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ACTIVE SCIENCE TO EMPOWER BEAUTY
JUVENESSENCE™
Youth in Full Power
Metabolism reactivation of aging cells

ALARIA ESCULENTA

The Arctic Winged Kelp

ALARIA ESCULENTA: a brown seaweed that gets its name from its Wing-shaped Ribbeb Thallus

- Alaria **can grow up** to **2 meters long** & **few dozen centimeters large**.
- It **lives in extreme conditions** in agitated but transparent waters, where **sea pressure can exceed 6T/m²**.
- It is made up of a **network of elastic fibers** (proteins & phycopolysaccharides), which:
  - **- preserves tissue integrity** against compression &
  - **- ensures cohesion & firmness of all the cell architecture**

  (similar role that **proteic fibers & elastin** play in the Skin).
- It is **sourced in Europe (France & Ireland)**

About The Arctic Winged Kelp

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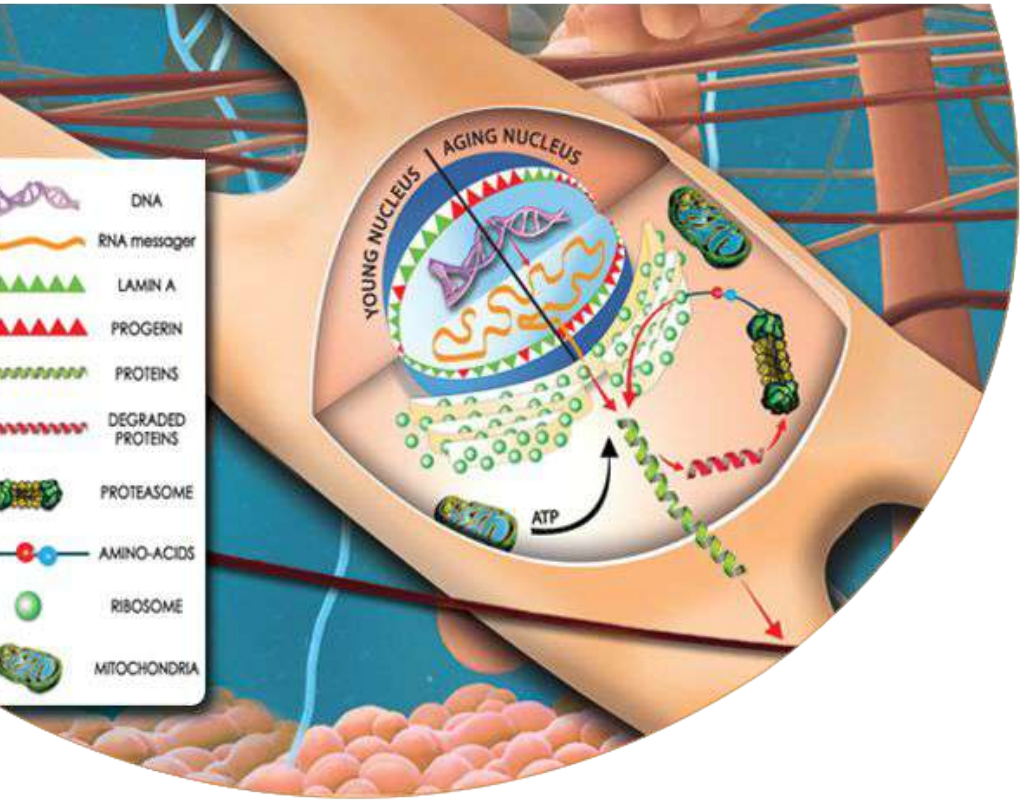
SKIN AGING: A COMPLEX MECHANISM

Involvement of nucleus membrane & some «cell actors»



SKIN PROTEINS SYNTHESIS ARE UNDER THE CONTROL OF DIFFERENT "CELL ACTORS": THE NUCLEUS, THE RIBOSOME & THE PROTEASOMES.

- Nucleus directs protein synthesis by synthesizing messenger RNA (mRNA) according to instructions provided by the DNA.
- The mRNA is then transported to the cytoplasm via the nuclear membranes pores.
- Once an mRNA molecule reaches the cytoplasm, ribosomes translate the mRNA's genetic message into the primary structure of a specific polypeptide & finally in proteins.
- Proteasomes insure the elimination of degraded proteins in amino acids in the aging cells.
- These could be used again as raw materials for the synthesis of new proteins.

Concept : The nucleus constitutes one of the first targets of aging. The premature aging disease is caused by constitutive production of Progerin, a degraded form of the nuclear architectural protein Lamin A.
Progerin protein is present at concentrations that are increasing in both Progeria disease & normal cells as they age. This protein is also linked to physiological aging. Progerin is found in higher quantity in keratinocytes & fibroblasts of older donors, than in young donors. "Progerin cells" exhibit extensive nuclear defects,which increase DNA damages & genes expression damages. At the organism level, an increase of progerin affects dermis & epidermis tissues.

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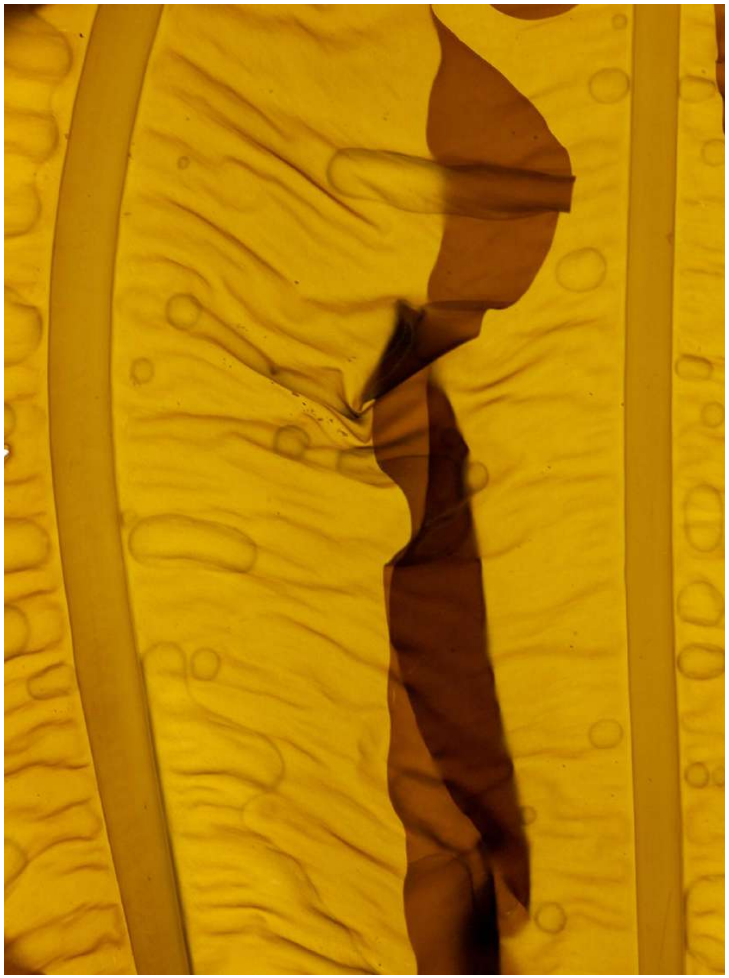
Characteristics



INCI name:	***Caprylic/Capric Triglyceride – Alaria esculenta extract***
Use level:	**1%**
Solubility:	**Liposoluble**
Form:	Liquid (pale yellow to dark green liquid)
Preservatives:	Free
Claims:	**Metabolism reactivation of aging cells**
Approvals:	CHINA COMPLIANT* · ECOCERT COSMETICS* · ECOCERT RAW MATERIAL COSMOS APPROVED* · NATRUE APPROVED · MB RSPO MASSBALANCE CERTIFIED · HALAL CERTIFIED*

ECOCERT Cosmetics: raw material approved by ECOCERT GREENLIFE, in conformity with Ecocert natural and organic cosmetic standard./ **COSMOS Approved**: raw material approved by ECOCERT GREENLIFE, in conformity with Cosmos standard. / **NATRUE Approved**: raw material certified according to Natrue standard. / **MassBalance Certified**: supplied under MassBalance certification BVC-RSPO-1-1972708497. Contributes to the production of certified sustainable palm oil. / **HALAL Certified**: BiotechMarine site & products have been audited & certified by HCS (Halal Certification Services) ; officially recognized by MUI (Indonesia), JAKIM (Malaysia), MUIS (Singapore), CICOT, and other respected Halal authorities around the world. ***China Compliant:** all INCI names are listed on the IECIC 2021

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Agenda

Introduction:

ALA*RIA ESCULENTA*

Skin aging: a complex mechanism

JUVENESSENCE™: Characteristics

- Reduction of accelerated aging protein: **Progerin**
- **Metabolism reactivation** of aging cells
- Regulation of **photo-aging mediators**
- **Anti-aging** efficiency: ***In vivo* studies**

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Reduction of
Accelerated Aging Protein:
Progerin

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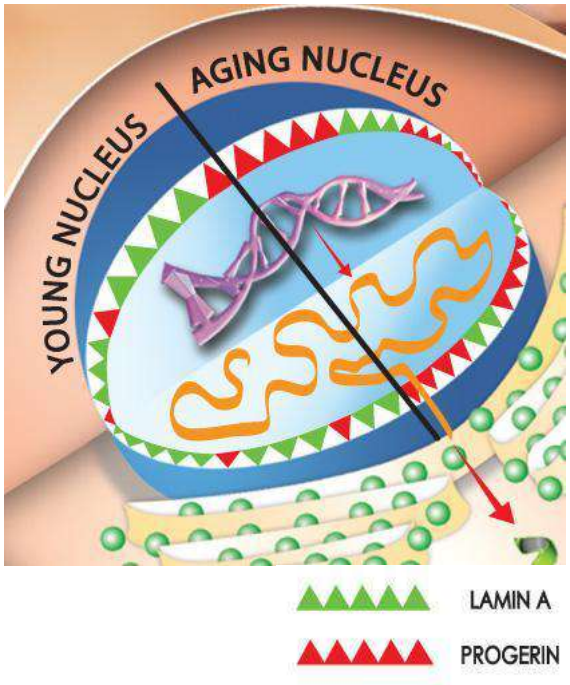
Reduction of the accelerated aging protein: PROGERIN

THE NUCLEUS:
One of the first targets of aging

Structural integrity of the nucleus

> This integrity plays an **essential role** in the adequate functioning of the cell, especially in:

- the **gene transcription**
- the **DNA replication**
- the **chromatin remodeling**



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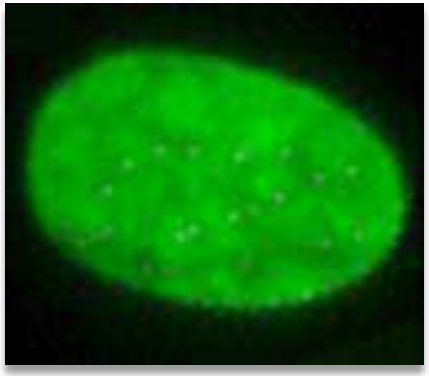
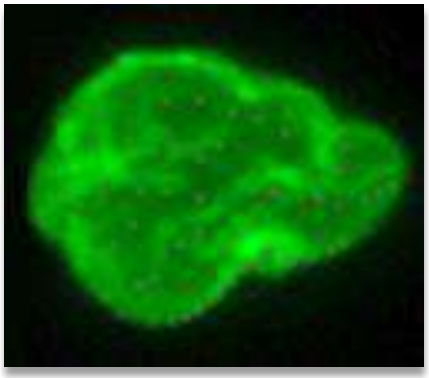
Reduction of the accelerated aging protein: PROGERIN

PROGERIN,
a degraded form of the nuclear architectural protein, Lamin A, is involved in the premature

A- Normal fibroblast nuclei

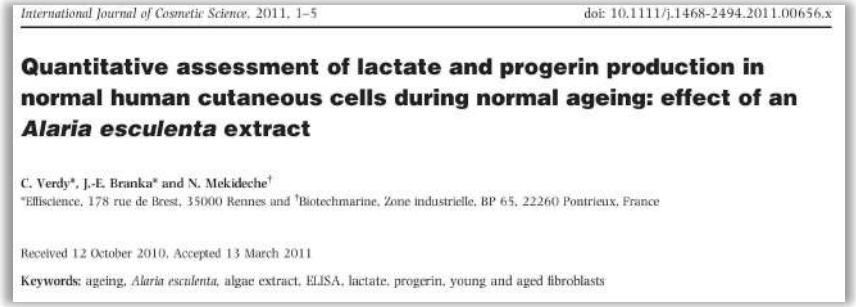


B- Progeria fibroblast nuclei



Publication by BiotechMarine

International Journal of Cosmetic Science, 2011, 1-5



International Journal of Cosmetic Science, 2011, 1–5 doi: 10.1111/j.1468-2494.2011.00656.x

Quantitative assessment of lactate and progerin production in normal human cutaneous cells during normal ageing: effect of an *Alaria esculenta* extract

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Received 12 October 2010, Accepted 13 March 2011
Keywords: ageing, *Alaria esculenta*, algae extract, ELISA, lactate, progerin, young and aged fibroblasts

In conclusion, we have demonstrated for the first time that it is **possible to quantify Progerin in human normal cutaneous cells.**

We have also shown than **in selected experimental conditions, Progerin production was linked to cell aging.**

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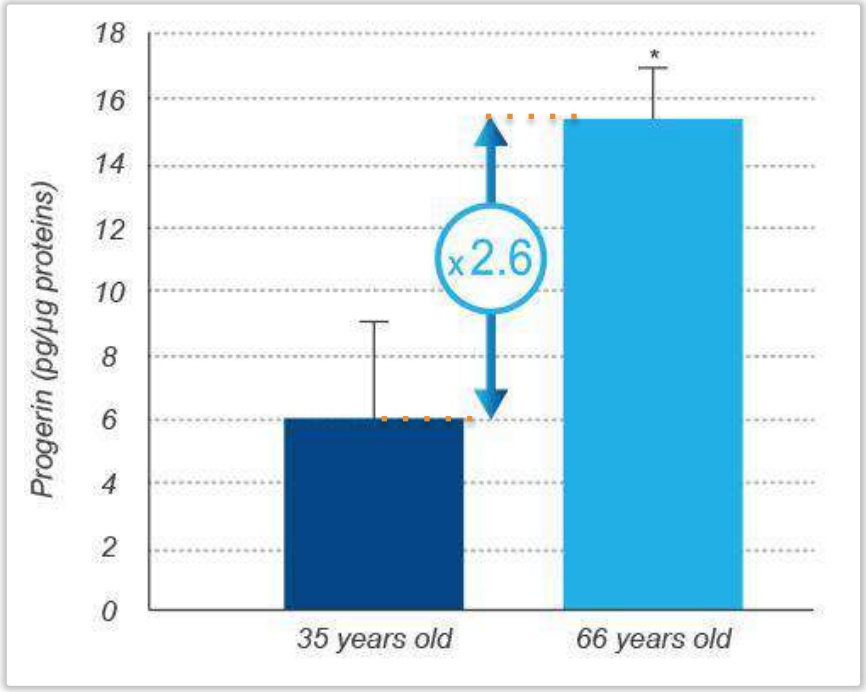
Reduction of the accelerated aging protein: PROGERIN

ASSESSMENT OF THE PROGERIN PRODUCTION in keratinocytes

Protocol

- **Human keratinocyte model, cultured in 3D**, from a *young subject* and a *mature subject* (from abdominal plasties, performed on women aged 35 to 66 years old)
- **Incubation of products** (3D models were incubated 96H)
- **Assessment of effects: Progerin assay** (ELISA)

Results



Explanation on the mechanism of action in Progerin regulation

Progerin is found in higher quantity in keratinocytes of older donors than those of young donors **(2.6 times higher)**

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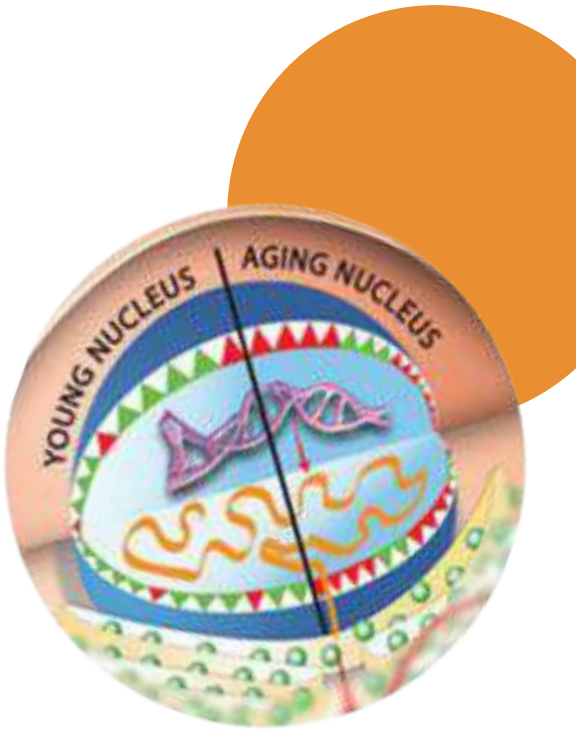
Reduction of the accelerated aging protein: PROGERIN

CONSEQUENCES OF PROGERIN ACCUMULATION

At nucleus level:

- Nucleus membrane integrity loss
- Nucleus malformation
- Increase of DNA alteration
- DNA repair process alteration
- Genes transcription process alteration



At the organism level, Progerin increase affects:

- Skin proteins
- Dermis & epidermis tissues

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Reduction of the accelerated aging protein: PROGERIN

ASSESSMENT OF **JUVENESSENCE™** ON THE PROGERIN PRODUCTION

Protocol

> Human keratinocytes model, cultured in 3D, from 'young' & 'mature' subjects
(from abdominal plasties, on women aged from 35 to 66 years old)

> Reference product: the Insuline
(increases the enzyme activity involved in the Progerin formation).

> Incubation of products (3D models were incubated 96H):

- **in the absence of the product** (control)
- **in the presence of the reference product**
- **in the presence of JUVENESSENCE™** at different concentration rates

> Assessment of effects:

Progerin dosage (by Elisa kit) & ***proteins dosage*** (Bradford)

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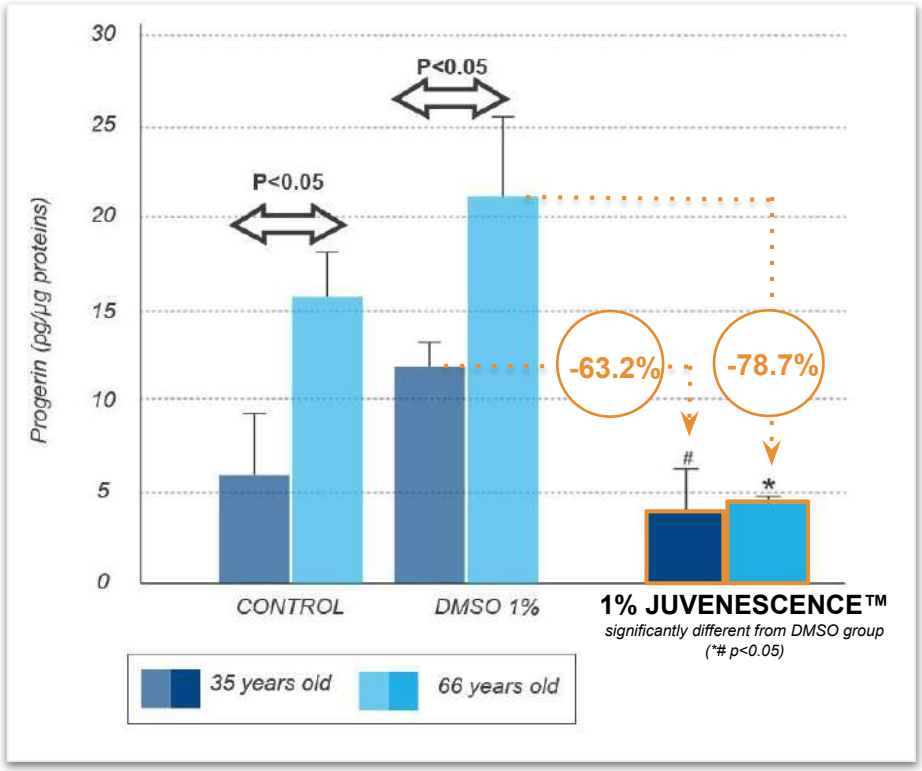
Reduction of the accelerated aging protein: PROGERIN

ASSESSMENT OF **JUVENESSENCE™** ON THE PROGERIN PRODUCTION

Results



At 1%, JUVENESSENCE™:

- **Down-regulates the Progerin production in aging cells:**

-78,7% *(66 years old donors)* **&** ***-63.2%*** *(35 years old donors)*

- **Brings Back the Progerin levels at the rates observed on young cells** (35 years old)

Summary

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Metabolism Reactivation
of Aging Cells

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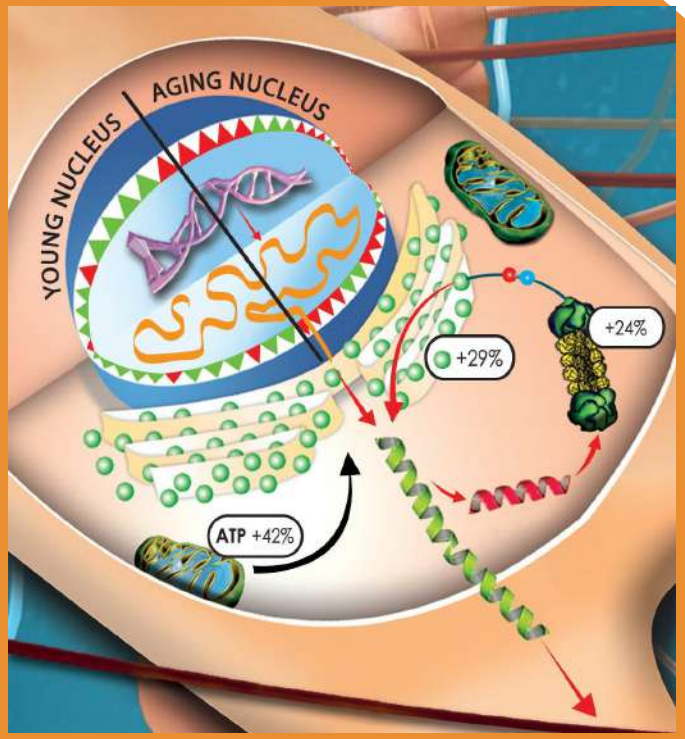
Metabolism reactivation of aging cells

All along the aging process, the ribosomal, mitochondrial & the proteasome activities are affected.

These decreases disturb the whole "skin cell machinery", and more specifically the skin protein synthesis mechanism, as well as the detoxification of aging proteins



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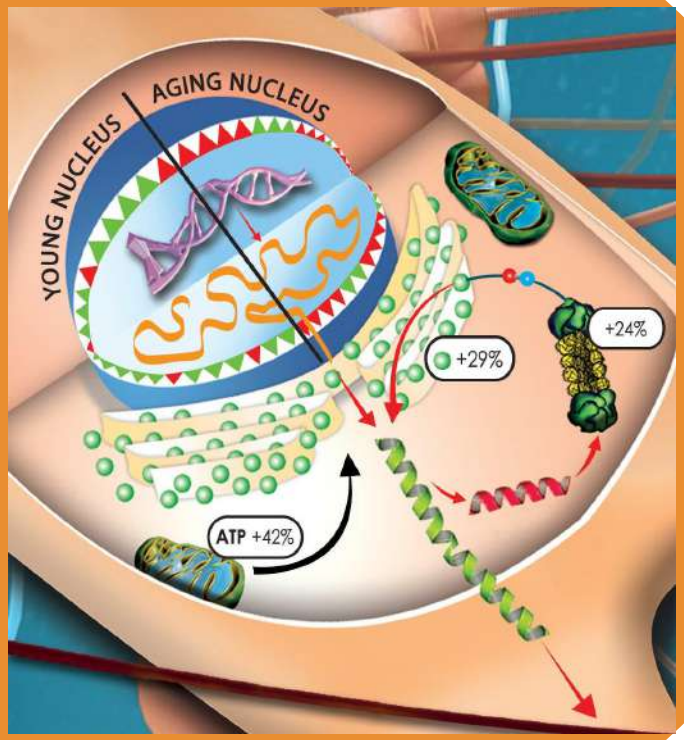
Metabolism reactivation of aging cells



JUVENESSENCE™ 1%
reactivates the metabolism of aging cells by stimulating:

- the detoxification (**+24%**),
- the mitochondrial activity (**ATP: +42%**),
- malate shuttles (**+31%**),
- cell viability (UVB): **+20%**,
- the ribosomal activity (**+29%**).

Summary

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Regulation of Photo-aging Mediators

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Regulation of photo-aging mediators & immunoregulation

Skin,

in direct contact with the environment, undergoes aging as a consequence of U.V. exposure & environmental damages.

- **Sunlight** has a profound effect on the skin **causing premature skin aging**.
- **Chronological aging & photo-aging** share fundamental molecular pathways.
- **UV** stimulates and activates various cells & tissues to produce & release cytokines that may play a **significant role in the process of photo-aging**.
- Indeed, **photo-aging is regulated by a delicate balance** between the deleterious effects of cytokines leading to photo-aging & their beneficial effects improving UV-caused damages.

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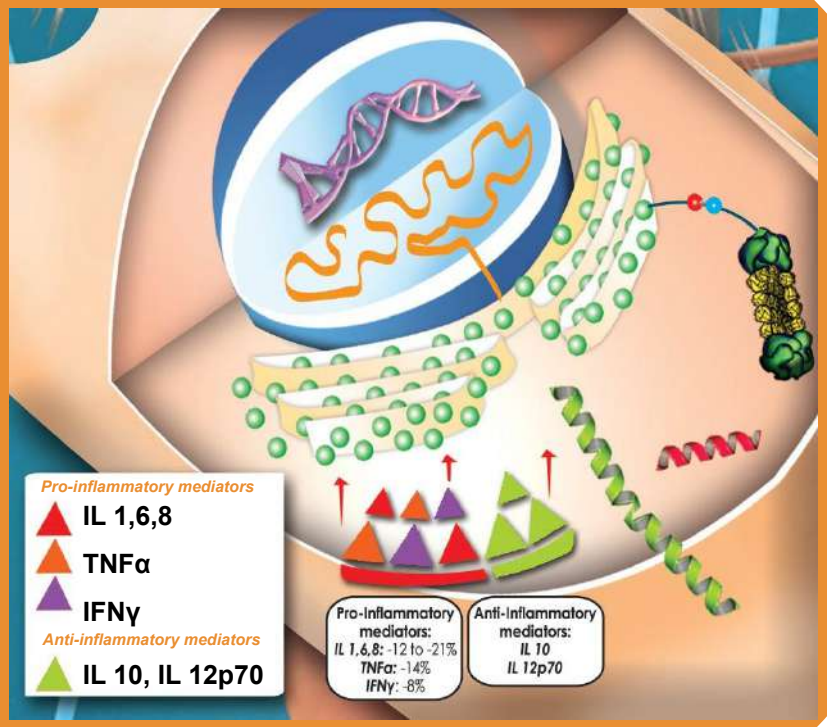
Regulation of photo-aging mediators for a soothing benefit

JUVENESSENCE™ 1%
plays an important role to **maintain homeostasis** in damaged skin by **regulating the production of mediators**, and more specifically:

Pro-inflammatory mediators:
- Interleukins 1, 6 & 8 (**-12%** to **-21%**)
- TNFα (**-14%**)
- IFNγ (**-8%**)

Anti-inflammatory mediators:
- Interleukins 10
- Interleukins 12p70



Summary

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In Vivo Studies

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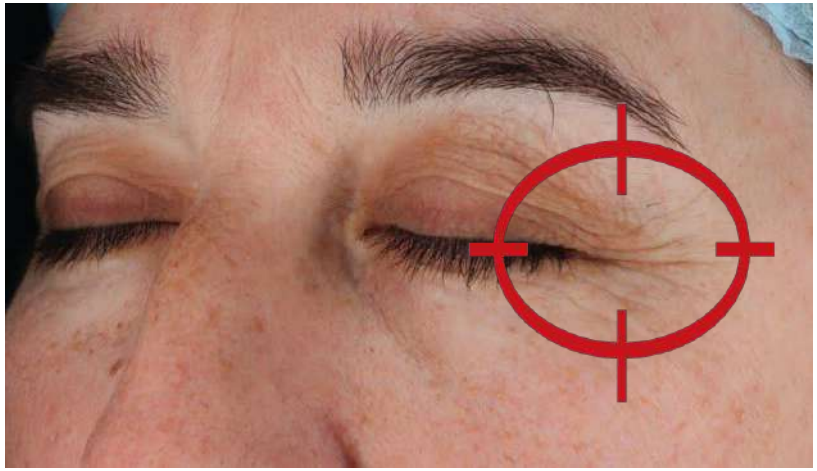
Anti-aging benefits - *In vivo* study 1/2

ASSESSMENT of the EFFICACY
of a cream containing **JUVENESSENCE™ 1%**,
applied **ON THE CROW'S FEET AREA**

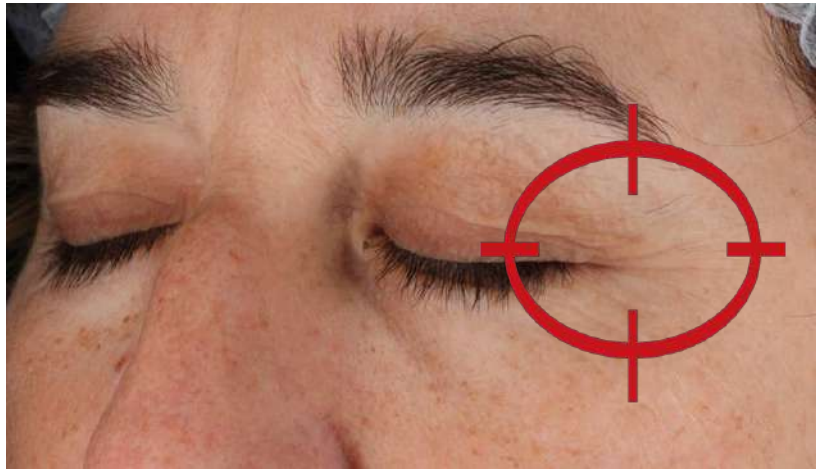
In vivo protocol

- Study during 28 days, on 20 subjects with periorbital wrinkles
- Average age: 50 years old
- Cream applied twice a day
- Measurements with **Cutometer**

*Subject at **D0***



*Subject at **D28***



CUTOMETER STUDY

Results

↗ **Firmness: +25% vs D0** & ↗ **Elasticity: +20% vs D0**

↘ of the **number of wrinkles** for **65%** of the volunteers & ↘ of the **depth of wrinkles** for **80%** of the volunteers

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Anti-aging benefits- *In vivo* study 2/2

ASSESSMENT of the EFFICACY
of a cream containing **JUVENESSENCE™ 1%**,
applied **ON THE CHEEKS**

In vivo protocol

- Study during 28 days, on 20 subjects with a skin lacking firmness
- Average age: 50 years old
- Cream applied twice a day, under dermatological control
- **At D28, assessment of skin quality & firmness** (self-evaluation)

*Results at **D28***

Dermatologist
Firmness: +10%

&

Self-evaluation
90% of the volunteers noticed an **increase in Firmness , after 28 days**

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Anti-aging benefits - SELF ASSESSMENT

% OF THE PANEL PERCEIVING A **BENEFIT**, **after 28 days of treatment**

*Results at **D28***

In vivo: crow's feet

Number of wrinkles: **65%**

Depth of wrinkles: **80%**

Radiance: **70%**

Hydration: **85%**

In vivo: cheeks

Skin Tonicity: **85%**

Firmness: **90%**

Skin Grain Improvement: **85%**

Hydration: **70%**

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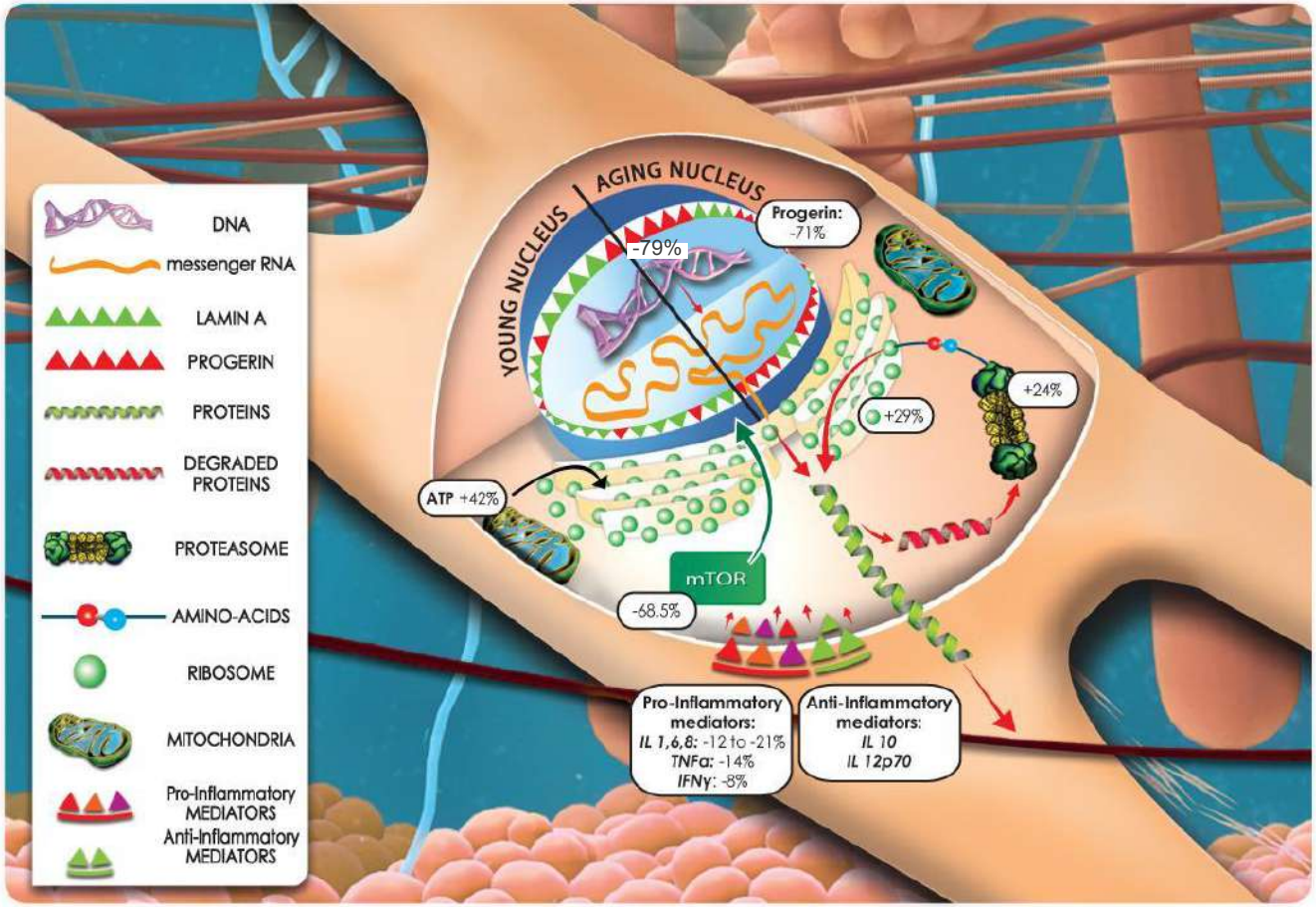
Summary



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Annexes

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ALARIA ESCULENTA, the Winged Kelp

Biogeography

Alaria esculenta is a kelp of Arctic/cold-temperature distribution, with its Southern distributional limit in the East-Atlantic at the coasts of Brittany and stretching northwards up to the coasts of Spitsbergen, as far as the winter sea ice. (Kraan et al, 2000). This species is common in the Faeroes, Greenland, Iceland (where it is eaten raw or cooked), and its absence in the southern North Sea and English Channel supports the hypothesis that the distribution of Alaria esculenta is temperature controlled. Indeed, its southernmost habitat in Europe is Brittany, France, in waters that do not exceed 16°C in temperature. (Munda, 1977)

This perennial seaweed, member of the Alariaceae family (Phylum Ocrophyta, Class Phaeophyceae), populates sublittoral zones, between 3-10 m depth on exposed rocky shores, while in very exposed sites, on vertical, very steep rocks it has been recorded at a 35 m depth.

Its growth is extremely fast, compared to other seaweeds, reaching up to 10 cm per day (Kraan and Guiry, 1998). Alaria esculenta can reach a length up to 6 m. Its short stipe gives rise to a long, thin, ribbon-like, wavy blade with a well-defined rib. The color is olive-green, and when touched, the blade feels satiny and flexible.

Life on the rocky shore

In some biotopes of the Arctic waters, Alaria forms large canopies, like other large laminarias do, often found in dense underwater forests. But this is usually not the case for Alaria in Britanny, where it is often accompanied by Laminaria flexicaulis and Corallina officinalis (Dizerbo, 1949). Here, it is exposed to violent surf and to the great pressure that waves create, of over 6t/m². More than any other seaweed, Alaria is conditioned to constant pounding by the waves. Holding on to the sloppy rocks, it can withstand the punishment that over 8000 waves dish out over the period of one day. On such a rocky shore, too dangerous to offer safe footing, only the seagulls enjoy the mad choreography that Alaria's ribbons engage into, back and forth on the surface of the water, tugged and tossed in a rhythm borrowed from the sea.

It is precisly this fate teasing dance, this constant movement of the algal fronds that enables Alaria to enhance photosynthesis, by reducing self-shading and generating light flecks. Contrary to what one might expect, productivity and growth reveal themselves very high on wave-beaten shores. The action of drag forces stimulates carbon uptake and seaweed competitors may be removed by whiplash, while the wave action can also reduce herbivore grazing (Lobban, 1994).

Both Alaria's chemistry and its morphology are adapted for living in the surf zone. The fertile pinnae in which the reproductive cells mature, are borne at the base of the frond, as this location is safer than the tips of the main blade. This is not the case for other rockweeds living higher on the shore and less subject to savage wave action, where the reproductive cells are formed at the tips of the fronds (Carson, 1998). Despite the apparent fragility of the translucent olive-green blade, its tissue, a web of elastic fibres, contains large amounts of alginic acid and its salts, which create a tensile strenght and elasticity able to withstand the pulling and pounding of the waves. It was shown that the blades of wave-exposed laminarias have greater proportions of polyguluronate (strong, rigid gels) than those of wave-sheltered specimens (Venegas et al 1993, Lobban, 1994) and large proportions of cell wall polysaccharids and different forms of these, with their distinctive gelling properties are found in different regions of seaweeds (blade vs stipe)

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ALARIA ESCULENTA, the Winged Kelp

The science drop

Alaria's biomass has been described as carbohydrate rich, with the main carbohydrate, alginate, accounting for 42% of the dry matter. (Schiener, 2013; Nwosu, 2010). In addition to structural carbohydrates, alginate and cellulose, laminarin, mannitol and fucoidan contents have also reported to comprise a significant proportion (Schiener, 2013).

The protein level is of about 11%, which is considerable for a brown seaweed, since, generally, brown seaweeds have lower protein levels than the red and green seaweeds.

High level of moist and ash (approx. 25 %) were measured during winter months in the same study. The ash content of Laminariales consist mainly of the ions- sodium, potassium, calcium, magnesium – with chloride and sulfate as the main counter-ions. Along with these macro-nutrients, trace metals such as Fe, Zn, Mn, Al and Cu have been identified. (Schiener, 2013; Schumacher, 2011)

Alaria esculenta was shown to be a species of high phenotypic plasticity, its acclimating capacities enabling it to respond to short-term stresses. As Wienke and Bischof show (Seaweed Biology, 2012), seaweeds populating a flexible environment require mechanisms of acclimation in order to set physiological performance to the variation of environmental requirements. Acclimation to UV exposure has been demonstrated in Alaria esculenta. Under repetead UV exposure, the competence of recovery from UV-induced photoinhibition increased after just a few exposure/recovery cycles, which might indicate an activation of different repair mechanisms, counteracting the impact of UV exposure by a faster replacement of damaged molecules. Moreover, the degree of inhibition became smaller, which might also be related to an activated ROS defense system counteracting UVB mediated oxidative stress, or the formation of UV-screening compounds, like phlorotannins. Indeed, an increase in the size of phlorotannin containg vesicles (physodes) was observed in Alaria esculenta after UVB exposure, indicating an induction of phlorotannin synthesis (C. Wienke and K. Bischof, 2012)

Seaweeds, as photosynthetic organisms, are exposed to a combination of light and high oxygen concentration at the origin of the formation of free radicals and other oxidative reagents. But the awareness of the lack of structural damage in their organs has led the scientific community to consider that their protection against oxidation comes from their natural content, or production under stress, in antioxidant substances. Indeed, macroalgae are particularly rich in natural antioxidants, e.g. phlorotannins, ascorbic acid, tocopherols and carotenoids. In a study carried on several Phaeophyta from the Brittany coast (Zubia et al, 2009) a significant correlation was found between the high antioxidant activity and the high phenolic content. Alaria esculenta was shown in this study to possess important antioxidant and antitumoral activities. Further anti-proliferative and anti-diabetic effects were discussed in a study by Nwosu et al, 2010. The multifunctional antioxidant activity of polyphenols was discussed by Wang et al 2008 in a study that invites to broadening the reflexion towards the research of synergistic effects of other active components than just phlorotannins, like maybe low-molecular-weight polysaccharides, proteins or peptides that could also contribute to the scavenging effect.

As determined for the Arctic Kongsfjord (Lippert et al 2001), Alaria esculenta is a species of utmost ecological relevance, hosting up to 51 epifauna species. Here, they act as food, habitat and nursery for multiple associated organisms (Fredersdorf et al 2009). On the Brittany coasts, the ecology of Alaria esculenta has not yet been precisely documented, but a close look at its growth sites would marvel the eye, for if at high tide nothing but Alaria's fronds show tossing in the wind, at low tide, when the waters retrieve, a kaleidoscope of colors and forms show their beauty along the rocks. The pink corallina algae, the electric blue Irish moss, the yellow sponge, brown rockweeds, or sea anemones like the solitary and very fashionable Sagartia elegans, all delicately stir with life, in a world of crushing force.

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ALARIA ESCULENTA, the Winged Kelp

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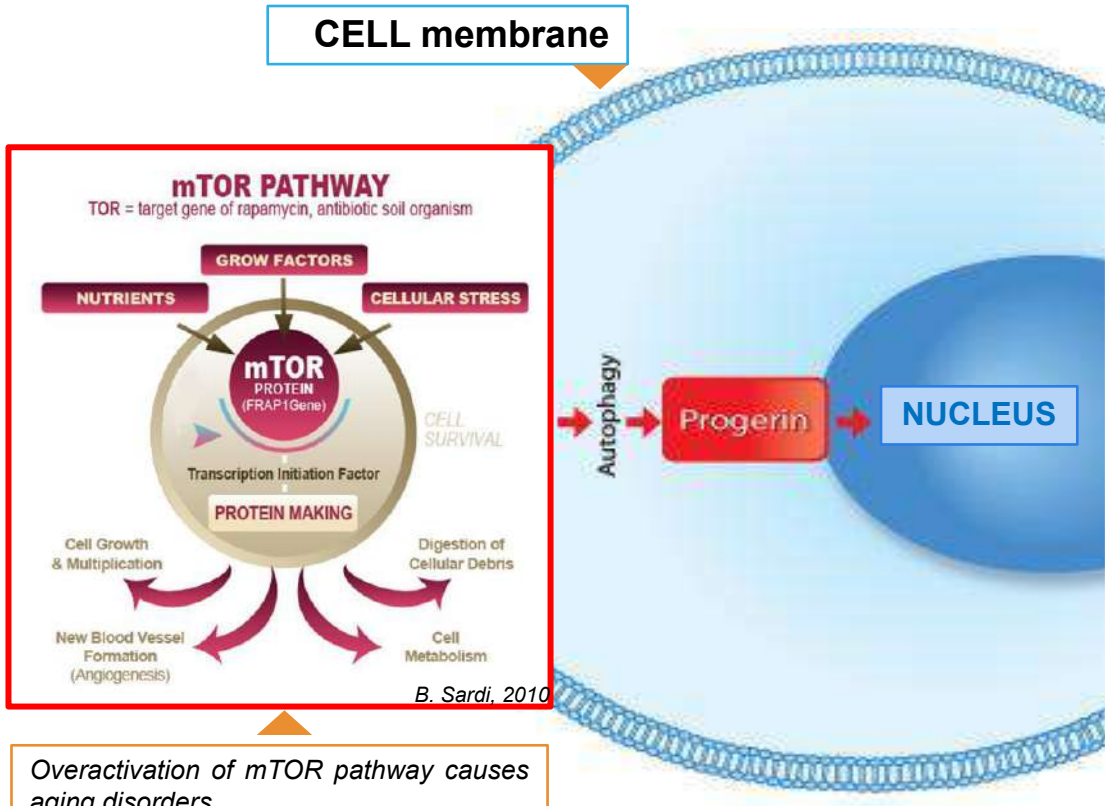
SENESCENCE AMPLIFIER & MTOR



Overactivation of mTOR pathway causes aging disorders.

*With age and stress, **mTOR production increases**, inhibiting the autophagy, the elimination of damaged proteins, as Progerin.*

mTOR protein
(Target Of Rapamycin mTOR): intracellular enzyme, discovered in 1994, having a kinase activity.

Essential role in the development & aging processes

Cellular Senescence
under the mTOR control.

A reduction of the activity of the mTOR signaling pathway increases the lifespan of various organisms

Activation by a membrane receptor, activated in its turn by signals (nutriments, stress…)

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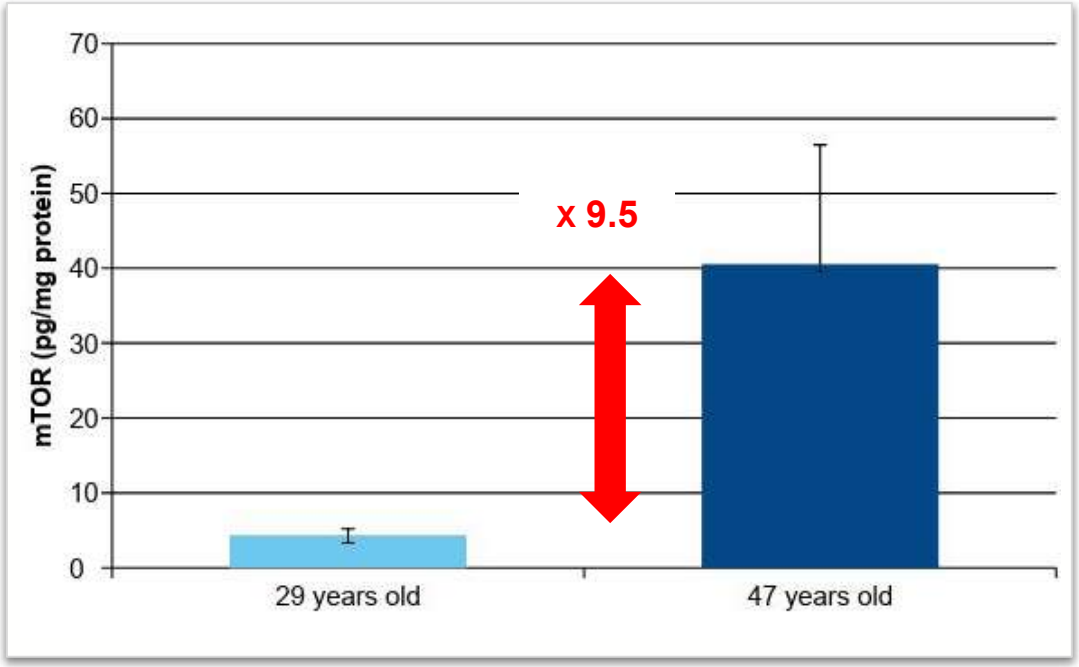
ACTIVITY OF JUVENESSENCE™ ON mTOR

Protocol

- Monolayer of human dermal fibroblasts: biopsies from women aged 29 & 47 years old
- Reference inhibitor: Rapamycin 100 nM (helps Progerin elimination from cells)
- Cells are incubated 96h, without the reference product, with the reference product, and with **JUVENESSENCE ™** (1% v/v)
- **Dosage of mTOR: quantification in cell medium by Elisa (specific)**

Results

Comparison of **mTOR production between young & mature human fibroblasts** in culture



mTOR production is significantly **superior** in **mature human cells** than in young ones.

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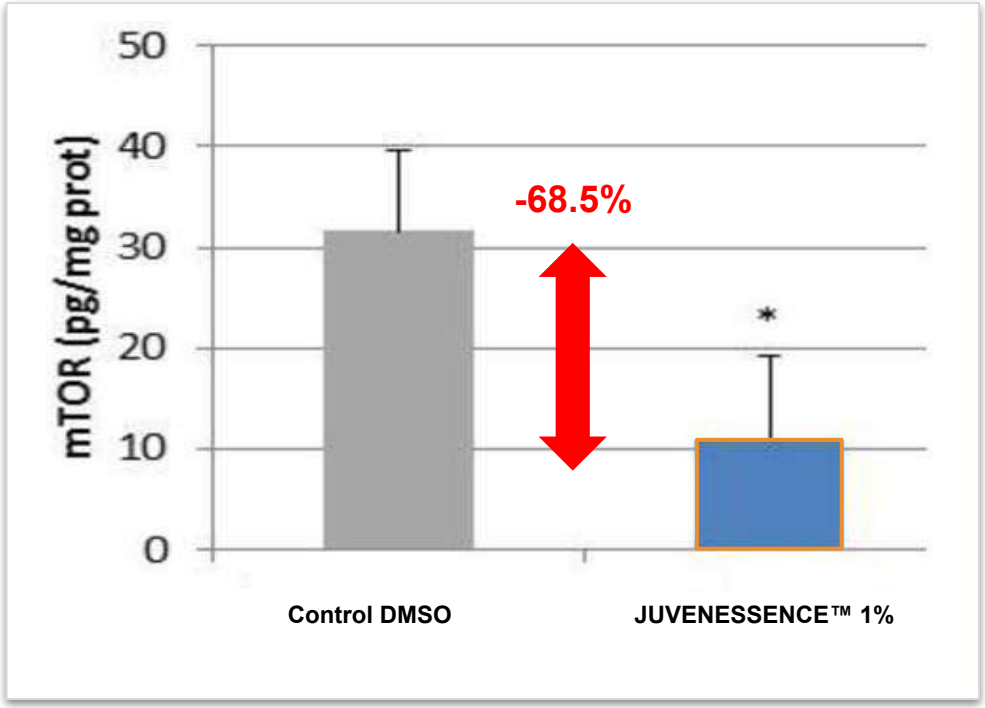
JUVENESSENCE™

ACTIVITY OF JUVENESSENCE™ ON mTOR

Results

Action of **JUVENESSENCE™ 1%** on mTOR production in mature human fibroblasts



JUVENESSENCE™ 1%
- **inhibits mTOR production: -68,5%**

Reference Rapamycin
- **inhibits mTOR production: -74%**

JUVENESSENCE™ 1% decreases significantly the mTOR production in **mature human cells.**

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