the determination of melanin in the cell lysates after 5 days of exposure to the extracts.



EphyLIFE drastically inhibits melanin content from a dose of **0,001%**

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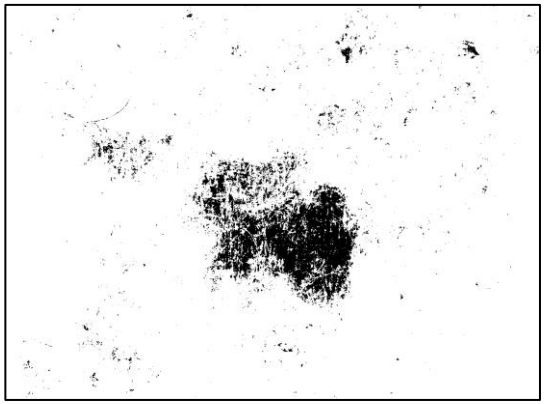
Clinical study at 28 days - FOCUS on Dark Spot

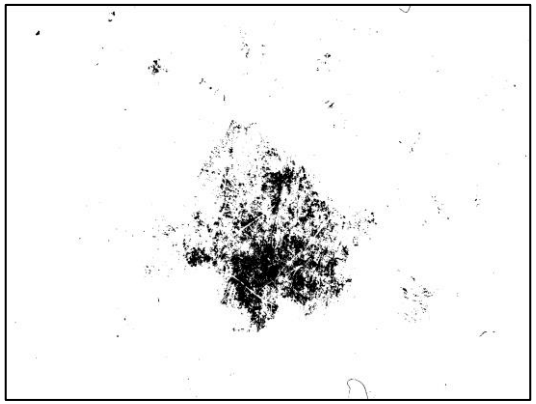
Description of volunteers

- 22 volunteers / Application: twice a day / Ephylife dosage: 1,5%

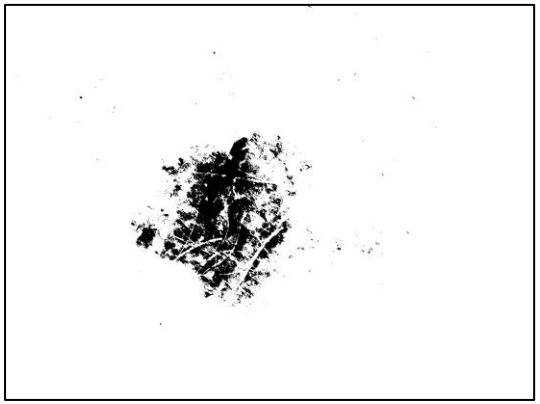
Day 1



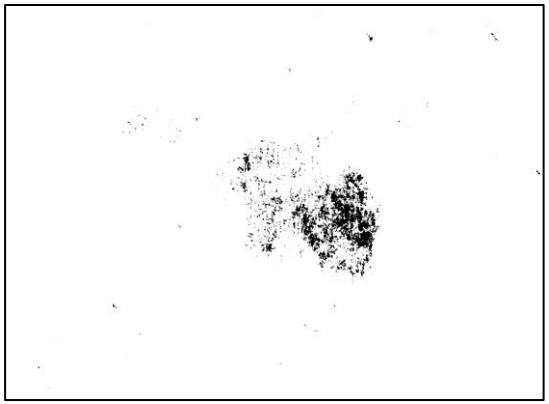
Day 1



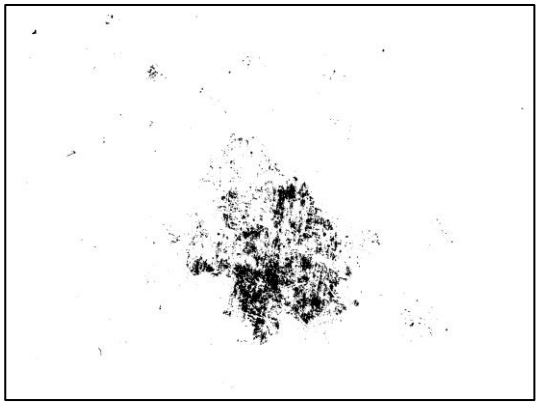
Day 1



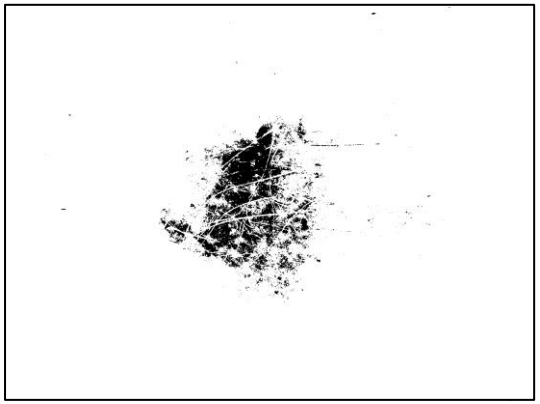
Day 28



Day 28



Day 28

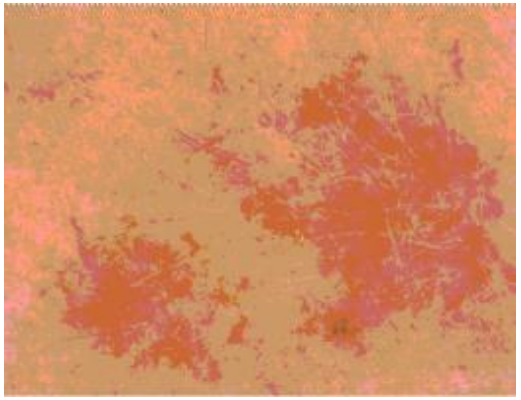


Clinical study at 28 days - Focus on Pigmentation Density

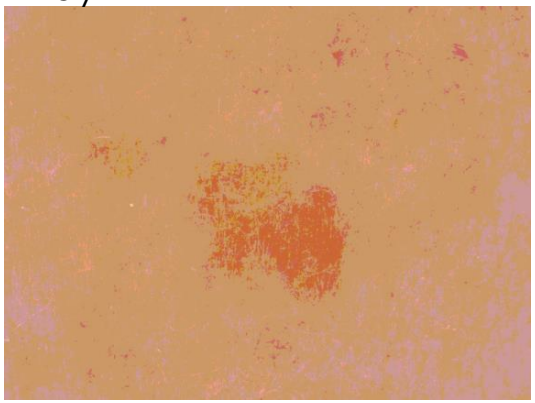
Description of volunteers

- 22 volunteers / Application: twice a day / Ephylife dosage: 1,5%

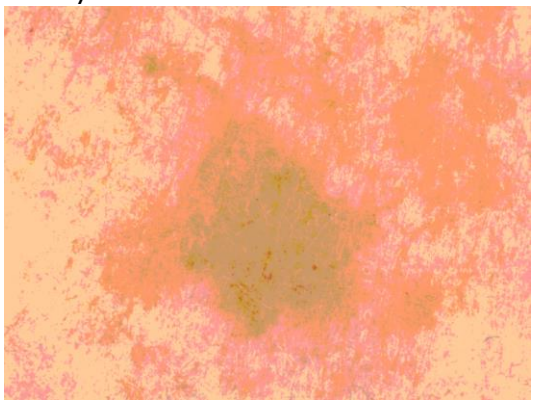
Day 1



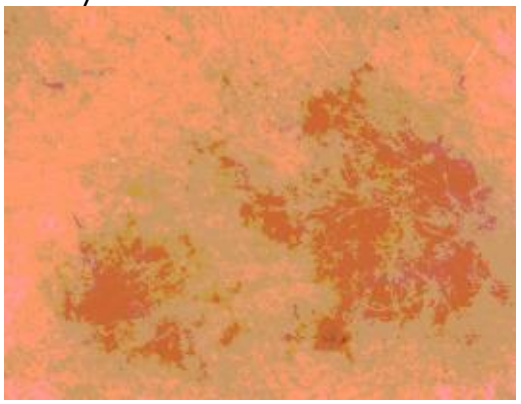
Day 1



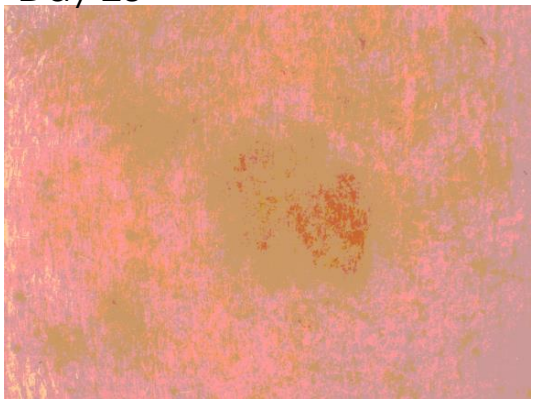
Day 1



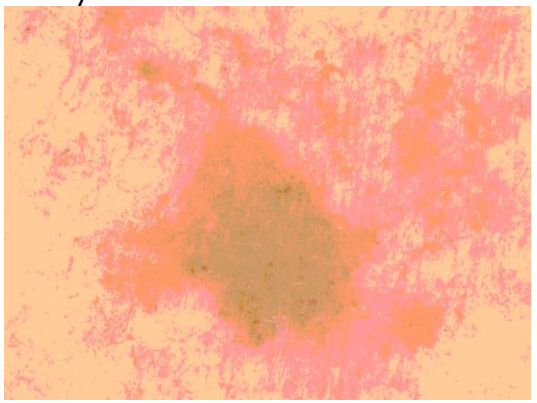
Day 28



Day 28



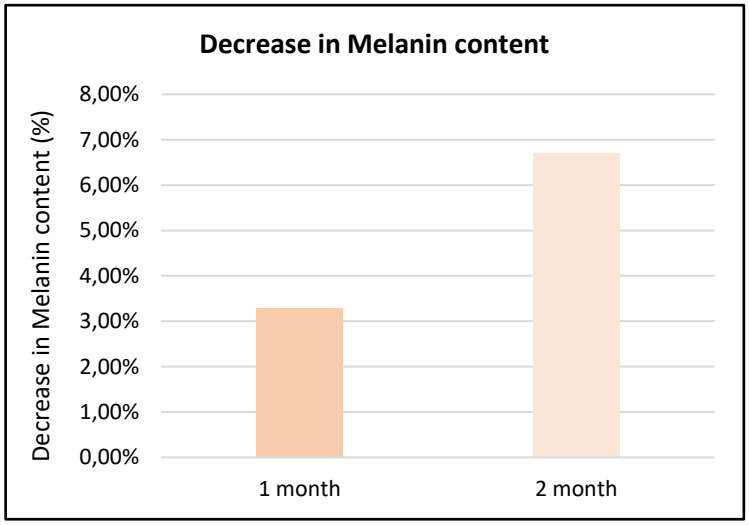
Day 28

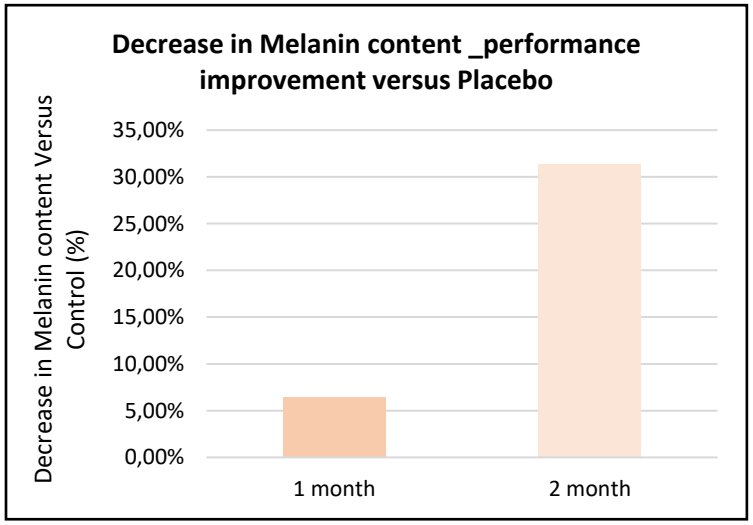


Clinical study at 56 days - Focus on Global Melanin Content

Description of volunteers

- 30 volunteers: 15 using Placebo & 15 using Placebo + Ephylife
- Application: twice a day / Ephylife dosage: 1,5%





100% of study volunteers saw their melanin content decrease at 28 and 56 days

Hallmarks of aging #9

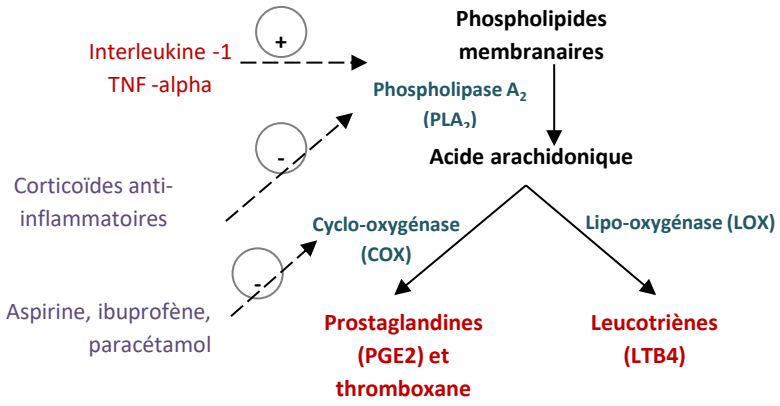
INFLAMMATION

MATERIAL AND METHOD :

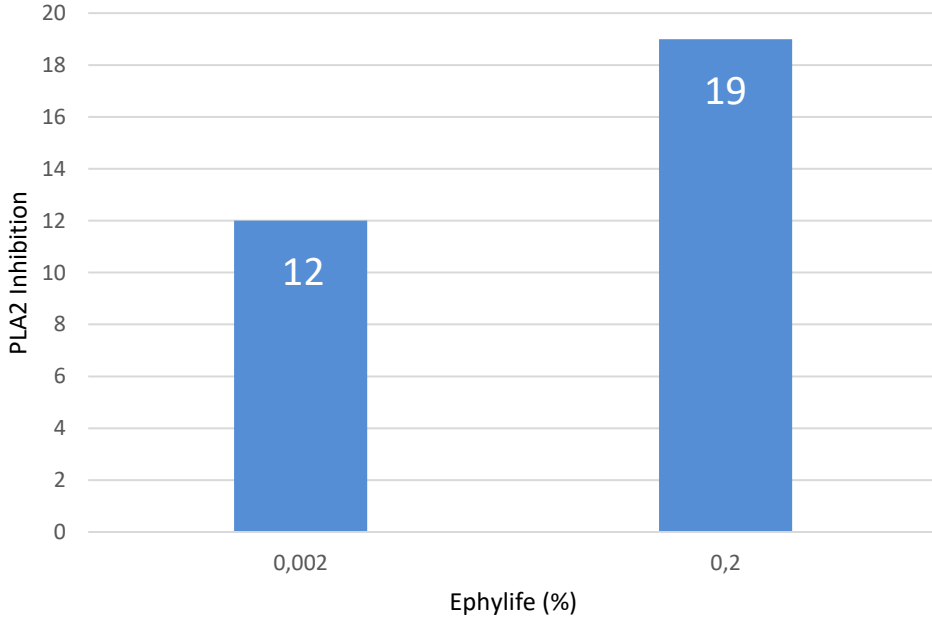
A soothing (anti-inflammatory) action can be demonstrated by the inhibition of the enzymes involved in the cascade of arachidonic acid, in particular phospholipase A2.

The inhibitory activity of phospholipase A2 is studied by enzymatic analysis Test, carried out in triplicate with controls.

.



Inhibition PLA2



EphyLIFE inhibits PLA2 by 12,0% at the 0,002% dose, and provides a further inhibition of 19,0% at the 0,2% dose.

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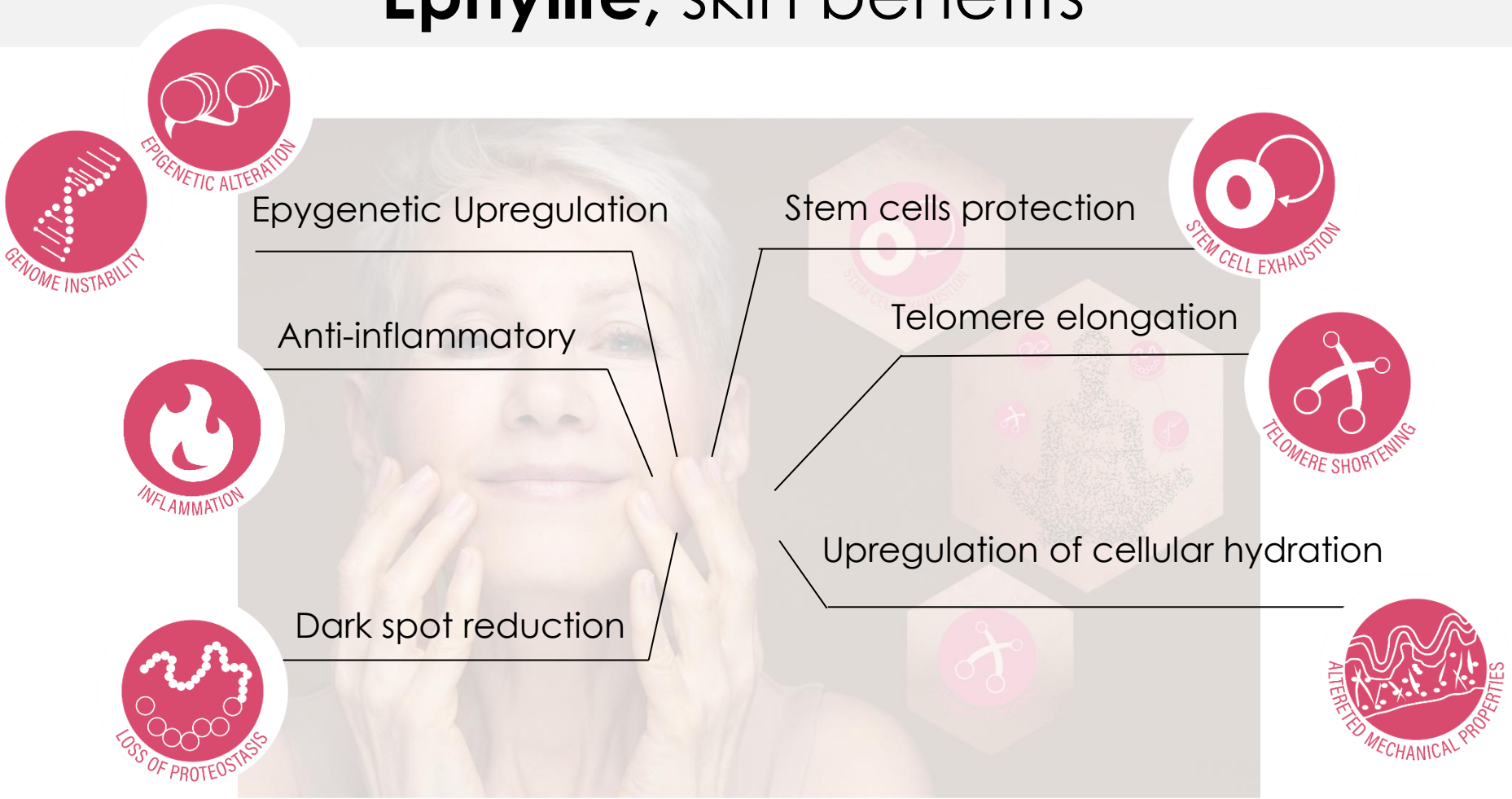
Ephylife, skin benefits



A road map to everyone true potential

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Ephylife, technical data sheet

- INCI: Glycerin, **Anacardium occidentale (Cashew) extract**
- CAS: 56-81-5 & 89958-30-5
- APPEARANCE: beige to yellowish liquid (Room Temperature)
- FORMULATION: Water-soluble
- STORE CONDITIONS: 18 months in a ventilated area
- DOSE OF USE: 1 - 2%
- TOLERANCE:
  - Skin irritation: non-irritating
  - Eye irritation: moderate irritation
  - Phototoxicity: not phototoxic
  - Mutagenicity (AMES): not mutagenic & not pro-mutagenic
  - Sensitization (HRIPT): non-sensitizing
- No Food Allergen or Cosmetic Allergen according to cosmetic regulation CE 1223/2009

EPHYLA
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